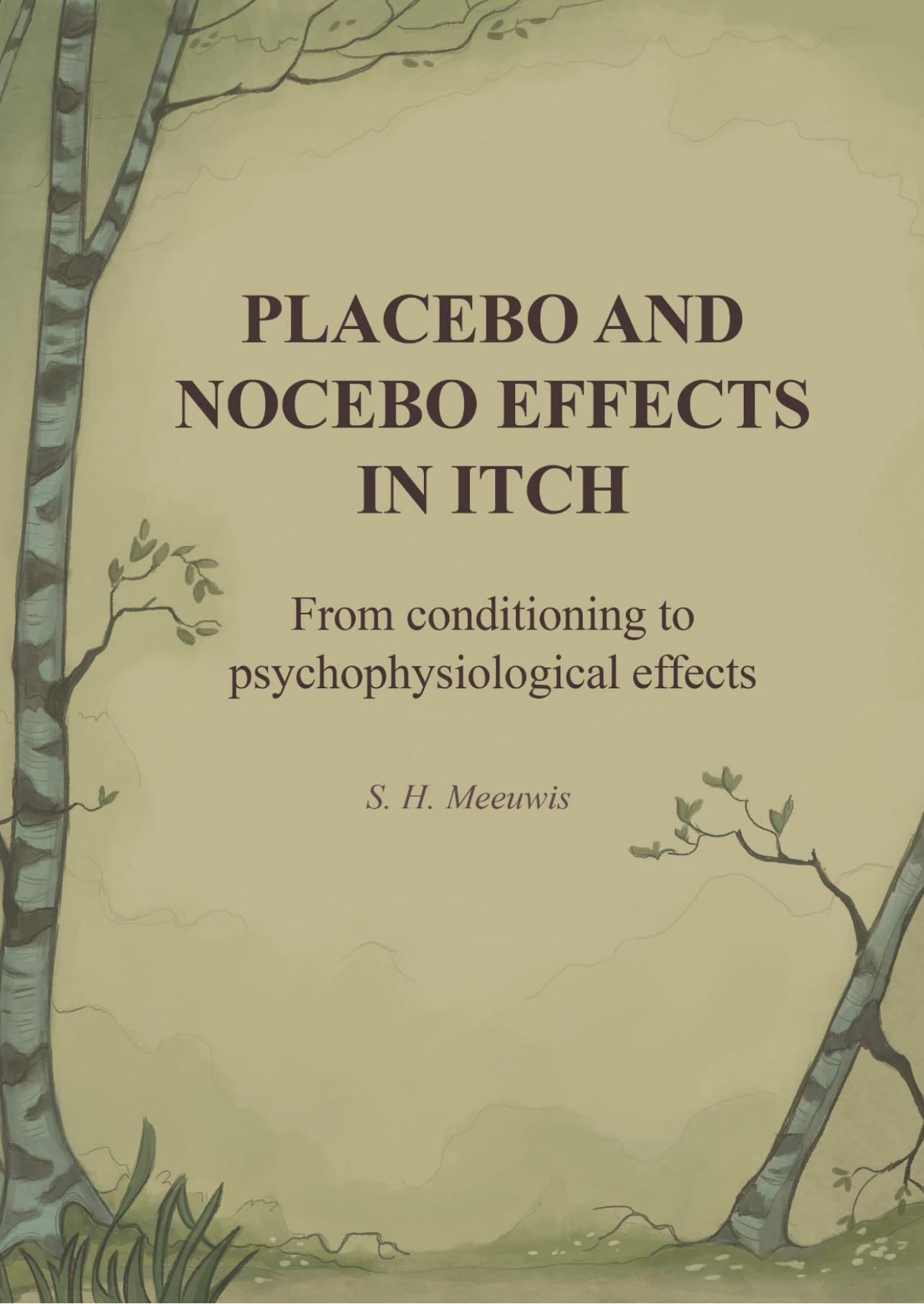

PLACEBO AND NOCEBO EFFECTS IN ITCH

From conditioning to psychophysiological effects

S. H. Meeuwis

Placebo and nocebo effects in itch

From conditioning to psychophysiological effects

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Chapter 1

General introduction

Placebos are inert substances (e.g., sugar pills) or other types of inert treatment forms [1]. Particularly in the field of medicine, placebos are used as a tool to which active pharmacological substances can be compared [2]. The rule of thumb involved is as follows: you have two groups of people ('A' and 'B'), give 'A' the real medicine, and give 'B' placebos instead – any improvement of 'A' over 'B' is indicative of whether the real medicine is effective [2,3]. At first glance, this appears very straightforward indeed. However, while simply looking at the difference between groups 'A' and 'B' in a clinical trial paints a clear picture of the efficacy of a specific medicine, it is also somewhat limited: this difference does not tell a patient exactly how much improvement to expect after taking said medication. For that, comparisons to a 'starting point' or baseline value are needed, and here the previously clear picture becomes blurry. Studies show improvement of symptoms within the control groups of clinical trials – so for the people who are taking placebos – across a wide range of medical conditions [4]. This type of improvement is generally attributed to contextual or nonspecific treatment factors. Moreover, these factors impact outcomes within the treatment groups of clinical trials as well: so the total improvement following medication use would then be the sum of the specific effects of the medication and the nonspecific treatment factors [2,3,5]. In reality this may be even more complex however. Research has shown that nonspecific treatment factors can interact with the efficacy of medication – and that the efficacy of medication can likewise impact nonspecific treatment factors, such as placebo effects [2-4].

Placebo and nocebo effects: concepts and definitions

Placebo effects are part of the nonspecific treatment factors that can impact or interact with the efficacy of medication, and may make up a significant portion of what makes a treatment effective. They are defined as beneficial treatment outcomes that cannot be attributed to active treatment components [6]. Rather, these effects are attributed to expectations of beneficial or positive treatment outcomes [7-11]. It is important to emphasize the difference between placebos and placebo effects: where *placebos* refer to inert treatments (e.g., sugar pills) that can be given to a person, *placebo effects* refer to *positive reactions* a person can show in response to inert substances or as part of active treatments, with these reactions being elicited by expectations of benefit [1,6]. Unravelling the specific mechanisms that underlie placebo effects and investigating their impact is important for two reasons: 1) this knowledge may help to improve research on the efficacy

of (new) treatments, and 2) knowing how placebo effects can be elicited may help to develop strategies to maximize them in clinical practice, which could then lead towards enhanced treatment outcomes, optimized medication use, and reduced side effect occurrences [6]. Placebo effects are attributed to expectancy and can be elicited by a variety of factors, for example, but not limited to: information about a treatment, previous experiences with treatments or otherwise learned associations of treatment and improvement, general beliefs about medicine, aspects of the patient-provider relationship, and other social or contextual cues [7-11]. On the opposite side of the spectrum are nocebo effects: negative or adverse treatment outcomes that are attributed to non-active treatment components [12,13]. Researchers have spent the last decades unravelling the mechanisms behind placebo and nocebo effects, and have identified three main mechanisms through which these effects may be induced: associative learning (i.e., conditioning), instructional learning (e.g., through verbal suggestions), and social or observational learning (e.g., by social cues in the environment) [14-16]. These different types of learning are proposed to shape an individual's expectations about treatment either positively (in case of a placebo effect) or negatively (in case of a nocebo effect). Theoretical models of the placebo effect, for example the response expectancy model [17] and the learning model of placebo effects [18], state that these modulated expectations can then influence the experience of symptoms of disease [19].

Both placebo and nocebo effects have been found to significantly impact health-related outcomes. For example, placebo effects have been found to reduce itch and other somatic symptoms such as pain, dyspnea, fatigue and nausea [20], and research shows that they can impact physiological parameters as well, for example, immune or endocrine responses [21-23]. Nocebo effects in contrast have been found to increase the experience of somatic symptoms, to result in increased side effects, or to result in reduced treatment efficacy [12,13]. One area in which placebo and nocebo effects may be relevant is that of dermatology [24].

Placebo and nocebo effects in dermatology: effects on itch

Itch, or pruritus, is commonly described as an unpleasant sensation that evokes the urge to scratch, and is considered chronic if it lasts for over six weeks [25-28]. This symptom is a key marker of most cutaneous conditions, for example allergic disorders, atopic dermatitis or eczema, urticaria, psoriasis, and lichen simplex [29, 30]. Listed as the fourth leading

world-wide cause of non-fatal disease burden, skin diseases have a major social, societal and economic impact [31]. Especially in skin diseases, the burden of itch is high, with an estimated lifetime prevalence of itch set at 100 percent for patients (whereas in the general population, estimated prevalence ranges from 7-22%) [32]. Finding strategies to reduce this burden of disease therefore remains a priority for scientific research. In addition, itch is a common symptom for non-dermatological conditions. It has been often reported in systemic, uraemic, neurological, or endocrine diseases (for example, kidney failure, multiple sclerosis, or diabetes mellitus), and is also prevalent in some psychiatric conditions [28,33,34]. For these conditions, itch often also has a considerable impact on quality of life and wellbeing of patients [34].

Depending on the origin of the itch sensations, different classifications can be identified [33,35]. A common classification of itch is by the pathway through which it is evoked: histaminergic, or non-histaminergic [36,37]. Although several signaling chemicals are known to evoke itch, histamine is investigated most frequently [27,28]. Treatment of histaminergic itch often consists of systemic treatment with H_1-receptor antagonists, commonly referred to as antihistamines, or topical agents such as corticosteroids. However, these treatments usually have low efficacy or result in significant side effects [33,37], thus increasing the need for formal investigation into approaches by which the efficacy of existing treatments may be enhanced. Moreover, considering the broad range of conditions for which itch occurs and its debilitating nature, it is important to find ways to reduce this symptom and thereby positively impact patients' wellbeing. One of the ways to do this is by strategically using placebo effect mechanisms [24].

Strategies for inducing placebo and nocebo effects

Studies on the phenomenon of 'contagious itch' – itch that is induced by visual, auditory or other contextual cues – show that social and psychological factors can play an active role in determining the severity of itch that is experienced by patients [38-40]. Contagious itch therefore may indicate that placebo and nocebo effects could potentially play a large role in itch. Moreover, while most studies on placebo and nocebo effects focus on pain and pain-related conditions (for a review of placebo and nocebo effects in pain see, for example, [41]), there is also evidence that these effects occur in itch. A meta-analysis shows that in control arms of clinical trials with patients suffering from dermatological conditions, over 30 percent of itch reduction can be attributed to placebo effects [42]. In addition, several

studies have investigated whether placebo and nocebo effects for itch can be experimentally elicited by associative or instructional learning.

Associative learning: classical and pharmacological conditioning

Originally described by Pavlov, classical conditioning entails the learning process by which (new) associations between stimuli are formed [43,44]. In short, an association is made by presenting an initially neutral stimulus (thereafter the conditioned stimulus, CS) together with an unconditioned stimulus (UCS) that is known to elicit a certain response (unconditioned response, UR). After the CS and UCS have been presented together, the CS will then elicit a response that is similar to the UR by itself, even when the UCS is not presented (this is known as the conditioned response, CR; see **Figure 1**) [22,44]. Studies show that these classical conditioning procedures can be used to modify itch levels in healthy volunteers. To illustrate, one recent study investigated effects of conditioning on itch by combining visual cues (i.e., various colors) with high and low levels of itch. After these CS's and UCS's were repeatedly paired together in a learning phase, the visual cues were then found to influence the amount of itch that participants reported when they were presented together with a medium level of itch during a testing (evocation) phase [45]. Learned associations like these could be what drives the placebo effect: positive expectations trigger actual symptom reduction when certain visual cues are presented. The type of classical conditioning that was investigated in the study described above [45] has been found to occur mostly on a cognitive level however – in other words, the association between CS and UCS needs to be made consciously by participants and, more importantly, the UCS consists of changes in itch that are made by manipulating the experimental procedure (i.e., exogenous change). Placebo effects may also be learned by conditioning responses on a more endogenous level: through classical conditioning of pharmacological responses [46,47]. In studies investigating conditioned pharmacological effects, the UCS typically is a substance known to elicit a certain physiological response, such as an active medicine [23,24]. For example, patients can learn that treatment cues (e.g., the color of a pill; CS) belonging to a pharmacologically active substance (painkiller, UCS) are associated with certain treatment effects (pain reduction; UR). These treatment cues could then prompt the same response (CR), which is what we know as the placebo effect (i.e., itch or pain reduction after taking a colored pill, even when active pharmacological substances are absent; see **Figure 1**).

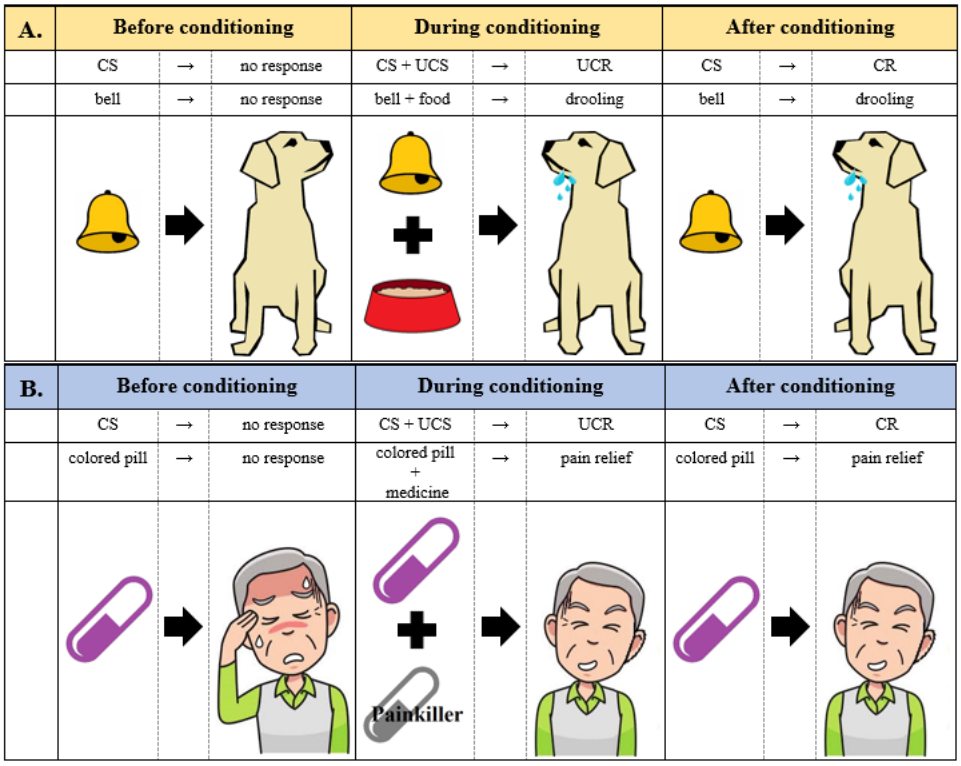

Figure 1. Schematic overview of (A) Pavlovian and (B) pharmacological conditioning: before conditioning, the (initially neutral) to-be conditioned stimulus (CS) causes no response. During conditioning, the CS is coupled with an unconditioned stimulus (UCS), that elicits an innate (unconditioned) response (UCR). After conditioning, the CS provokes a similar (conditioned) response (CR), even in the absence of the UCS.

Research demonstrates that it is possible to modulate immune functioning with pharmacological conditioning [47-50]. For example, studies show that, after having been conditioned with the immunosuppressive drug cyclosporine A as UCS, a saccharin solution can significantly reduce blood serum levels of interleukins, and thereby reduce the physiological response to immune challenges (e.g., viruses or allergens) in animal and human models of allergy [49,50]. Likewise, this type of conditioning can also increase immune responses, for example when a CS is coupled with a substance that challenges or sensitizes immune functioning, such as an allergen. Allergic responses were found to be sensitive to these conditioning effects [51-53]. For example, cases are known where patients who are allergic to roses have developed allergic asthma attack when presented

with artificial roses [54]. Such learned allergic responses to inert stimuli may exacerbate existing allergic disorders, which could be interpreted as a nocebo effect. There is also evidence that conditioned immunosuppression can be used to reduce allergic symptoms that are elicited through histaminergic pathways [55,56]. Goebel and colleagues [55] found that conditioning with the antihistamine desloratadine (UCS) could influence the basophil response to dust mite allergens on a level comparable to actual drug effects in humans, although they did show that effects of conditioning on self-reported allergic symptoms (including itch) and skin response to dust mite were less evident.

A second study complemented these findings by showing that allergic symptoms and physical responses to histamine reduced in both the conditioned and sham-conditioned (i.e., receiving a CS without UCS) groups compared to a natural history (i.e., no intervention) group [56]. Given that not only the conditioned group but also the sham-conditioned group showed reduced symptoms, it is likely that these reductions can be attributed to factors other than pharmacological conditioning [56]. Taken together, these two studies show mixed evidence for the efficacy of antipruritic conditioning of the effects of antihistamines for allergy [55,56]. However, as noted by the authors of both studies, a number of factors may have impacted study findings, such as elicitation of symptoms through non-histaminergic pathways, regression to the mean, receiving an intervention (regardless of this being a sham or active intervention), or potentially, participants' own previous experiences with antihistamines. More research is needed in order to unravel whether conditioning of antihistamines is possible in humans.

Instructional learning: the impact of positive and negative verbal suggestions

Where conditioning may largely rely on one's ability to associate one cue with another (which would then impact expectancy), another type of placebo and nocebo effect induction often used in laboratory settings is to alter expectancy by instructional learning. This type of learning does not rely on prior experience (as associative learning does) or on observations of others (as social learning does), but on communication of information or advice [57-61]. Placebo and nocebo effects can be elicited by instructional learning, for example when suggestive information is given about the effectiveness of a certain treatment (i.e., verbal suggestions) [14,16,22]. These suggestions can elicit positive or negative expectations, which would then in turn impact symptom perception [14]. Previous studies have shown that verbal suggestions can influence somatic symptoms, for example of pain,

fatigue, and nausea [20]. Evidence for the efficacy of verbal suggestions in itch varies: some studies show that placebo and nocebo effects in itch can be elicited by verbal suggestions [62-65], whereas others show mixed evidence, with suggestions eliciting nocebo but not placebo effects [66], or fail to show effects on itch of verbal suggestions alone [45,67,68]. Moreover, the methods used to elicit itch vary across studies and only a few have investigated histaminergic itch induction. A single study indicated that nocebo responses to verbal suggestions in physical responses to histamine (i.e., wheal or flare response) could be provoked [63], however, most studies report finding no significant changes in physical parameters following verbal suggestions [62,67,69]. Considering the mixed evidence, more research is needed to investigate whether placebo and nocebo effects could be induced for itch specifically through verbal suggestions.

Across studies variations in the type of verbal suggestions that are employed to elicit placebo and nocebo effects are found. For example, some studies give suggestions of high or low itch because of changes in the pain or itch induction method (also known as placebo- and nocebo-like responses) [45,66,70], whereas others give suggestions about a dummy treatment (e.g., an inert cream) provided alongside the pain or itch induction [62,67,69]. More research is needed in order to identify how and under which circumstances verbal suggestions may elicit placebo and nocebo effects. It should be clarified what type of information and which environmental cues can elicit placebo effects, as this knowledge could be used in clinical practice by health care providers, for instance to maximize positive expectations while informing patients who start new treatments. Knowing which manner of information provision may or may not be helpful could then be used to improve patient-provider communication, and by that enhance placebo effects and prevent nocebo effects in clinical practice.

Open-label placebo effects

Knowing how to best inform patients about treatments, and using this knowledge to improve patient-provider communication may be a potential strategy for utilizing placebo and nocebo effects mechanisms to improve healthcare. At a first glance it appears that this might in fact be the only way to use the knowledge on placebo and nocebo effects in clinical practice in an ethical and non-deceptive way [71,72]. After all, any use of inert substances or covert changes in medication dosages – which are common techniques used to study placebo and nocebo responses in laboratory experiments – would be considered

unethical in clinical practice as these involve deception [73]. Patients should be fully informed about which treatment they receive, and any attempt to circumvent this could harm a patient or challenge their autonomy. Because of this, the means by which placebo and nocebo effects are traditionally investigated in the laboratory (i.e. by providing inert substances under guise of an active treatment) cannot be immediately translated to clinical practice. In the last decade, however, research has shown that it may be possible to induce placebo effects in clinical practice without involving deception [74-76].

It has been found that providing inert pills to patients alongside a rationale that explains how subsequently elicited placebo effects could impact symptomatology can reduce self-reported symptoms for patients suffering from irritable bowel syndrome [77], chronic low back pain [78], attention deficit hyperactivity disorder [79], and allergic rhinitis [80,81]. Most of these aptly dubbed 'open-label' placebo effects are elicited on top of treatment as usual. Because of this it remains unclear by which aspects (or combinations thereof) open-label placebo effects are elicited [75]. For example, it may be possible that the effects of the open-label placebos are evoked by the provided explanation (instructional learning), or that the inert pills alone are enough to elicit improvements in symptomatology (through classical conditioning mechanisms). However, it may be equally likely that the open-label placebo could interact with treatment as usual and that this may enhance those pharmacological effects. For example, it may be possible that the open-label rationale (i.e. explaining the role of learning and expectations) interacts with expectations about or the pharmacological effects of treatment as usual, or that it impacts other components of treatment (e.g., patients' belief in treatment efficacy) and influences symptomatology through those components. Therefore more research into the specific mechanisms of open-label placebo effects is necessary. Likewise, the efficacy of open-label placebos for histamine-induced itch is unclear. Considering that placebo effects for itch appear to be substantial, it may be of interest to investigate them in an open-label (placebo) context as well. Finally, no study to date has investigated whether pharmacological conditioning of antihistamines for itch may be effective in an open-label context.

In short, placebo and nocebo effects can be elicited through various pathways, among which pharmacological conditioning and verbal suggestions. Previous literature indicates that placebo and nocebo effects may be relevant for the field of dermatology and itch. However, little is known about whether pharmacological conditioning with antihistamines may impact itch specifically, and evidence for the influence of verbal suggestions (providing either positive or negative information) on itch is oftentimes mixed. In addition,

placebo and nocebo effects for itch have not yet been investigated in an open-label context, either in case of placebo effects elicited through pharmacological conditioning, or placebo and nocebo effects elicited through verbal suggestions. Doing so may be a first step towards therapeutic application of placebo and nocebo effects and may help in improving existing treatments for itch.

The current dissertation

In this dissertation, placebo and nocebo effect inductions for histaminergic itch are investigated using multiple approaches in various randomized controlled studies, i.e., pharmacological conditioning and positive and negative verbal suggestions in both an open-label context as well as a closed-label context (i.e., concealed, with participants not knowing about the placebo or nocebo effect induction). Moreover, effects of these methods on other (psycho)physiological responses to histamine are addressed. An overview of the outline of this dissertation is provided in **Figure 2.**

In **Chapter 2**, studies using experimental placebo and nocebo effect induction methods within the field of dermatology are systematically reviewed. Evidence for placebo and nocebo effects elicited in cutaneous conditions, in symptoms of the skin and mucous membranes associated with itch, and in relevant experimental animal and human models is summarized. The impact of different placebo and nocebo effect induction methods on three broad categories of outcomes (self-reported, physiological, and behavioral) is reviewed and differential aspects of studies (i.e., different designs) are compared. Potential implications for clinical practice are discussed.

In **Chapter 3**, the design and results of a randomized controlled study are presented and discussed. In this study, the possibility of pharmacologically conditioning the antipruritic effects of antihistamines in healthy volunteers is assessed. Moreover, the potential of non-concealed, or open-label, use of conditioning for influencing itch is explored for the first time. Effects of (open-label) conditioning on other (psycho)physiological parameters are assessed, and the role of individual characteristics (e.g., expectations, personality) in eliciting placebo effects for itch is explored.

Chapter 4 describes the first in a series of three randomized controlled studies investigating the efficacy of open-label suggestions for itch. In this study, the effects of open-label positive verbal suggestions about the itch induction method are investigated for

itch and other responses to histamine in healthy volunteers and compared to neutral instructions.

In **Chapter 5**, a study is presented that was conducted as a follow-up study to the one described in Chapter 4. In this follow-up study, we assessed whether verbal suggestions about an inert substance (i.e., a sham tonic) can influence itch and other responses to histamine in healthy volunteers. Effects of positive verbal suggestions and negative verbal suggestions are compared. Moreover, the efficacy of verbal suggestions in influencing itch is assessed for both an open-label context and a closed-label (i.e., concealed) context.

The final of the three studies on the efficacy of open-label suggestions is described in **Chapter 6**. Here, it is investigated whether open-label and closed-label positive and negative suggestions can influence itch in healthy volunteers, when those suggestions are about side effects rather than treatment effects. As in the previous studies, the effects of the suggestions on itch and other responses to histamine are assessed.

Chapter 7 is the summary and main discussion of this dissertation. Here, the results of the conducted studies are summarized and discussed in light of other work in this field and possible clinical implications.

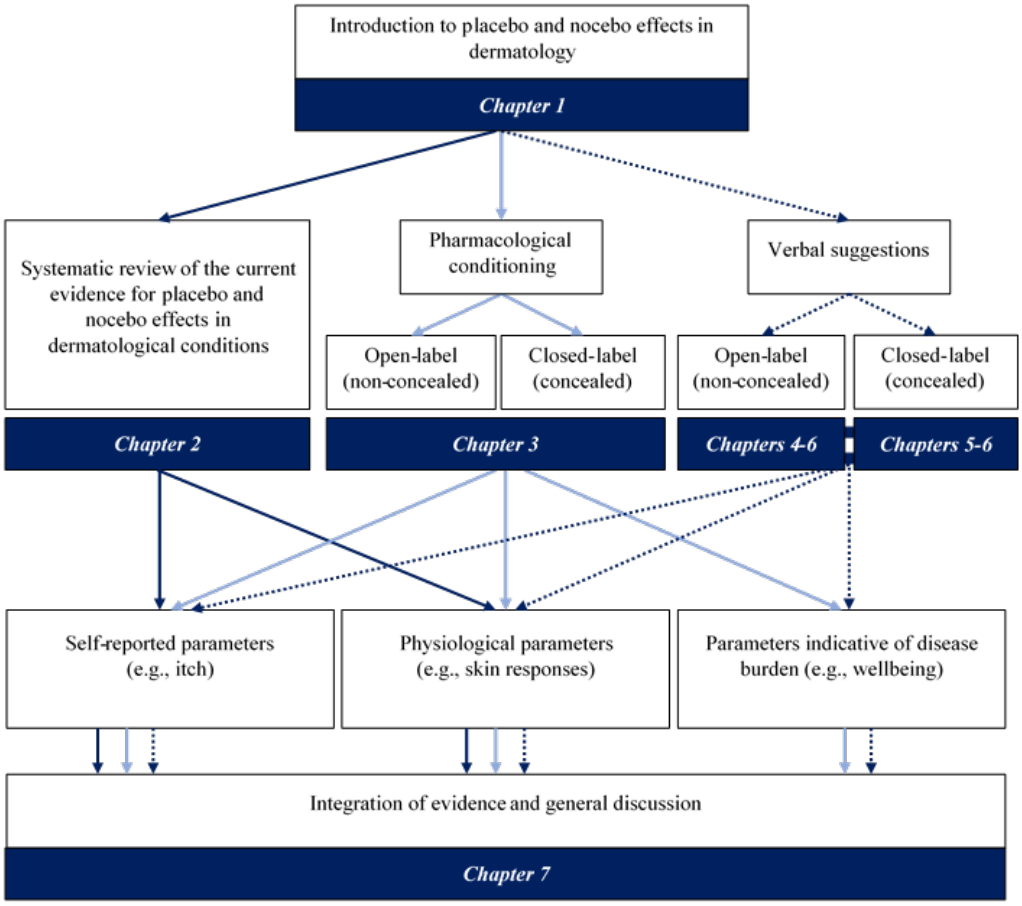

Figure 2. The outline of this dissertation and a brief overview of the topics within each chapter.

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Chapter 2

Placebo and nocebo effects across itch and dermatological conditions: a systematic review

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ABSTRACT

Placebo and nocebo effects have a large influence on somatic symptoms such as pain. For itch and other dermatological symptoms these effects have been far less investigated. The current review systematically integrates for the first time evidence from both animal (mainly rodents, but also non-human primates) and human trials on the elicitation of placebo and nocebo responses in itch, itch-related symptoms and conditions of the skin and mucous membranes, and related immune outcomes (e.g., histamine). Thirty-one animal studies, twenty-one human studies with healthy participants, and thirty-four human studies with patients were included. Overall, studies consistently show that placebo and nocebo effects can be induced by various methods (e.g., suggestions, conditioning and social cues), despite a high level of heterogeneity across studies. Effects of verbal suggestions were found consistently across subjective (e.g., itch in humans) and behavioral (e.g., scratching in animals) parameters, whereas conditioning was likely to impact physiological parameters under certain conditions (e.g., more pronounced conditioning of histamine levels in stressed rodents). Brain areas responsible for processing of itch were associated with nocebo effects in itch. Future research should investigate how variations in methods may impact placebo and nocebo effects, and whether all symptoms and conditions can be influenced equally.

INTRODUCTION

Placebo and nocebo effects are known to influence symptom severity and treatment efficacy in various medical symptoms and conditions [1-4]. Placebo effects can be described as beneficial effects that are not due to a (pharmacologically) active treatment component, but are rather elicited by contextual cues, or by positive expectations regarding treatment outcomes [5,6]. Nocebo effects are adverse treatment outcomes (e.g., increased side effects, reduced treatment efficacy) elicited by non-active treatment components [5]. Studies show that placebo and nocebo effects can be experimentally induced by, among other things, conditioning (associative learning), expectancy manipulations through providing positive or negative information (verbal suggestions) about treatment outcomes (instructional learning), or by social cues (e.g., learning by observing others) [6-8]. In addition, some work suggests that placebo effects may still occur when it is known that a placebo is given (open-label placebo) [9-13].

Placebo and nocebo effects have been found to impact various somatic symptoms such as pain and itch [3]. Itch is a key symptom of many dermatological conditions [14,15], has a high impact on patients' quality of life and has high economic costs [16-18]. The estimated lifetime prevalence of itch in the general population is 7-22%, and in patients with a skin disease estimates are set on 100% [19]. Most often, itch is evoked in the skin by mediators (e.g., histamine) eliciting changes in the chemical environment that are detected by C nociceptive fibers (capable of transmitting noxious stimuli, including itch and pain) to regions in the brain stem, the thalamus, somatosensory cortex, as well as areas involving emotion and reward [20]. A meta-analysis shows that at least 30 percent of itch reduction in randomized controlled trials can be explained by placebo effects [21]. Research shows that such placebo effects may occur through top-down processes stemming from brain regions involved in planning, emotion regulation, as well as brain regions specific to the symptom or condition for which they occur, and that they can moreover be evoked by expectations regarding treatment outcomes [22,23].

Most studies demonstrate that placebo and nocebo effects can be induced by verbal suggestions, for example, for self-reported symptoms of itch. There is some evidence, however, that these effects can also be elicited for physiological parameters related to itch, for instance, for wheal or flare responses to histamine [24]. Literature moreover shows that conditioning can influence immune parameters in animal models and human populations [25-27]. As such, conditioning may potentially be used to influence the immune pathways

underlying itch and cutaneous conditions as well. Although narrative reviews emphasize the impact of placebo and nocebo effects on itch [3,7,8], a systematic overview of studies investigating placebo and nocebo effects, which also encompasses the immunomodulatory aspects of these effects, has not been provided yet. Providing such an overview could provide new insights in the consistency of placebo and nocebo effects found across induction methods, clinical conditions, and symptoms. The current review therefore aims to summarize the available knowledge of placebo and nocebo effects that were experimentally elicited in controlled trials in cutaneous conditions, in symptoms of the skin or atopic symptoms of the mucous membranes that are associated with itch, as well as in related experimental human (i.e. healthy participants) or animal models.

RESULTS

Search results and study characteristics

An overview of the literature search and number of articles in each step of the selection procedure can be found in **Figure 1**. In total, the literature search identified 16.440 unique studies, of which 79 were considered eligible for inclusion. An additional 7 studies were identified by screening the reference lists of the included studies, bringing the total to 86 articles that were included in this review (k=31 animal and k=55 human studies). Articles that were identified through reference lists did not have keywords listed online, or provided no online abstract and were therefore not found in the systematic search. A semi-quantitative overview of effects for each induction method and outcome type is provided in **Table 1** (with a graphical representation and short summary being given in **Supplementary Figure S1 and Supplementary Table S1,** respectively). An extensive overview of the study characteristics and a short summary of results is presented for animal and human studies separately (with human studies further split into healthy volunteers and patient studies) in **Supplementary Tables S2, S3 and S4**.

Risk of bias assessment

An overview of the risk of bias assessment outcomes is provided separately for animal and human studies, in **Supplementary Figures S2-S5**. The quality of the 86 included studies varied. None of the included animal studies met all criteria for risk of bias, most often due to a lack of important information to decide risk of bias. For human studies, more information was provided, and risk of bias was lower. In general, no differences in risk of bias were detected between studies that reported null findings and studies that reported significant findings. Studies on verbal suggestions combined with hypnosis more often had a selection bias compared to the other studies – participants who were highly hypnotizable were often selected, which may have increased bias in the study findings. In addition, some studies on verbal suggestions had high risk of bias for blinding, mostly due to the personnel that assessed outcomes not being blinded to allocated groups.

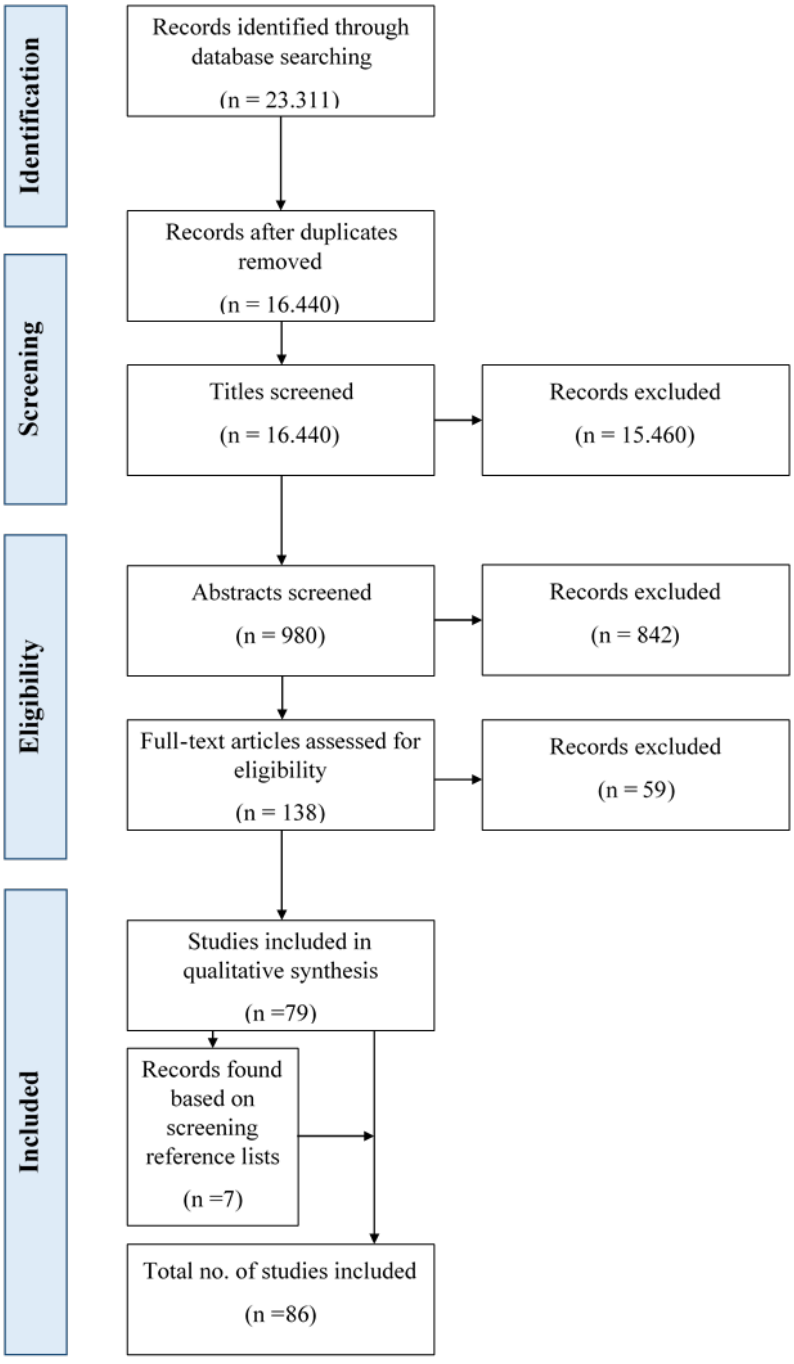

Figure 1. Flowchart for the selection of articles to be included in the systematic review.

Table 1. Semi-quantitative overview of outcomes for each study, separated for animal studies, and human studies subdivided in healthy participant and patient samples.

Sample type	Type of induction	Mechanism(s)	Outcome category	Outcome classification	
				Confirmation of hypothesis*	**Non-confirmation of hypothesis**
Animal	**Placebo**	Conditioned immunosuppression	Behavioral	Saccharin preference ratio [28,30,115-118]	-
			Physiological	DTH response to SRBC: hemagglutination titers [115,117,118], paw swelling [29], footpad swelling [30], plasma glucocorticoids [30], DNCB-induced ear swelling [116], left/right ear weight ratio [119], leukocyte migration inhibitory factor [119] Sensitization to corticosterone: DTH-induced paw swelling [28]	-
	Nocebo	Conditioned allergic responses and anaphylactic shock	Behavioral	Saccharin preference ratio [120-122], Anaphylactic shock behavior [120,121], breathing pattern indicative of asthmatic attack [32], asthma attack [123], lung anaphylactic response [33]	Breathing pattern indicative of asthmatic attack [31], rearing behavior [33], freely acting plus maze behavior [33]
			Physiological	Plasma histamine levels [34-39,124,125], lung tissue histamine levels [34], rat mast cell protease II [126], plasma cortisol levels [37], plethysmographic amplitude [127], corticosterone levels [33]	Plasma histamine levels [34], bronchoalveolar lavage fluid [34], respiratory resistance [35]
		Operant conditioning	Behavioral	Scratching behavior [41]	Scratching behavior [40]
		Social induction	Behavioral	Scratching behavior [42-44], attention (looking behavior) [45]	Scratching behavior [45], attention (looking behavior) [43]
Sample type	**Type of induction**	**Mechanism(s)**	**Outcome category**	**Outcome classification**	
				Confirmation of hypothesis*	**Non-confirmation of hypothesis**
Healthy participants	**Placebo**	Verbal suggestions + hypnosis	Self-reported	Laser-induced and histaminergic pain [46]	
			Physiological	Wheal area (Prausnitz-Küstner reaction to horse serum) [47], wheal area to histamine [48], titration gradients [48], histaminergic flare [46,51], Mantoux skin response [51]	Titration endpoint data [48], wheal/erythema ratio [49,50], histamine wheal [51], erythema [52], skin thickness [52]

Table 1. *continued (2 of 3).*

Sample type	Type of induction	Mechanism(s)	Outcome category	Outcome classification	
				Confirmation of hypothesis*	**Non-confirmation of hypothesis**
		Verbal suggestions	Self-reported	Expected wheal area [53], self-rated itch in response to histamine [54], expected itch [55,56], expected pain [55,56], expected fatigue [55], self-rated pain during CPT [56], mechanically induced itch [58], electrically induced itch [58], chemically induced itch (histamine) [58], mechanically induced pain [58], electrically induced pain [58], chemically induced pain (histamine) [58]	**Itch + anxiety levels [57] not described** Self-rated itch [55,56], self-rated pain [55], self-rated fatigue [55], physical sensitivity [55], chemically induced itch (histamine) [58]
			Physiological	Heart rate [53], histamine wheal area [57], histaminergic flare [57]	Histamine wheal area [53,54,56], heart rate variability [53], heart rate [55], skin conductance [55], skin temperature [56]
		Verbal suggestions (OL)	Self-reported	Expected itch [59]	Self-rated itch in response to histamine [59], self-reported skin response [59]
			Physiological	-	Wheal area [59], flare area [59]
		Conditioning (+ VS)	Self-reported	Electrically induced itch [60]	-
	Nocebo	Verbal suggestions	Self-reported	Self-rated itch [24], self-rated unpleasantness [24]	-
			Physiological	Wheal intensity (NaCl) [24], histaminergic flare intensity [24]	-
		Conditioning (+ VS)	Self-reported	Self-rated itch [61,62]	-
			Physiological	fMRI: activity in contralateral rolandic operculum [62], functional coupling between periaqueductal gray [62]	-
		Social induction	Self-reported	Self-rated itch [63-65]	-
			Behavioral	Scratching [63-65]	-
				fMRI: activation of major areas of itch matrix (thalamus, primary somatosensory cortex, premotor cortex, insula) [63]	-

Sample type	Type of induction	Mechanism(s)	Outcome category	Outcome classification	
				Confirmation of hypothesis*	**Non-confirmation of hypothesis**
Patients	**Placebo**	Verbal suggestions + hypnosis	Self-reported	Cutaneous pain threshold [66], atopic eczema symptoms [66], retrospectively assessed symptoms of allergy [67], nasal flow symptoms during challenge (NPT) [67], self-rated itch [68]	Daily self-report symptoms of allergy [67], nasal flow symptoms after challenge (NPT) [67]

Table 1. *continued (3 of 3).*

Sample type	Type of induction	Mechanism(s)	Outcome category	Outcome classification	
				Confirmation of hypothesis*	**Non-confirmation of hypothesis**
			Physiological	Skin temperature [69], skinfold thickness [69], allergen-induced wheal size [70], allergen induced flare size [70], histaminergic flare size [128], number of warts**** [71,72,74]	Allergen-induced wheal size [70], allergen induced flare size [70], histamine wheal size [128,129], histaminergic flare [129], phosphate dilutions [129], number of urticaria wheals [68], clinical psoriasis severity [73], delayed blanch of skin [130], histaminergic flare [130], white line response [130]
		Verbal suggestions (OL, +inert pill)	Self-reported	Allergic symptoms composite score [75,76]	Allergic symptoms: separate scores [75]
		Pharmacological conditioning / conditioned dose reduction	Self-reported	Psoriasis severity scale [79]	Allergic symptoms [77], Allergic symptoms in response to NPT [78]
			Physiological	Psoriasis relapse [79], basophil activation [77]	Wheal size in response to allergens [77,78], blood count & flow cytometry [77]
		Conditioning (+ VS)	Self-reported	Pain in response to electrical stimulation [80]	-
		Social induction	Physiological	Wheal size in response to histamine [81]	-
	Nocebo	Verbal suggestions + hypnosis	Physiological	Skin temperature [82]	-
		Verbal suggestions	Self-reported	Self-rated itch [83]	-
			Physiological	Conductance – thoracic gas volume ratio [85,86], airway resistance [85,86] fMRI: increase in dorsolateral prefrontal cortex, caudate, and intraparietal sulcus [83]	Wright-McKerrow Peak Flow Meter outcomes [84], maximum expiratory flow rate [84], respiratory pattern [84]
		Conditioning [A]	Self-reported		Subjective allergic symptoms [87,88]
			Physiological	Peak nasal inspiratory flow [87], blood serum histamine levels [87], nasal tryptase levels [88]	Wheal size in response to sham allergen [89]
		Social induction	Self-reported	Self-rated itch [92-95]	Self-rated itch (HC only) [93]
			Behavioral	Scratching behavior [92-95], allergic symptoms [96]	-
			Physiological	Breathing frequency [96] fMRI: higher activity in SMA, the left ventral stratium and higher right OFC activation [95]	Airflow [96], tidal volume [96], inspiratory time [96], respiratory resistance [96]

Note (table 1). * A confirmation of hypothesis is defined as a significant ($p<.05$) difference of the experimental group(s) with a) included control groups or b) a baseline measurement, that indicates successful placebo or nocebo induction in line with the proposed hypothesis (e.g., increased paw swelling following conditioning of a CS with antigens [animals], or itch reduction following suggestions of lower itch, or itch exacerbation following suggestions of an increase in itch [humans]). This included studies for which effects were conditional (e.g., depending on stress or isolation [animals], effects depending on depth of hypnosis [humans]). ** A non-confirmation of hypothesis is defined as either a non-significant ($p>.05$) difference of the experimental group(s) with a) included control groups or b) a baseline measurement, or a significant in the opposite direction of the proposed hypothesis.

CPT = cold pressor task, HC = healthy controls, OL= open-label, NPT = nasal provocation test, VS = verbal suggestions.

[A] Two out of five studies (Jordan, 1972, and Robertson, 1975) compared the efficacy of conditioning scratch responses for patients and healthy controls. While it was concluded that scratch responses were more easily conditioned in patients, no remarks regarding the efficacy of conditioning itself were made (i.e. no comparisons with control groups / a non-conditioned state). As such, these were not counted amongst the proportional positive results (confirmed hypotheses) in the table.

Animal studies

Of all thirty-one animal studies, most investigated effects in rodents (guinea pigs k=12; rats k=11; mice k=4; both rat and mice k=1) or non-human primates (k=3; exclusively included in studies on social induction of scratching behavior). The number of animals included in each experiment ranged from 5 to 96. Three studies did not report sample size. Most (k=18; 58%) included male samples exclusively, followed by studies that included both sexes (k=5; 16%) or females exclusively (k=4; 13%). A minority (k=4; 13%) did not report the sex of the animals. Most animal studies were conducted before 1990 (k=19; 61%), and only a few took place within the last 10 years (2010-2019: k=3; 10%).

1. Placebo effects

1.1. Conditioned immunosuppression

Eight studies investigated whether allergic responses could be suppressed by conditioning of a neutral stimulus (or conditioned stimulus, CS; e.g., a saccharin solution or an odor) with a pharmacological drug (unconditioned stimulus; UCS) in rodent models of delayed-type hypersensitivity responses. Saccharin preference ratio (i.e. behavioral parameter – the amount of saccharin that was ingested by the animal in a subsequent testing phase following conditioning) was reduced in all studies (k=6) that assessed this parameter. Evidence of conditioned immunosuppression was found for most physiological parameters (i.e., for hemagglutination titers, ear or paw swelling, and leukocyte migration to the area of antigen injection). Conditioning did not affect paw swelling when dexamethasone was used as UCS [28]. One study found extinction of conditioned responses following the first of three re-exposures [29]. Moreover, one study indicated that conditioned effects are dependent on the induction of stress [30], suggesting that conditioned responses may be context-specific.

2. Nocebo effects

2.1. Conditioned allergic responses and anaphylactic shock

Twelve studies investigated whether an allergic response could be learned through conditioning in rodent models by pairing a cue (the CS, for example, an odor) with an

allergen or substance for which animals were previously sensitized. Behavioral parameters were influenced in 5 of the 7 studies that assessed them: saccharin preference ratio decreased following conditioning in all studies (k=3), whereas behavior indicating anaphylactic shock or asthmatic attack increased in 2 of 4 studies. In the two studies that overall reported null effects, behavior indicating an asthmatic attack remained unchanged in one study [31], while another found conditional effects: exposure to the CS led towards asthmatic attacks – but only when animals were stressed [32]. It was demonstrated that freely-acting behavior (e.g., rearing, locomotion) did not change following conditioning [33]. Changes in physiological parameters were found following conditioning, which were indicative of an allergic response (i.e., increases in histamine serum levels, Rat Mast Cell Protease II, or lung tissue histamine levels; increased plethysmographic amplitude, and respiratory resistance, see also **Table 1)**. Two studies failed to find effects on (secondary) physiological outcomes [34,35]. Others showed mixed evidence for conditioned histamine release in rodents: it was shown that effects depended on handling-induced stress [36,37], fasting stress [35], anesthetization [34], or receiving medication such as diazepam [38] or dexamethasone [39]. For example, conditioned histamine release occurred exclusively in stressed animals.

2.2. (Operant) Conditioning of scratch responses

Two studies described a series of experiments, in which it was investigated whether scratching behavior could be operationally conditioned by reinforcing bouts of scratching with food [40,41]. One study found scratching to be less readily conditioned compared to rearing or washing [40], while the other found that scratching could be increased through operant conditioning – with the behavior being more easily conditioned when an itchy stimulus (i.e. collar) was present [41].

2.3. Social induction

Four studies investigated whether scratching behavior could be contagious in animals (k=1 in rodents, k=3 in non-human primates). The most common designs consisted of either observing a live same-species animal, or of observing videos in which scratching behavior was displayed. Two studies found that scratching behavior in observers (i.e., animals that watched others scratching) increased [42,43], while two other studies found that scratching did not increase following observation of another animal scratching [44,45].

Healthy volunteers

Of the 21 studies with healthy volunteers, most studies included both males and females (k=16; 77%). Two studies (9%) were stratified by sex (50:50 distribution in experimental groups) or investigated females exclusively (k=3; 14%). Sample sizes ranged between 10 to 159 healthy volunteers. Most studies were conducted in the past 10 years (2010-2019: k=13, 64%).

1. Placebo effects

In total, fourteen studies were included that investigated placebo effects by verbal suggestions. A single study investigated the induction of placebo effects by conditioning combined with verbal suggestions (described in subsection '2.4.1.2. Conditioning').

1.1. Verbal suggestions

Across studies, a further subdivision could be made for studies that induced placebo effects by: a combination of verbal suggestions and hypnosis (k=7), by verbal suggestions exclusively (k=6), or by open-label verbal suggestions (k=1). Three of the seven studies that provided positive verbal suggestions with hypnosis demonstrated improvement in self-reported (i.e., pain induced by laser and histamine skin prick tests, [46]) and physiological parameters (i.e., skin responses to histamine and horse serum such as wheal and flare, titration gradient and endpoints, pain-related brain potentials) [46-48]. One study found that effects on wheal area were a function of the depth of the trance induced by hypnosis [47]. In addition, four studies compared suggestions of decreased responses to antigens or histamine for one arm with suggestions of increased responses for the other arm within subjects. All four studies included or divided participants on being highly hypnotizable [49-52]. Only one study reported significant differences in skin thickness following suggestions at certain dilution strengths of the test substance [51]. The others reported no effects. Four studies investigated placebo effect induction by positive verbal suggestions exclusively. Expected itch, pain or skin responses were reduced following positive suggestions in all studies [53-56]. Three studies assessed histamine-induced itch [54-56], but only one of these found lower itch following suggestions [54]. Positive suggestions reduced pain during a cold-pressor task in one study [56], but not in another [55]. Wheal area was not affected by suggestions in any study. Two studies compared positive suggestions with negative suggestions [57,58]: overall, findings were mixed. In one study, suggestions of high and

low itch or pain were able to respectively enhance and decrease self-reported parameters of itch and pain after mechanical and electrical stimulation, but suggestions of low itch did not reduce histamine-induced itch [58]. In another study, physiological parameters (i.e. flare, wheal) differed between positive and negative suggestions groups, but no differences were found compared to a neutral control group [57]. Finally, a single study investigated whether open-label positive verbal suggestions could induce positive expectations and placebo effects for itch compared to a neutral control [59]. Suggestions decreased itch expectations, but not itch. No effects on physical skin response (histaminergic flare (area), skin temperature, wheal area) were found.

1.2. Conditioning

A single study investigated placebo effect induction by conditioning, verbal suggestions, and by combining suggestions and conditioning. While no significant reduction in electrically induced itch was found following conditioning exclusively or following verbal suggestions exclusively, a combination of the two did result in reduced itch levels [60].

2. Nocebo effects

In total, seven studies investigated nocebo effects in healthy volunteers. Nocebo effects were induced by verbal suggestions (k=1), conditioning (k=1), a combination of verbal suggestions and conditioning (k=2; described in the subsection '2.4.2.2. conditioning'[1]), or by social cues (k=3; contagious itch).

2.1. Verbal suggestions

In the study that focused exclusively on suggestions-induced nocebo, participants received information (verbal suggestions) about the severity to which they would respond to histamine and saline skin prick tests [24]. Itch, unpleasantness of the test, and wheal diameter were higher in response to saline, and the histaminergic flare (measured by diameter) was greater following negative suggestions [24].

[1] This includes the study of Bartels et al. (2014) that is also described under subsection '2.4.1. placebo effects', as both placebo and nocebo effects were investigated within this study.

2.2. Conditioning

Three studies investigated nocebo effect induction by conditioning. One study demonstrated successful nocebo effect induction by conditioning for itch. Moreover, the study showed that these learned responses could be reversed by positive suggestions, and demonstrated generalization of effects from electrical to histamine-induced itch [61]. Two studies found that conditioning and verbal suggestions could both increase itch [60,62]. In addition, one of these reported that a combination was most effective to induce nocebo effects [60]. Using functional magnetic resonance imaging (fMRI), increased activity was found in the contralateral Rolandic operculum, and increased functional coupling was found between the insula and the periaqueductal gray (PAG), all areas involved in the somatosensory processing of histaminergic itch [62].

2.3. Social induction

Three studies investigated whether itch could be induced by social or contextual factors in healthy participants, using a variety of methods to induce itch sensations: videos of people scratching [63], slideshows of itch-related pictures [64], or itch suggestions during music, which were presented either sub- or supra-liminally [65]. Itch and scratching behavior were increased in 2 of 3 studies [63,64]. In the remaining study, findings were mixed: itch and scratching were increased only when suggestions were presented supra-liminally during music, but not when presented super-liminally [65]. Watching itch-inducing videos moreover activated major areas of the itch matrix (thalamus, primary somatosensory cortex, premotor cortex (BA6), and insula) as demonstrated through fMRI [63].

Patients

In the 34 studies on placebo and nocebo effects within patient samples, the investigated medical conditions were: allergic rhinitis (including, but not limited to, hay fever and dust mite allergy) (k=10; 29%), atopic dermatitis (k=9; 26%), allergic asthma (or other lung problems associated with irritation by allergens, e.g., bronchitis) (k=6; 18%), warts (k=3; 9%), psoriasis (k=2; 6%), chronic urticaria (k=1; 3%), lichen simplex (k=1; 3%), multiple conditions combined (k=1; 3%), or unspecified skin diseases (k=1; 3%). Most studies included both male and female patients (k=23; 67%), but some did not describe sample sex (k=11; 33%). The majority of studies took place either within the last ten years (2010-2019: k=9, 27%) or before 1970 (k=8, 24%).

1. Placebo effects

In total, nineteen studies investigated placebo effects in patient samples. Placebo effects were elicited by positive verbal suggestions and hypnosis (k=12), by open-label suggestions (k=2), by conditioning (k=4) or by social induction (k=1).

1.1. Verbal suggestions

Across studies investigating placebo effect induction by suggestions, medical conditions investigated were: allergy (k=4), warts (k=3), allergic asthma (k=2), atopic dermatitis (k=2), chronic urticaria (k=1), psoriasis (k=1), and multiple conditions combined (k=1). In the twelve studies on suggestions and hypnosis, eleven provided suggestions of non-responding (e.g., to allergens) or symptom relief. Four studies investigated self-reported symptoms, with three demonstrating significant induction of placebo effects (in one of these studies, effects were found exclusively when symptoms were assessed retrospectively) [66-68]. Physiological parameters (e.g., clinical symptoms of skin conditions, such as wheals or warts) were assessed in 10 studies, and were generally reduced following suggestions and hypnosis in 3 studies [69-71]. In the other 7 studies, no or mixed evidence was found. One study gave suggestions of improvement for one side of the body and concluded that any observed improvement was on that side, however, no data or statistical tests were reported [72]. Some studies noted that symptoms improved only when deep hypnosis was achieved [73,74]. Finally, two studies investigated whether open-label placebo effects could be induced for allergic rhinitis [75,76]. A briefing about the placebo effect was given together with inert pills (in addition to treatment as usual) in one study [75]. In the other, both separate and combined effects of the briefing and the inert pills were examined [76]. Open-label placebo effects were induced for allergic symptoms in both studies. Moreover, while the inert pills reduced allergic symptoms, no additional effect of the open-label briefing was found [76].

1.2. Conditioning

Medical conditions investigated were allergy (k=2), psoriasis (k=1), and atopic dermatitis (k=1). Studies on conditioning placebo effects in patient samples could be further subdivided into pharmacological conditioning (k=2), conditioned dose reduction (k=1), or suggestions and conditioning (k=1). In the two studies on placebo effects by pharmacological (antihistamine) conditioning for allergic rhinitis, no effects on subjective symptoms or wheal size were found [77,78]. Basophil activation after exposure to allergens

was reduced, however, which is indicative of conditioned immunosuppression [77]. In the single study that investigated conditioned dose reduction (i.e., using conditioning principles to partially replace medication by placebo), findings were mixed: although conditioned dose reduction prevented psoriasis relapse overall, significant improvement in symptoms was demonstrated only in one of two research sites [79]. Finally, a single study investigated whether verbal suggestions, conditioning, or a combination of both could influence electrically-induced pain in atopic dermatitis and healthy controls [80]. Verbal suggestions, but not conditioning, reduced pain in both atopic dermatitis and healthy controls. Moreover, a combination of suggestions and conditioning was most effective.

1.3. Social induction

A single study assessed whether advertising of antihistamine brands would influence drug efficacy (defined as % decrease in wheal) in allergic vs. non-allergic participants [81]. Two types of advertisements were shown, one where only brand A (the antihistamine used in the study) was promoted, and one where brand B was promoted as working faster than A. Decreased efficacy was found for allergic participants at 60 minutes following antihistamine use when brand A was promoted, compared to when brand B was promoted. For non-allergic participants, increased efficacy was found when brand A was promoted at 120 minutes following antihistamine use.

2. Nocebo effects

In total, fifteen studies investigated nocebo effects in patient samples. Nocebo effects were elicited by negative verbal suggestions (k=5), by conditioning (k=5), or by social induction (k=5).

2.1. Verbal suggestions

Across studies investigating nocebo effect induction by suggestions, medical conditions examined were: atopic dermatitis (k=2), allergic asthma (k=2), and other lung problems related to irritants or allergens (k=1). One study investigated negative verbal suggestions with hypnosis, and four investigated negative verbal suggestions exclusively. Following suggestions and hypnosis, higher skin temperature was found in both atopic dermatitis and healthy controls [82]. Another study in atopic dermatitis investigated nocebo effects induction by suggestions exclusively, and found that this increased self-reported itch [83].

Moreover, fMRI signal increased following suggestions in the dorsolateral prefrontal cortex, caudate, and intraparietal sulcus – all regions involved in motivational and cognitive processing, and all regions that respond when real allergens are presented [83]. Finally, three studies investigated effects of negative suggestions on physiological parameters representing airway reactivity [84-86]. One study failed to find effects of negative suggestions on physiological parameters (i.e., respiratory pattern, maximum expiratory flow) in bronchial asthma [84]. In the other two studies, suggestions did elicit significant changes in physiological parameters (i.e., airway resistance, thoratic gas volume, conductance-thoratic gas volume ratio) indicative of bronchoconstriction [85,86]. Moreover, positive suggestions (i.e., that a bronchodilator was given) reversed these effects [86].

2.2. Conditioning

Five studies investigated whether nocebo effects could be induced by conditioning in allergic rhinitis (k=3), atopic dermatitis (k=1), and lichen simplex (k=1). No effects of conditioning on self-reported allergic symptoms were found [87,88]. Physiological parameters (i.e., peak nasal inspiratory flow, histamine level, nasal tryptase level) increased following conditioning in 2 studies [87,88], while another failed to find effects (i.e., for wheal response to sham allergens) [89]. Generally, conditioned effects were stronger when the number of acquisition trials increased, and effects were prone to extinction [87]. Finally, for patients with atopic dermatitis and lichen simplex, conditioning led to a higher number of scratch responses compared to healthy controls [90,91].

2.3. Social induction

Five studies investigated whether symptoms such as itch could be induced socially (e.g., contagious itch, induced by a lecture on itch, scratching videos, or pictures of allergens) in atopic dermatitis (k=3), non-specified skin diseases (k=1) or allergic asthma (k=1). Three studies compared patients with healthy controls. Self-reported parameters (i.e. itch, asthma symptoms composite score) and scratching behavior were increased following social induction in all studies that measured these outcomes. Both self-reported and behavioral parameters increased more for patients compared to healthy controls [92-95]. Moreover, fMRI data showed that activation of the supplementary motor area, the left ventral striatum and the right orbitofrontal cortex increased following an itch video compared to a control video – all regions that are particularly associated with the desire to scratch in itch [95].

While breathing frequency increased in response to allergen pictures in allergic asthma, no changes were detected for other (physiological) respiratory parameters [96].

DISCUSSION

This review summarizes the available knowledge on experimentally induced placebo and nocebo effects in cutaneous conditions, and symptoms of the skin or atopic symptoms of the mucous membranes associated with itch, in relevant animal or human models (i.e., healthy participants and patients). In general, considerable evidence is provided for placebo and nocebo effects in medical conditions and symptoms relevant to the field of dermatology. Placebo and nocebo effects were elicited in self-reported and behavioral parameters related to symptoms (e.g., itch, allergic symptoms or other self-reported symptoms, scratching behavior). Effects could also be induced for physiological parameters, most notably when (pharmacological) conditioning or a combination of suggestions and conditioning were used. Generally, findings were less consistent for physiological parameters than for self-reported or behavioral parameters. The findings illustrate that placebo and nocebo effects can be induced through similar mechanisms across animal studies, studies using healthy volunteers, and studies with patients, despite a high level of heterogeneity across studies.

Animal studies show that both placebo and nocebo effects may be elicited through associative learning (conditioning). It was demonstrated that allergic reactions can be conditioned, which is indicative of a nocebo effect. Likewise, placebo effects were shown in rodent models of allergy (i.e. modelled hypersensitivity responses), as demonstrated by studies investigating conditioned immunosuppression. However, the methods used within these studies were very diverse. For example, the way in which hypersensitivity is modeled in rodents differed, as did the conditioning paradigms used: both CS and UCS were heterogeneous amongst studies, the number of acquisition and evocation sessions varied, and the specific control groups differed between studies (see **Supplementary Table S2**). There was consistency in behavioral outcomes, but physiological outcome parameters varied depending on the specific sensitization method and unconditioned stimulus that were used. Overall, the studies illustrate that learned placebo effects are moreover sensitive to the context (they may not be elicited when the context changes) and are prone to extinction [29,30,34-39]. In the future, research may consider systematically investigating which

conditioning paradigms are most effective. Moreover, replication and generalization of the conditioning paradigms used in previous studies may be considered.

Of all human studies included in the review, the outcome parameters used were most consistent in studies with healthy participants – with self-reported measures of itch and physiological outcomes of wheal and flare responses to histamine being most often assessed [97,98]. Most models with healthy participants simulate cutaneous conditions by mechanical, electrical, and chemical (i.e., histamine) stimulation of the skin. Effects were found most consistently for self-reported outcomes such as itch, and behavioral outcomes such as scratching. Physiological outcomes, on the other hand, were less consistently influenced. In patient samples, similar trends in study outcomes were observed, with self-reported and scratching behavior generally more likely to be affected than physiological parameters. Most studies investigated – and found placebo and nocebo effects for – atopic dermatitis and allergic rhinitis, with only a small body of research done on placebo and nocebo effects in other conditions (e.g., psoriasis, chronic urticaria, and other skin diseases). Future research may consider replicating these findings, as well as extending them to other dermatological conditions, in order to assess similarity of effect sizes for different symptom etiologies. It should be noted that the manner of placebo and nocebo effect induction varied a lot across human trials (both for healthy participants and patients). Overall, different mechanisms (i.e., verbal suggestions, conditioning, social induction) were used to elicit placebo and nocebo effects – furthermore, even in case of similar mechanisms, other variances in the study design (e.g., type of instructions, dissimilarities in conditioning paradigm) may complicate the comparability of placebo and nocebo effect sizes across studies. In trials with patients, an additional confounding factor is added by heterogeneity across medical conditions and condition-dependent outcome parameters.

Finally, few studies have investigated neurological pathways and brain areas that are involved in placebo and nocebo effects for dermatological symptoms such as itch. Placebo and nocebo effects may modulate itch through top-down processing in brain areas related to the specific condition or symptom in which they emerge [23]. Indeed, work on itch shows that brain areas likely involved in nocebo responding are those that are responsible for somatosensory processing of itch or are otherwise related to the itch-scratch cycle as well [62,63,83,95]. Caution is needed in interpreting these findings, however, as only nocebo effects have been investigated. Moreover, of the four studies on brain processing of nocebo effects in itch, two were investigating contagious itch. Mirror neurons (i.e., activated when mirroring facial expressions for affective or empathetic purposes) have been proposed to

play a role in eliciting contagious itch [99]. It unclear whether or how this may relate to nocebo effects induced by other means. In addition, brain processing of placebo effects in itch have not yet been investigated. Future research may aim to further identify brain regions of interest for both placebo and nocebo effects processing.

It has been proposed previously that verbal suggestions are more likely to elicit effects on self-reported outcomes in humans – either alone, or in combination with conditioning [3,8,98], whereas for physiological outcome parameters, (pharmacological) conditioning may be more likely to elicit effects. The studies included in the current review likewise underline this notion. Moreover, findings show that cues from the social environment may impact the experience of symptoms. Most evidence stems from the induction of contagious itch in experimental settings, for instance, while listening to a lecture or watching videos of people scratching. Research on the extent to which these concepts may translate towards clinical practice, or on how such cues may impact symptom experience in daily life, is lacking. Future research may consider further investigating the influence of social and contextual cues on treatment efficacy in clinical populations. In addition, future research may further investigate which (combination of) mechanisms would be most effective in inducing placebo and nocebo effects for a variety of symptoms across dermatological conditions. Clinical relevance and applicability may be considered here, and the mechanisms that are most promising to establish longer-term effects should have precedence over those that appear to elicit short-term changes. Conditioned dose reduction may be a promising approach, as this method is based on conditioning principles [100], could be considered most directly applicable in clinical practice [101], and has been found to be as effective as full medication doses – not just in psoriasis, but also in other conditions such as attention-deficit hyperactivity disorder [79,102]. Likewise, open-label placebo effect induction may be investigated further in the future. Even though this has been investigated only infrequently in relation to dermatological symptoms or conditions [59,75,76,103,104], research from various other fields further supports the notion that placebo effects can be elicited even when it is known that an inert substance is given [9-13]. Information derived from these studies may pave the way for new therapeutic possibilities, for example the development of psychoeducation regarding the role of expectations and learning in health and disease, or the development of a training specifically targeting the patients' expectations of treatment, and in turn treatment effects. Open-label placebo effects may be a way to ethically apply placebo and nocebo effects in clinical practice [105]. The available body of evidence for open-label placebo effects within dermatology is currently

limited, however, and more research is necessary as a consequence, especially in patient populations.

In addition to utilizing placebo effects in clinical practice, attention should be given to the occurrence of nocebo effects as well. The current review demonstrates that these can be evoked by a variety of methods, and attention should be given to ways to reduce their impact in clinical practice. Some work already shows that previously learned nocebo effects for itch can be reduced by a combination of suggestions and counterconditioning [61]. Studies in other research areas (e.g., in the field of pain) also show promising results for such methods [106]. Suggestions and counterconditioning may, for example, be used to reduce the occurrence of unwanted side effects, or to counter diminished treatment efficacy due to previously learned negative associations [106]. The efficacy of these methods in reducing nocebo effects for itch-related symptoms of the skin and mucous membranes should be researched more extensively in the future.

Placebo and nocebo effects in symptoms and medical conditions are known to vary between individuals. For example, a study investigating pharmacological conditioning of anti-allergic effects demonstrated that symptoms in both conditioned and sham-conditioned groups were likely influenced by the participants' own expectations and cognitions, as these differed from a natural history group [78]. Likewise, there is evidence that individual characteristics, such as personality characteristics and polymorphisms in genetic markers, may impact placebo and nocebo effects [7,107-110], although evidence for these specific predictors of placebo and nocebo effects within the field of dermatology is limited and mixed [8]. Of the studies included in the current review, few investigated predictive factors for placebo or nocebo responding. Some work illustrated that placebo and nocebo responses may have occurred in subgroups only, such as highly hypnotizable or suggestible individuals [73,74]. Likewise, the individual characteristics of the person who is providing information about a treatment (e.g., warmth and competence of a health care provider) may impact the size of effects [24]. Future research could aim to further investigate what factors may impact placebo and nocebo effects in order to provide a more complete and structured picture of under which circumstances these effects are likely to be most strong.

Limitations of the current review were the heterogeneity of the included studies, which prevented a meta-analysis of study results. In addition, some studies have demonstrated high risk of bias, most notably in inclusion of participants (studies on hypnosis selected on high hypnotizability), or in blinding (experimenters providing verbal suggestions were not

blinded and often examined outcomes as well). Moreover, in most articles that described animal research, information needed to rate bias was lacking. As a result, most studies were rated as being unclear on bias. In addition, sample sizes reported in most studies included in this review are small. As such, effects that are small may not have been detected in these studies. Finally, some of the included studies describe experimentally elicited pain. These tests were incidentally included as they occurred alongside an itch induction test or in a relevant patient sample. However, the review did not systematically include pain-induction tests, so the number of studies finding placebo and nocebo effects for pain, as described here, might not reflect the actual incidence of placebo and nocebo effects studied within the field of pain. For a review on those studies see, for example, Peerdeman and colleagues [7].

Overall, this review provides considerable evidence for placebo and nocebo effects within dermatological conditions, specifically for itch and other symptoms of the skin and mucous membranes associated with itch. Such effects can be elicited using various methods, most importantly, by using verbal suggestions, conditioning, or social induction. Some caution is needed in translating this work to clinical practice and more research is needed for a more robust foundation upon which clinical applications may be built. First and foremost, it is important to structurally investigate how variations in induction methods may impact placebo and nocebo effects, and whether all symptoms and medical conditions may be influenced similarly by placebo and nocebo effects elicited through these induction methods. Second, the impact of external factors (e.g., predictors such as suggestibility) on placebo and nocebo effects should be investigated more extensively. Finally, more research is needed to implement this knowledge about placebo and nocebo effects in clinical practice: clinical trials may further explore whether conditioning may be used to maximize placebo effects and minimize nocebo effects in clinical practice, to enhance treatment efficacy, reduce medication intake, and enhance patients' quality of life.

MATERIALS AND METHODS

A complete overview of the methods for the systematic review is provided in the **Supplementary Material**. In short, this review was conducted in accordance with the PRISMA statement on systematic reviews [111] and pre-registered in Prospero (PROSPERO 2018: CRD42018096636). Articles were included in the review if they (1) were conducted in healthy volunteers, animals, or patients with chronic or acute itch associated with a dermatological condition, or associated with (atopic) symptoms of the

skin or the mucous membranes related to itch; (2) investigated experimentally-induced placebo or nocebo effects (e.g., elicitation of effects through conditioning, or social or verbal expectation induction methods such as suggestions); (3) were written in English, Dutch or German; (4) presented new data; and (5) assessed outcomes including – but not limited to – perceived itch, behavioral measures related to itch (e.g., scratching behavior), self-reported symptoms (e.g., allergic or atopic symptoms), extent of neurogenic inflammation, or itch-related inflammatory markers (e.g., histamine, substance P). Articles were excluded when data was presented on a case-by-case descriptive level or when total sample size was $n<5$.

PubMed, PsycInfo, and Embase databases were searched for relevant articles on May 8, 2018. Two independent raters (SM, CvL) screened titles for the inclusion criteria. Next, the two raters assessed abstracts and full-texts for eligibility, using a hierarchical approach. Discrepancies between the two raters were resolved by discussion with a third independent rater (HvM). The reference lists from the included articles were checked for additional relevant articles by both independent raters. Data from the included articles were extracted by one rater (SM) using a piloted form. Two independent raters (SM, KB) assessed risk of bias of each study using the Cochrane risk of bias tool [112]. The SYRCLE risk of bias tool was used for articles describing animal research [113], as were the guidelines described by O'Connor and Sargeant [114].

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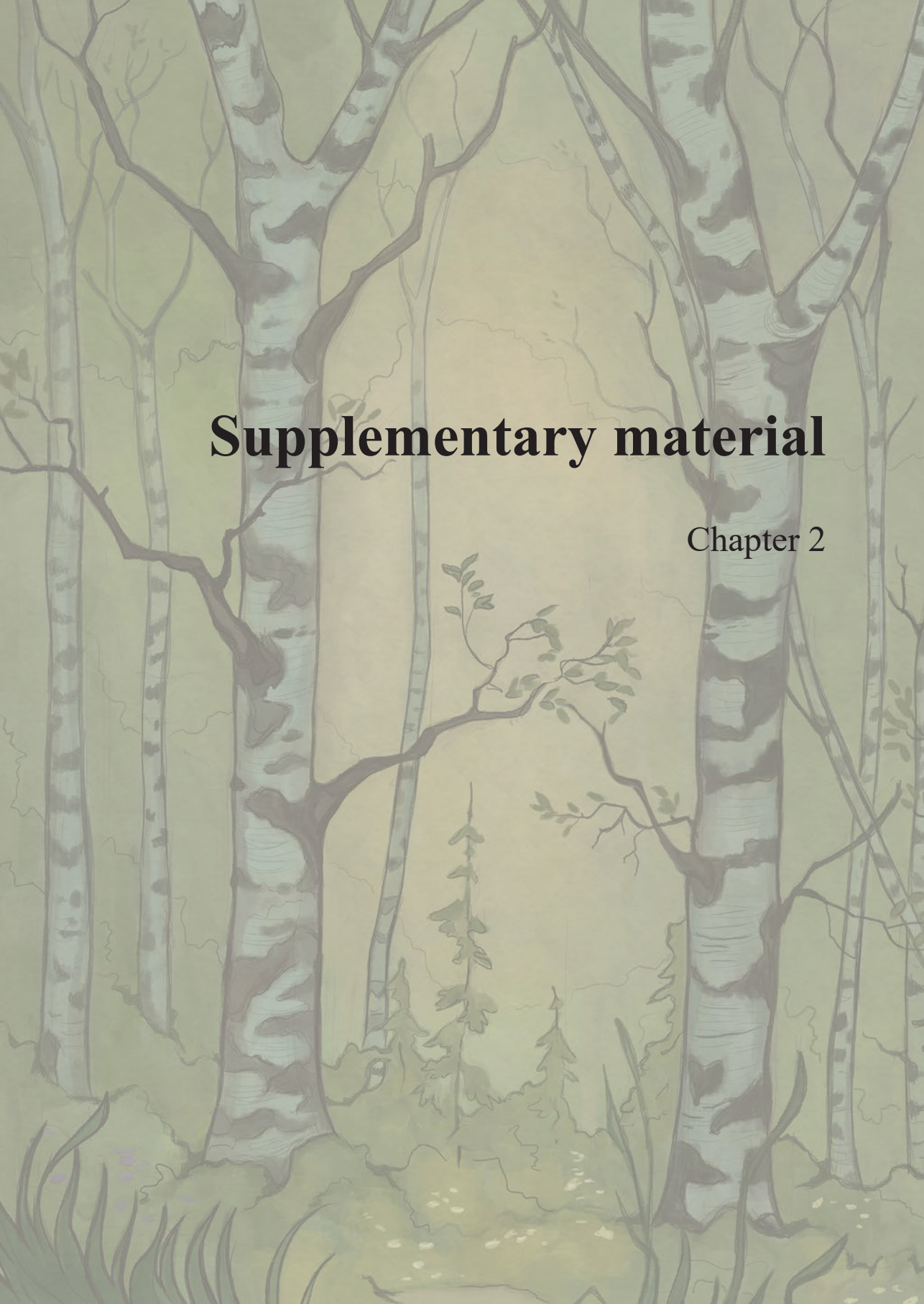


Supplementary material

Chapter 2

SUPPLEMENTARY METHODS

Elaboration on the search strategy

The inclusion criteria were transformed into a systematic search strategy consisting of Medical Subject Headings (MeSH) and title or abstract text words (tiab), that combined placebo or nocebo induction-related terms with (1) methods of experimental itch elicitation, (2) dermatological conditions associated with itch, or (3) eligible types of outcome parameters. The search was conducted in the PubMed, PsycInfo, and Embase databases on May 8th 2018, with search strategies for PsycInfo and Embase being derived from the PubMed strategy (see **Supplementary Figure S6** for the search strategy).

Parameters extracted from the articles

The following categories of parameters were extracted from the articles: 1) self-reported parameters (when self-report measures were not directly related to the intended placebo or nocebo induction, the experimental model used or the medical condition assessed, they were not included); 2) behavioral parameters; and 3) physiological parameters. In addition, descriptives of the included articles were extracted by piloted forms.

Risk of Bias assessment

The SYRCLE risk of bias tool was used by two independent raters (SM, KB) for articles describing animal research [1] together with the guidelines provided by O'Connor and Sargeant [2]. Assessed criteria were: selection bias (clarity of the description in regard to a. random sequence generation, b., baseline characteristics of animals, and c. concealment of group allocation), performance bias (clarity of description for a. random housing of animals, and b. the blinding of personnel and participants), detection bias (clarity of the description of the blinding of outcome assessments), attrition bias (description of incomplete outcome data), reporting bias (whether selective reporting occurred), and other bias (not specified before). Risk of bias of each human study was assessed using the Cochrane risk of bias tool [3]. Ratings of both independent raters were compared, and discrepancies between ratings were resolved by discussion. The following criteria were assessed: selection bias (clarity of the description in regard to a. random sequence generation, and b. concealment of group allocation), performance bias (clarity of description for the blinding of personnel and participants), detection bias (clarity of the description of the blinding of outcome assessments), attrition bias (description of

incomplete outcome data), reporting bias (whether selective reporting occurred), and other bias (not specified before). For both human and animal studies, each category was scored as ‘low RoB’ when the provided information was enough to suspect low bias, ‘high RoB’ when information was mentioned that would incur bias (e.g., insufficient blinding), or ‘unclear RoB’ when information was not clearly provided. Risk of bias analyses were descriptive: no further steps were undertaken to conduct sensitivity analyses or to exclude studies based on risk of bias ratings.

Supplementary Table S1. Brief summary of the methods and results of the included studies.

Population	Total no. of studies	Type of induction	Learning mechanism(s)	Outcome measure(s) category	Proportion of hypo-theses confirmed/k studies per outcome	% confirmed
Animals	8	Placebo	Conditioning	Behavioral	6 / 6	100
				Physiological	8 / 8	100
	17	Nocebo	Conditioning	Behavioral	5 / 7	71
				Physiological	10 / 11	91
	2	Nocebo	Operant conditioning	Behavioral	1 / 2	50
	4	Nocebo	Social induction	Behavioral	2 / 4	50
Healthy participants	7	Placebo	Verbal suggestions + hypnosis	Self-reported	1 / 1	100
				Physiological	4 / 7	57
	6	Placebo	Verbal suggestions	Self-reported	2 / 5	40
				Physiological	1 / 6	17
	1	OL placebo	Verbal suggestions	Self-reported	0 / 1	0
				Physiological	0 / 1	0
	2	Placebo	Conditioning (+ verbal suggestions)	Self-reported	2 / 2	100
	1	Nocebo	Verbal suggestions	Self-reported	1 / 1	100
				Physiological	1 / 1	100
	1	Nocebo	Conditioning (+ verbal suggestions)	Self-reported	3 / 3	100
				Physiological	1 / 1	100
	3	Nocebo	Social induction (e.g., contagious itch by video of people scratching)	Self-reported	3 / 3	100
				Behavioral	3 / 3	100
Patients	12	Placebo	Verbal suggestions + hypnosis	Self-reported	3 / 4	75
				Physiological	3 / 10	30
	2	OL placebo	Verbal suggestions (+ inert pill)	Self-reported	2 / 2	100
	3	Placebo	Pharmacological conditioning / conditioned dose reduction	Self-reported	1 / 3	33
				Physiological	2 / 3	67
	1	Placebo	Conditioning (+ verbal suggestions)	Self-reported	1 / 1	100
	1	Placebo	Social induction (advertising)	Physiological	1 / 1	100
	1	Nocebo	Verbal suggestions + hypnosis	Physiological	1 / 1	100
	4	Nocebo	Verbal suggestions	Self-reported	1 / 1	100
				Physiological	3 / 4	75
	5	Nocebo	Conditioning [a]	Self-reported	0 / 2	0
				Physiological	2 / 3	67
	5	Nocebo	Social induction (e.g., contagious itch by video of people scratching)	Self-reported	5 / 5	100
				Behavioral	4 / 4	100
				Physiological	0 / 1	0

General summary of the proportion of positive results for the studies above (summarized across type of induction and learning mechanisms involved)

Population	Total no. of studies	Type of induction	Learning mechanism(s)	Outcome measure(s) category	Proportion of hypo-theses confirmed/k studies per outcome	% con-firmed
Animals	31	All types	All mechanisms	Behavioral	14 / 19	74
				Physiological	18 / 19	95
Healthy participants	21	All types	All mechanisms	Self-reported	10 / 14	71
				Behavioral	3 / 3	100
				Physiological	7 / 16	44
Patients	34	All types	All mechanisms	Self-reported	13 / 18	72
				Behavioral	4 / 4	100
				Physiological	12 / 23	52

Note. Total no. of studies is the number of studies on the same type of induction (e.g., placebo). The 'k studies per outcome' in the column of proportion of hypotheses confirmed refers to the number of studies measuring that specific category of outcomes. Some studies report multiple experiments (e.g., follow-up experiments) or multiple outcomes of the same type (e.g., more than 1 self-report measure). For these studies, a hypothesis was considered confirmed when the average across experiments or outcomes indicated effective placebo or nocebo induction.

OL = open-label (non-concealed placebo effect induction). [a] Two out of five studies (Jordan, 1972, and Robertson, 1975) compared the efficacy of conditioning scratch responses for patients and healthy controls. While it was concluded that scratch responses were more easily conditioned in patients, no remarks regarding the efficacy of conditioning itself were made (i.e. no comparisons with control groups / a non-conditioned state). As such, these were not counted amongst the proportional positive results (confirmed hypotheses) in the table.

Supplementary Table S2. Overview of animal studies.

Author, year	*Subjects species (n, sex)*	*Study design*	*Category*	*Induction method type*	*Experimental conditions*	*Short summary of study findings*
I. Conditioned immunosuppression						
Ader, 1975 [4]	Charles River rats (96, male)	Between-subjects	Conditioned immunosuppression	Conditioning of a saccharin drinking solution (SAC, conditioned stimulus; CS) and a cyclophosphamide injection (CY, unconditioned stimulus; UCS), as tested by a delayed-type hypersensitivity response (DTH) sensitization to sheep red blood cells (SRBC)	1) Conditioned groups: a) CS_0 (not re-exposed to CS) b) CS_1 (conditioned, re-exposed once) c) CS_2 (conditioned, re-exposed twice) d) UCS (re-exposed once to UCS) 2) Non-conditioned control 3) Placebo group	▪ SAC preference in CS_1 and CS_2 groups: ↓ ▪ DTH response to SRBC: hemagglutination (antibody) titers in CS_1 and CS_2 groups: ↓
Bovbjerg, 1987 [5]	Mice (61, male)	Randomized controlled trial, between-subjects	Conditioned immunosuppression	Conditioning of SAC (CS) and CY (UCS), as tested by a DTH sensitization to SRBC	1) Conditioned groups: a) CS_r (experimental) b) CS_{0r} (not re-exposed) c) UCS (re-exposed to UCS) 2) Non-conditioned control 3) Placebo group	DTH response to SRBC: paw swelling in CS_r groups vs. controls: ▪ 1st exposure ↓, ▪ 2nd + 3rd exposures ↑
Exton, 2000 [6] *Experiment 1*	Dark agouti rats (30, male)	Randomized controlled trial, between-subjects	Conditioned immunosuppression	Conditioning of SAC (CS) and cyclosporine-A (CSA; UCS), as tested by a DTH sensitization to 2,4-dinitrochlorobenzene (DNCB)	1) conditioning 2) sham-conditioned 3) CSA exposed	▪ SAC preference after conditioning: ↓ ▪ DNCB-induced ear swelling after conditioning: ↓
Exton, 2000 [6] *Experiment 2*	Dark agouti rats (30, male)	Randomized controlled trial, between-subjects	Conditioned immunosuppression *following splenic denervation*	Conditioning of SAC (CS) and CSA (UCS), as tested by a DTH sensitization to DNCB	1) Sham conditioned, sham denervated 2) Sham conditioned, denervated 3) Conditioned, not denervated 4) Conditioned, denervated 5) CSA treated, denervated	▪ SAC preference after conditioning: ↓ ▪ DNCB-induced ear swelling after conditioning: ↓ ▪ Effects of splenic denervation on conditioning: n.s.
Kelley, 1985 [7] *Experiment 1*	Balb/c mice (60, female)	Between-subjects	*n/a*	Testing of the effects of lithium (LiCl) on DTH response, as tested by a DTH sensitization to SRBC	1) Immunized animals 2) Non-immunized animals	▪ No effects of LiCl on DTH-induced footpad swelling (as assessed by footpad thickness)

Supplementary Table S2 *(continued 2/11)*

Author, year	*Subjects species (n, sex)*	*Study design*	*Category*	*Induction method type*	*Experimental conditions*	*Short summary of study findings*
Kelley, 1985 [7] *Experiment 2*	Balb/c mice (33, female)	Between-subjects	Conditioned immunosuppression	Conditioning of SAC (CS) and an injection of LiCl (UCS), as tested by a DTH sensitization to SRBC	1) Placebo: SAC + saline, immunized 2) Non-conditioned: water + LiCl, immunized 3) Conditioned: SAC + LiCl, immunized 4) Placebo: SAC + saline, non-immunized 5) Non-conditioned: water + LiCl, non-immunized 6) Conditioned: SAC + LiCl, non-immunized	▪ SAC preference (measured by SAC intake) in immunized conditioned animals: ↓ ▪ DTH-induced footpad swelling (as assessed by footpad thickness) in immunized conditioned animals: ↓
Kelley, 1985 [7] *Experiment 3*	Balb/c mice (30, female)	Between-subjects	*n/a*	Testing whether conditioned effects on DTH in experiment 3 could have been caused by water deprivation, as tested by a DTH sensitization to SRBC	1) Water-deprived, DTH tested, immunized 2) Non-water-deprived, DTH tested, immunized 3) Water-deprived, DTH tested, non-immunized 4) Non-water-deprived, DTH tested, non-immunized	▪ Differences in DTH-induced footpad swelling (as assessed by footpad thickness) between groups: n.s.
Kelley, 1985 [7] *Experiment 4*	Balb/c mice (24, female)	Between-subjects	Conditioned immunosuppression	Conditioning of SAC (CS) and an injection of LiCl (UCS), as tested by a DTH sensitization to SRBC	1) Placebo: SAC + saline, immunized 2) Non-conditioned: water + LiCl, immunized 3) Conditioned: SAC + LiCl, immunized 4) Placebo: SAC + saline, non-immunized 5) Non-conditioned: water + LiCl, non-immunized 6) Conditioned: SAC + LiCl, non-immunized	▪ SAC preference (measured by SAC intake) in immunized conditioned animals: ↓ Plasma glucocorticoids in immunized conditioned animals: ↑
Mei, 2000 [8]	LACA mice & Wistar rats (36, male mice & female rats)	Randomized controlled trial, between-subjects	Conditioned immunosuppression	Conditioning of camphor odor (CAM, CS) and an intraperitoneal injection of CY (UCS), as tested by a DTH sensitization to DNCB	1) CR (conditioned) 2) UCR control 3) DTH control 4) DTH- control 5) CY control 6) CAM control	▪ Left/right ear weight ratio (as index of DTH response) after conditioning: ↓ Leukocyte migration inhibition (assessed by leukocyte migration inhibitory factor/LMIF) after conditioning: ↑

Supplementary Table S2 *(continued 3/11)*

Author, year	*Subjects species (n, sex)*	*Study design*	*Category*	*Induction method type*	*Experimental conditions*	*Short summary of study findings*
Rogers, 1976 [9]	Sprague-Dowley rats (80, male)	Between-subjects	Conditioned immunosuppression	Conditioning of SAC (CS) and an intraperitoneal injection of CY (UCS), as tested by a DTH sensitization to SRBC	1) conditioned (n=50): a) CS_0: no re-exposure b) CS_1: re-exposure once c) CS_2: re-exposed twice d) UCS: re-exposed to UCS only 2) non-conditioned (n=10) 3) placebo (PLA; n=10)	SAC preference in conditioned (CS_i) groups: ↓ ▪ DTH (assessed by hemagglutination/antibody titers) ↓ in CS_2 and UCS, compared to CS_1, CS_0 an control groups
Roudebush, 1991 [10]	Balb/c mice (*n* unknown, male)	Randomized controlled trial, within-between-subjects	Conditioned immunosuppression	Conditioning of SAC (CS) and CY or dexamethasone (DEX; UCS), as tested by a DTH sensitization to corticosterone	1) DTH negative 2) DTH positive 3) Conditioned 4) Non-conditioned 4) Other controls	Saccharin preference after conditioning with CY and DEX ↓ DTH-induced paw swelling after conditioning when CY=UCS ↓; when DEX=UCS n.s.
Wayner, 1978 [11] *Experiment 1*	Wistar derived albino rats (84, male)	Between-subjects	Conditioned immunosuppression	Conditioning of SAC (CS) and CY (UCS), as tested by a DTH sensitization to SRBC *In experiment 1, animals were sensitized to a T-cell **dependent** antigen: sheep red blood cells (SRBC)*	1) Conditioned: a. UCS: re-exposed to UCS once b. CS_0: not re-exposed to CS or UCS c. CS_1: CS re-exposed once d. CS_2: CS re-exposed twice e. CS_3: CS re-exposed thrice 2) Non-conditioned 3) PLA	▪ SAC preference ↓ in conditioned groups compared to baseline DTH response to SRBC – hemagglutination (antibody) titers: ▪ CS_1 & CS_2 ↓ than NC & CS_0; ▪ CS_1 & CS_2 compared to UCS: n.s.; CS_3 compared to all: n.s.
Wayner, 1978 [11] *Experiment 2*	Wistar derived albino rats (84, male)	Between-subjects	Conditioned immunosuppression	Conditioning of SAC (CS) and CY (UCS), as tested by a DTH sensitization to Bruscella (B.) Abortus *In experiment 2, animals were sensitized to a T-cell **independent** antigen: B. Abortus*	1) Conditioned: a. UCS: re-exposed to UCS once b. CS_0: not re-exposed to CS or UCS c. CS_1: CS re-exposed once d. CS_2: CS re-exposed twice e. CS_3: CS re-exposed thrice 2) Non-conditioned control 3) PLA	▪ SAC preference ↓ in conditioned groups compared to baseline DTH response to B. Abortus – hemagglutination (antibody) titers: ▪ *UCS excluded from analysis.* ▪ PLA + CS_3 ↑ than other groups ▪ NC, CS_0, CS_1, CS_2: n.s.

Supplementary Table S2 *(continued 4/11)*

Author, year	*Subjects species (n, sex)*	*Study design*	*Category*	*Induction method type*	*Experimental conditions*	*Short summary of study findings*
II. Conditioned allergic response						
Dark, 1987 [12] *Experiment 1*	Guinea pigs (24, male)	Randomized controlled trial, between-subjects	Conditioned allergic response	Conditioning of bovine serum albumin (BSA; UCS) to odor of dimethylsulfide or triethylamine (CS+/CS-)	1) stressed conditioned 2) non-stressed conditioned	Histamine release (assessed by blood serum level): ▪ stressed: post-conditioning ↑ ▪ non-stressed: n.s.
Dark, 1987 [12] *Experiment 2*	Guinea pigs (15, male)	Randomized controlled trial, between-subjects	Conditioned allergic response	Conditioning of BSA (UCS) to odor dimethylsulfide (CS)	1) conditioned 2) unpaired	Histamine release (assessed by blood serum level): ▪ post-conditioning: ↑
Djuric, 1987 [13] *Experiment 1*	Wistar rats (66, female)	Randomized controlled trial, between-subjects	Conditioned allergic response	Conditioning of ovalbumin (OA; UCS) to saccharin (CS)	Control groups 1) non-immunized SAC-OA 2) non-immunized SAC-OA with pre-exposure 3) immunized SAC-OA with pre-exposure 4) immunized, OA, no CS 5) immunized, SAC, no UCS Conditioned groups 6) immunized, SAC-OA (2mg) 7) immunized, SAC-OA (1mg, late evocation) 8) immunized, SAC-OA (1mg)	▪ Anaphylactic shock behavior post-conditioning: ↑ ▪ SAC preference post-conditioning: ↓
Djuric, 1987 [13] *Experiment 2*	Wistar rats (36, female)	Randomized controlled trial, between-subjects	Conditioned allergic response	Conditioning of OA (UCS) to saccharin (CS) for different retention periods	1) water-OA, no CS, 4 weeks rentention 2) water-OA, no CS, 8 weeks rentention 3) SAC + OA (2mg), 4 weeks rentention 4) SAC + OA (2mg), 8 weeks rentention	▪ Anaphylactic shock behavior after conditioning: ↑ ▪ SAC preference after conditioning: ↓ ▪ Effects of rentention period: n.s.
Djuric, 1988 [14]	Wistar rats (73, 53% male)	Between-subjects	Conditioned allergic response	Conditioning of OA (UCS) to saccharin (CS) with different dosages of OA	1) 0.0 mg OA (control) 2) 0.5 mg OA ($CS_{0.5}$) 3) 1.0 mg OA ($CS_{1.0}$) 4) 2.0 mg OA ($CS_{2.0}$) 5) 3.0 mg OA ($CS_{3.0}$)	▪ Anaphylactic shock behavior after conditioning: ↑ in parallel with the increase in OA dosage ▪ SAC preference following conditioning: ↓ as function of OA dosage

Supplementary Table S2 *(continued 5/11)*

Author, year	*Subjects species (n, sex)*	*Study design*	*Category*	*Induction method type*	*Experimental conditions*	*Short summary of study findings*
Irie, 2001 [15] *Experiment 1*	Guinea pigs (20, male)	Randomized controlled trial, between-subjects	Conditioned allergic response	Conditioning of OA (UCS) to an odor of dimethylsulfide (CS)	1) conditioned group: CS-UCS paired presentation 2) unconditioned group: unpaired presentation of CS or UCS	▪ Plasma histamine levels following conditioning: ↑
Irie, 2001 [15] *Experiment 2*	Guinea pigs (37, male)	Randomized controlled trial, between-subjects	Conditioned allergic response *(with and without anesthesia)*	Conditioning of OA (UCS) to an odor of dimethylsulfide (DMS; CS) or triethylamine (TEA, CS)	1) Conditioned, CS exposed (DMS), no UCS (antigen) 2) Conditioned, CS exposed (TEA), no UCS 3) Conditioned, saline exposed, no UCS 4) Unconditioned, CS exposed, no UCS 5) Conditioned, UCS exposed 6) Conditioned, CS+UCS exposed	▪ Plasma histamine levels: n.s. ▪ Bronchoalveolar lavage fluid (BALF) histamine levels: n.s. ▪ Lung tissue histamine levels: ↑ in DMS group compared to TEA group
Irie, 2002a [16] *Part 1*	Guinea pigs (34, male)	Randomized controlled trial, between-subjects	Conditioned allergic response *(with and without fasting stress induction)*	Conditioning of ovalbumin (UCS) to an odor of dimethylsulfide (CS)	1) Conditioned + feeding 2) Conditioned + fasting 3) Control (uncoupled UCS/CS presentation + feeding)	▪ Plasma histamine levels following conditioning: ↑ for fasting groups only
Irie, 2002a [16] *Part 2*	See Irie, 2002a part 1	Controlled trial, between-subjects (repeat of part 1 after 1 month, in which animals were reconditioned)	Conditioned allergic response *(with and without fasting stress induction)*	Conditioning of ovalbumin (UCS) to an odor of dimethylsulfide (CS)	1) Conditioned + fasting 2) Conditioned + feeding 6) Control (uncoupled UCS/CS presentation + feeding) *Feeding and fasting animals were switched compared to part 1*	▪ Plasma histamine levels following conditioning: n.s. ▪ Respiratory resistance: n.s. ▪
Irie, 2002b [17]	Guinea pigs (40, male)	Randomized controlled trial, between-subjects	Conditioned allergic response *(with and without isolation)*	Conditioning of ovalbumin (UCS) to an odor of dimethylsulfide (CS)	1) Conditioned, alone (acq), paired (evoc) 2) Conditioned, alone (acq), alone (evoc) 3) Conditioned, paired (acq), alone (evoc) 4) Conditioned, paired (acq),paired (evoc) 5) Control, paired	▪ Plasma histamine levels following conditioning: ↑ in groups 1, 2, 4 ▪ Plasma histamine levels in group 4 ↑ than in group 1, 3 and control ▪ Main effect of isolation: n.s.
Irie, 2004 [18]	Guinea pigs (24, male)	Randomized controlled trial, between-subjects	Conditioned allergic response *(with and without diazepam administration)*	Conditioning of ovalbumin (UCS) to an odor of dimethylsulfide (CS)	1) Conditioned, diazepam given on evocation day 1+2, saline on 3 2) Conditioned, saline given on evocation day 1, diazepam on 2+3	▪ Plasma histamine levels on day 1 following conditioning: ↑ only after saline, ▪ Group differences on evocation day 2+3: n.s.

Supplementary Table S2 *(continued 6/11)*

Author, year	*Subjects species (n, sex)*	*Study design*	*Category*	*Induction method type*	*Experimental conditions*	*Short summary of study findings*
MacQueen, 1989 [19]	Rats (43, sex not described)	Randomized controlled trial, between-subjects	Conditioned allergic response	Conditioning of a subcutaneous injection of egg albumin (EA, UCS) to an audiovisual cue (AV, CS)	1) Paired (conditioned) 2) Unpaired control 3) Negative control (placebo only) 4) Positive control (AV+EA)	▪ Rat Mast Cell Protease II (RMCP II) after conditioning: ↑
Markovic, 1988[20] *Experiment 1*	Wistar rats (24, female)	Randomized controlled trial, between-subjects	Conditioned allergic response	Conditioning of ovalbumin (OA, UCS) given intraperitoneally (ip) to a saccharin solution (SAC, CS)	1) CS-US group (immunized, CS-UCS) 2) Non-immunized rats, presented with a CS-UCS pairing (n.i. control) 3) US-only control (immunized , no CS) 4) CS-only control (immunized , no UCS)	▪ SAC preference after conditioning: ↓
Markovic, 1988[20] *Experiment 2*	Wistar rats (46, female)	Randomized controlled trial, between-subjects	Conditioned allergic response	Conditioning of OA (UCS) given intraveneously (iv) to a saccharin solution (SAC, CS)	1) CS-US group (immunized, CS-UCS) 2) Non-immunized rats, presented with a CS-UCS pairing (n.i. control) 3) US-only control (immunized , no CS) 4) CS-only control (immunized , no UCS)	▪ SAC preference after conditioning: ↓
Markovic, 1988[20] *Experiment 3*	Wistar rats (49, female)	Randomized controlled trial, between-subjects	Conditioned allergic response	Conditioning of OA (UCS) to an isotonic 3.16% sodium saccharin solution (CS) that was injected iv together with UCS	1) CS-US group (immunized, CS-UCS) 2) Non-immunized rats, presented with a CS-UCS pairing (n.i. control) 3) US-only control (immunized , no CS) 4) CS-only control (immunized , no UCS)	▪ SAC preference after conditioning: ↓
Peeke, 1987a [21]	Guinea pigs (24, male)	Randomized controlled trial, within-between-subjects	Conditioned allergic response *(with and without stress induction)*	Conditioning of bovine serum albumin (BSA; UCS) to an odor (triethylamine or dimethylsuldife; 50/50 rate for being CS+ or CS-)	1) Handled (stressed) conditioned 2) Non-handled conditioned	▪ Difference between CS+ and CS- presentation: ▪ plasma histamine level ↑ for CS+ in handled group; in non-handled group n.s. ▪ plasma cortisol levels ↑ for CS+ in handled group on 1 out of 3 extinction trials. For non-handled group, n.s.

Supplementary Table S2 *(continued 7/11)*

Author, year	*Subjects species (n, sex)*	*Study design*	*Category*	*Induction method type*	*Experimental conditions*	*Short summary of study findings*
Peeke, 1987b [22]	Guinea pigs (23)	Randomized controlled trial, within-between-subjects	Conditioned allergic response *(with and without pre-treatment with dexamethasone (DEX))*	Conditioning of nebulized bovine serum albumin (BSA; UCS) to an odor (dimethylsulfide, CS)	1) Conditioned with DEX 2) Conditioned w/o DEX 3) Control	▪ plasma histamine level ↑ in conditioned compared to control; with DEX ↑ than w/o. ▪ plasma cortisol levels ↓ in DEX pretreated compared to non-pretreated groups.
Russell, 1984 [23]	Guinea pigs (8, male)	Within-subjects	Conditioned allergic response	Conditioning to an injection of bovine serum albumin (BSA) in the footpad to an odor (triethylamine or dimethylsuldife; 50/50 rate for being CS+ or CS-)	None	Difference between CS+ and CS- presentation: ▪ plasma histamine level ↑
III. Conditioned lung anaphylactic shock						
Justesen, 1970 [24]	Guinea pigs (25, male)	Between-subjects	Conditioned asthmatic response	Conditioning of inhalation of an aerosol (nebulized protein solution, UCS) to aspects of the nebulizer process (i.e., sound, room; CS)	1) Conditioned animals 2) Naïve controls	▪ On 2nd day of conditioning: 3 out of 16 conditioned animals developed a response (asthmatic attack as measured by plethysmographic amplitude). ▪ On 3rd day of conditioning: 10 out of 16 conditioned animals developed response. ▪ On 4th day of conditioning: 16 out of 16 conditioned animals developed response.
Noelpp , 1951a [25]	Guinea pigs (8, sex not described)	Within-subjects	Conditioned lung anaphylactic response	Conditioning of an aerosol (allergen; UCS) to a sound (sound of aerosol nebulizer; CS) in 2 series of 5 acquisitions followed by 1 CS-exposure only (evocation).	None	▪ Asthmatic attack (marked by a change in breathing patterns) after 5 acquisitions: n.s. (1 out of 8 animals responded) ▪ Asthmatic attack after 10 acquisitions: n.s. (3 out of 8 animals responded)

Supplementary Table S2 *(continued 8/11)*

Author, year	*Subjects species (n, sex)*	*Study design*	*Category*	*Induction method type*	*Experimental conditions*	*Short summary of study findings*
Noelpp, 1951b [26]	Guinea pigs (28, male & female)	Between-subjects	Conditioned lung anaphylactic response	Conditioning of an aerosol (allergen; UCS) to a sound (sound of aerosol nebulizer; CS) in series of 5 acquisitions followed by 1 CS-exposure only (evocation).	1) Light-stressed animals 2) Non light-stressed animals	▪ For asthmatic attack (marked by a change in breathing patterns): Conditioning in stressed group ↑ than non-stressed. ▪ In stressed group 14/14 animals developed asthmatic breathing patterns upon CS-exposure. ▪ Of the non-stressed animals, 2/14 showed a clear response, 3/14 hints of a response (5/14 total).
Ottenberg, 1958 [27]	Guinea pigs (30, male)	Randomized controlled trial, between-subjects	Conditioned lung anaphylactic response	Conditioning of a fine mist of a dilute solution of egg white (UCS) to location (CS).	1) conditioned 2) control	▪ Asthma attacks (marked by use of accessory muscles, gasping, coughing, and pronounced respiratory distress) following egg-white spray: ↑ ▪ *After 3rd time exposure; 20% experienced attacks. During the extinction phase all of this 20 per cent (6 animals) had asthmatic attacks without the presence of egg-white spray. Four animals continued to have attacks through 9 trials. Extinction was apparent in all after 13 trials.*
Palermo-Neto, 2000 [28] *Experiment 1*	Wistar rats (60, male)	Randomized controlled trial, between-subjects	Conditioned lung anaphylactic response	Conditioning of ovalbumin (OA, UCS) as an aerosol to an audiovisual cue (AV, CS)	1) P_1 – paired (conditioned) 2) NC_1 – negative control 3) PC_1 – positive control (exp.1)	▪ Lung anaphylactic response (LAR; as behavior scored by observer on a 0-5 scale) following conditioning in P_1: ↑ ▪ Rearing n.s. ▪ Locomotion frequency: PC_1 ↓ than NC_1; P_1 & NC_1 n.s. ▪ Locomotion, rearing and the plus-maze data following conditioning: n.s. ▪ Corticosterone levels following CS-exposure in PC_1: ↑

Supplementary Table S2 *(continued 9/11)*

Author, year	*Subjects species (n, sex)*	*Study design*	*Category*	*Induction method type*	*Experimental conditions*	*Short summary of study findings*
Palermo-Neto, 2000 [28] *Experiment 2*	Wistar rats (40, male)	Randomized controlled trial, between-subjects	Conditioned lung anaphylactic response	Conditioning of OA (UCS) as an aerosol to an audiovisual cue (AV, CS)	1) NC_2 – negative control 2) PC_2 – positive control (exp.2) 3) U_2 – unpaired (CS & UCS presented separately) 4) N_2 – naïve control	▪ Rearing: n.s. ▪ Locomotion frequency: PC_2 ↓ than U_2, NC_2 or N_2 ▪ Plus-maze data: % of open arm exploration and time spent in open-arm ↓ in PC_2 compared to U_2, NC_2 or N_2 ▪ Corticosterone levels following CS-exposure in PC_2 ↑ *CS exposure per se induced effects in plus maze data and corticosterone levels; not conditioning*
IV. Operant conditioning of scratching response						
Morgan, 1979 [29] *Experiment 1*	Lister hooded rats (12, female)	Randomized controlled trial, between-subjects	Operant conditioning of scratching behavior	When desirable behavior was presented, a lever with food was presented. A concurrent variable-interval schedule was used.	1) wash-scratch behavior conditioned 2) wash-rear behavior conditioned 3) wash-scratch yoked control 4) wash-rear yoked control	▪ Scratching after operant conditioning: ↑ in wash-scratch than in the wash-rear group. ▪ Comparison with yoked control: n.s.
Morgan, 1979 [29] *Experiment 2*	Lister hooded rats (10, female)	Randomized controlled trial, between-subjects	Operant conditioning of scratching behavior	When desirable behavior was presented, a lever with food was presented. A concurrent variable-interval schedule was used.	1) wash-scratch behavior conditioned 2) wash-rear behavior conditioned 3) wash-scratch yoked control 4) wash-rear yoked control	▪ Scratching after operant conditioning: n.s.
Pearce, 1978 [30] *Experiment 1*	Lister rats (12, male)	Randomized controlled trial, between-subjects	Operant conditioning of scratching behavior	Animals were rewarded with food pellets when showing a bout of scratching behavior. Prior to experiment, animals were food deprived.	1) Scratching conditioned 2) Yoked control (to scratching group) 3) Lever press control	▪ Scratching behavior ↑ in scratching conditioning and lever press groups compared to control.
Pearce, 1978 [30] *Experiment 2*	Lister rats (8, male)	Randomized controlled trial, between-subjects	-	Preparation of experiment 3; testing effects of a collar on scratching. No conditioning took place.	-	-

Supplementary Table S2 *(continued 10/11)*

Author, year	*Subjects species (n, sex)*	*Study design*	*Category*	*Induction method type*	*Experimental conditions*	*Short summary of study findings*
Pearce, 1978 [30] *Experiment 3*	Lister rats (24, male)	Randomized controlled trial, between-subjects	Operant conditioning of scratching behavior	Animals were rewarded with food pellets when showing a bout of scratching behavior. Prior to experiment, animals were food deprived. *The effects of a collar on operant conditioning were tested.*	1) Scratching conditioned; with collar 2) Yoked control (to scratching group w collar) 3) Scratching conditioned; w/o collar 4) Yoked control (to scratching group w/o collar)	▪ Scratching behavior ↑ in scratching conditioning groups; with collar ↑ compared to w/o collar
Pearce, 1978 [30] *Experiment 4*	Lister rats (12, male)	Randomized controlled trial, between-subjects	Operant conditioning of scratching behavior	The extinction of previously learned scratching bouts was tested. *Conditioned animals of experiment 3 were used.*	1) Scratching conditioned; *collar removed* 2) Scratching conditioned; w/o collar	▪ Difference in scratching behavior between extinction with and w/o collar: n.s.
V. Social induction (contagious scratching)						
Feneran, 2013 [31] *Experiment 1*	Rhesus macaques (16, male)	Within-subjects observational	Social induction (contagious scratching)	Monkeys were paired with another and each pair was observed during 2x20min. All occurrences of scratching were recorded	None	Scratching after cagemate scratched (time elapsed between scratching bout of cagemate and target monkey): ↑
Feneran, 2013 [31] *Experiment 2*	Rhesus macaques (10, male)	Within-subjects, counterbalanced	Social induction (contagious scratching)	Videotapes of monkeys scratching in experiment 1 were presented (also neutral, and passive controls). Scratching in response was measured	None	Scratching episodes when presented with 'scratch video': ↑
Nakayama, 2004 [32]	Japanese monkeys (5, 40% male)	Within-subjects	Social induction (contagious scratching)	The transferability of scratching from a stranger to a target was tested in a time-series experiment. Three test conditions were used that varied as a function of the placement of the stranger and the visibility of the target by conspecific observers: the stranger, no stranger, and obstructed view conditions. Each monkey served as target for 10 trials.	None	▪ Main effect of target behavior (stranger's scratching): n.s. (marginal ↑ scratching). ▪ Scratching by conspecific observers ↑ in the stranger condition than in the no stranger and obstructed view conditions. Target's behavior: effects on scratching by conspecific observers ↑ only in the stranger condition

Supplementary Table S2 *(continued 11/11)*

Author, year	*Subjects species (n, sex)*	*Study design*	*Category*	*Induction method type*	*Experimental conditions*	*Short summary of study findings*
						▪
Whitehouse, 2016 [33]	Barbary macaques (6, 33.3% male)	Within-subjects	Social induction (contagious scratching)	10 scratching videos and 10 neutral videos were displayed, with half featured a familiar individual and half featured an unfamiliar individual. Each video was composed of five unique occurrences of scratching (or other neutral behavior) from a single individual.	None	▪ Influence of videos on scratching rate n.s. ▪ Attention to video ↑ for scratching video compared to neutral. ▪ Attention to video ↑ for familiar compared to unfamiliar individual.
Yu, 2017 [34] *Experiment 1*	Mice (14-16)	Randomized controlled trial, between-subjects	Social induction (contagious scratching)	Mice with excessive spontaneous scratching due to chronic itch were used as demonstrators; naïve mice were observers. Mice that did not scratch excessively were used as control demonstrators	1) Scratching group (demonstrators) 2) Control demonstrators 3) Scratching observers 4) Control observers	▪ Scratching behavior ↑ in scratching observers, in control observers n.s. ▪ Looking behavior n.s.
Yu, 2017 [34] *Experiment 2*	Mice (*n* unknown, sex unknown)	Randomized controlled trial, between-subjects	Social induction (contagious scratching)	The dispensability of auditory and olfactory cues for contagious scratching was tested by placing mice in front of a screen displaying a conspecific with scratching behavior. As a control, a video of a mouse that ambulated without scratching was displayed.	1) Scratching observers 2) Control observers	▪ Scratching behavior ↑ in scratching observers, in control observers n.s. ▪ Looking behavior n.s.

Note. acq = acquisition (of conditioned response); AV = audiovisual cue; BALF = bronchoalveolar lavage fluid; B. Abortus = Bruscella Abortus; BSA = bovine serum albumin; CAM = camphor odor; CS = conditioned stimulus; CSA = cyclosporine-A; CY = cyclophosphamide; DEX = dexamethasone; DMS = dimethylsulfide; DNCB = 2,4-dinitrochlorobenzene; DTH = delayed-type hypersensitivity response; EA = egg albumin; evoc = evocation (of conditioned response); ip = intraperitoneally; iv = intravenously; LAR = lung anaphylactic response; LiCl = lithium; LMIF = leukocyte migration inhibitory factor; NC = negative control; n.i. = non-immunized; n.s. = non-significant; OA = ovalbumin; P = paired; PC = positive control; PLA = placebo; SAC = saccharin drinking solution; SRBC = sheep red blood cells; TEA = triethylamine; CS = unconditioned stimulus

Supplementary Table S3. Overview of studies with healthy participants.

Author, year	*Subjects N (sex, age)*	*Study design*	*Category / type of comparison*	*Induction method type*	*Experimental conditions*	*Short summary of study findings*
I. Placebo induction (regular)						
a. By verbal suggestions and hypnosis						
Black, 1963b [35]	14	Within-subjects	Placebo	Verbal suggestions (VS) about decrease in response to allergen (horse serum), and hypnosis	None	Wheal area (Prausnitz-Küstner reaction to horse serum): ▪ ↓ following VS (as a function of depth of trance after hypnosis)
Laidlaw, 1996 [36]	38 (34.2% male, M_{age}= 39.2)	Within-subjects	Placebo	Participants were asked to use their imagination to become nonreactive to skin tests. Various possibilities were given under hypnosis for calming skin reactions to histamine (e.g., imagining a protective layer on or changes in the skin). In addition, direct VS of nonreactivity, coolness and dissociation were given.	None	Within-group pretest, control & intervention comparisons: ▪ Wheal areas following intervention compared to control: ↓ ▪ Titration gradients following intervention compared to control: ↓ ▪ Titration endpoint data: n.s.
Locke, 1987 [37]	12 *+ 30 non-hospitalized controls*	Within-subjects	Nocebo vs. placebo	Participants received one of the following verbal suggestions: 1) that the right arm (RA) would show an enhanced response to one of seven antigens (tetanus toxoid, diptheria toxoid, Streptococcus, tuberculin, Candida. Trichophyton, and Proteus), and the left arm (LA) was control; 2) that RA would show a suppressed response, LA control; 3) that RA was control, LA enhanced; or 4) RA control, LA suppressed. Suggestions were given under hypnosis. Audiotape reinforcement was practiced prior to testing.	1) RA enhanced 2) RA suppressed 3) LA enhanced 4) LA suppressed 5) 30 control subjects	▪ Skin test responses (measured as wheal/erythema (flare) ratio) after hypnosis and VS between groups differences: n.s.

Supplementary Table S3 *(continued 2/8)*

Author, year	*Subjects N (sex, age)*	*Study design*	*Category / type of comparison*	*Induction method type*	*Experimental conditions*	*Short summary of study findings*
Locke, 1994 [38]	24 (45.8% male, M_{age}= 22.0) *Participants were pre-selected on the basis of being able to influence skin temperature under hypnosis*	Within-subjects	Nocebo vs. placebo	Participants were told that highly hypnotizable persons are usually able to increase and decrease their peripheral skin temperature during hypnosis using images associated with cold or warmth. The images of immersing the hand in hot water and ice water were used unless the subjects offered particularly vivid images of their own. Participants were then given suggestions to influence their skin response to varicella-zoster (VZ) antigen. Suggestions were given under hypnosis.	1) No hypnosis 2) Hypnosis, no suggestions 3) Hypnosis, suggestions to suppress 4) Hypnosis, suggestions to enhance	▪ Skin response to VZ antigen (swelling/induration) after hypnosis and VS between groups differences: n.s.
Zachariae, 1989 [39]	18 (72.2% male) *Experimental group was preselected for being highly susceptible for hypnosis*	Between-subjects	Placebo vs. nocebo	Suggestions (VS) were given that the reaction to histamine / Mantoux test would be less than before for the right arm, and that the reaction for the left arm would increase. VS were given under hypnosis. Participants were given an audio tape containing hypnotic induction and guided imagery instructions repeating the VS. They were instructed to use the tape twice daily during 72h.	1) highly hypnotizable 2) controls	▪ Histamine flare ↓ following positive VS, and ↑ for negative VS. ▪ Histamine wheal n.s. (for pos. vs. neg.) <u>Mantoux skin responses:</u> ▪ Difference between positive VS and negative VS areas ↑ for both erythema size and induration ▪ Differences between positive VS and negative VS in the control group: n.s.
Zachariae, 1990 [40]	10 (50.0% male) *Preselected for being highly susceptible for hypnosis*	Within-subjects	Placebo	VS of analgesia in the right hand and arm were given.	None	▪ After hypnosis/suggestions: mean reduction in pain (from laser stimulation & from histamine skin prick) was 71.7% ▪ Laser-induced pain related brain potentials ↓during hypnosis/suggestions ▪ Flare response to histamine ↓ during hypnosis/suggestions compared to pre- and post-measurements

Supplementary Table S3 *(continued 3/8)*

Author, year	*Subjects N (sex, age)*	*Study design*	*Category / type of comparison*	*Induction method type*	*Experimental conditions*	*Short summary of study findings*
Zachariae, 1993 [41]	20 (60.0% male, M_{age}= 29.5)	Within-subjects	Placebo vs. nocebo	In a 2x2 design, participants had dinitrochlorobenzene (DNCB) and diphenylcyclopropenone (DCP) placed on either the left or right arm for a delayed-type hypersensitivity response (DTH). Participants had their arms marked with red (indicating the reaction should be increased) and blue (indicating the reaction should be decreased) (balanced).	None	▪ Effect of suggestions on DTH skin responses (erythema, skin thickness): n.s.
b. By verbal suggestions only						
Darragh, 2013 [42]	58 (27.6% male, M_{age}= 20.3)	Randomized-controlled between-subjects	Placebo	VS about to-be-expected skin response to histamine after application of a (sham) antihistamine cream	1) Placebo VS 2) Control	▪ Expected wheal area: ↓ ▪ Wheal area following VS: n.s. ▪ Heart rate (HR) following VS: ↓ ▪ Heart rate variability (HRV) following VS: n.s.
Darragh, 2015 [43]	50 (21.0% male, M_{age}= 22.0)	Within-subjects, counterbalanced	Placebo	VS about to-be-expected skin response to histamine after application of a (sham) antihistamine cream	1) control first, VS second 2) VS first, control second	▪ Itch levels in response to histamine skin prick following VS: ↓ at 1, 3 & 5min post histamine; n.s. at 7min ▪ Wheal response to histamine following VS: n.s.
Howe, 2017 [44]	159	Randomized controlled between-subjects	Nocebo vs. placebo	VS about either positive or negative effect of a lotion on itching; combined with either low or high experimenter warmth and competence (2x2x2 design)	1) Positive VS, high warmth, high competence 2) Positive VS, high warmth, low competence 3) Positive VS, low warmth, high competence 4) Positive VS, low warmth, low competence 5) Negative VS, high warmth, high competence 6) Negative VS, high warmth, low competence 7) Negative VS, low warmth, high competence 8) Negative VS, low warmth, low competence 9) Neutral suggestions, high warmth + competence	▪ Itch and anxiety not described ▪ Wheal and flare after positive VS compared to negative VS: ↓ ▪ Wheal and flare following positive/negative VS compared to neutral: n.s.

Supplementary Table S3 *(continued 4/8)*

Author, year	*Subjects N (sex, age)*	*Study design*	*Category / type of comparison*	*Induction method type*	*Experimental conditions*	*Short summary of study findings*
Meeuwis, 2018 [45]	92 (18.5% male, M_{age}= 21.3)	Randomized controlled between-subjects	Open-label placebo	Participants were given positive verbal suggestions that a histamine test would elicit little itch. They were told that expectations play a large role in how itch is experienced, and that the suggestion provided would likely already cause them to experience little itch. A control group was given no suggestions.	1) Open-label verbal suggestions 2) Control	After suggestions: ▪ Expected itch: ↓ ▪ Self-rated itch: n.s. ▪ Self-reported skin response: marginally ↓ ▪ Physical skin response: n.s.
Peerdeman, 2015 [46]	116 (29.0% male, M_{age}= 21.8)	Randomized controlled between-subjects	Placebo	Positive VS were given with and without imagery. Participants were given an inert pill and were told that people become less sensitive to physical sensations after taking the substance. For positive imagery, people were told to imagine their best possible health.	1) VS 2) Imagery 3) VS + imagery 4) Control	▪ Expectations of pain, itch, and fatigue ↓ following VS and imagery ▪ Physical sensitivity (pain, itch, fatigue combination score): n.s. ▪ Separate scores for pain, itch, fatigue: n.s. ▪ HR, and skin conductance: n.s.
Skvortsova, 2018 [47]	108 (all female, M_{age}= 22.1)	Randomized controlled between-subjects	Placebo	Positive verbal suggestions (VS) were given about a nasal spray (2x2 design). The spray contained either oxytocin (OXY) or saline. Participants received suggestions that the spray would decrease cold-water induced pain and histamine-induced itch, or neutral instructions.	1) No VS, no OXY 2) No VS, OXY 3) VS, no OXY 4) VS, OXY	▪ Expected pain & itch ↓ following VS compared to neutral. OXY and VS x OXY effects n.s. ▪ Pain (cold pressor task) ↓ following VS. OXY and VS x OXY effects n.s. ▪ Itch in response to histamine n.s. ▪ Wheal response to histamine & skin temperature following histamine: n.s.
Van Laarhoven, 2011 [48] ***Part 1***	105 (all female, M_{age}= 21.8)	Randomized controlled between-subjects	Nocebo vs. placebo	Suggestions for either pain or itch were given about electrical (dermal) stimuli. Participants were told that 95% of people experience pain/itch from the stimuli to induce high expectations. In control conditions, it was told that very few (5%) experience pain/itch to induce low expectations.	1) itch nocebo (high expectation), 2) itch nocebo control (low expectation), 3) pain nocebo (high expectation), 4)pain nocebo control (low expectation)	▪ Mechanically, electrically and chemically induced itch ↑ in itch nocebo compared to placebo control ▪ Mechanically, electrically and chemically induced pain ↑ in pain nocebo compared to placebo control

Supplementary Table S3 *(continued 5/8)*

Author, year	*Subjects N (sex, age)*	*Study design*	*Category / type of comparison*	*Induction method type*	*Experimental conditions*	*Short summary of study findings*
Van Laarhoven, 2011 [48] ***Part 2***	69 (from part 1)	Randomized controlled between-subjects	Placebo (*follows nocebo VS*)	Suggestions for low pain and itch were given about histamine iontophoresis. Participants were told that a substance was added to iontophoresis that would reduce itch / pain, so that nearly all participants would not experience itch / pain anymore. In control condition, it was told that nearly all participants experience itch / pain from the stimuli.	1) itch placebo 2) itch placebo control 3) pain placebo 4) pain placebo control	▪ Histamine induced pain & itch: effect of VS n.s.
c, By conditioning only						
No studies						
d, By a combination of verbal suggestions and conditioning						
Bartels, 2014 [49]	95 (23% male, M_{age}= 22.7 ± 3.2)	Randomized controlled between-subjects	Nocebo & placebo (*mixed presentation*)	Verbal suggestions (VS) about to-be-expected itch levels during presentation of colored lights, and conditioning of itch level (unconditioned stimulus; UCS) to differently colored lights (conditioned stimulus; CS)	1) VS + conditioning 2) VS 3) Conditioning 4) Control	Itch intensity during electric stimulation (assessed as change from baseline): ▪ Nocebo conditioning + VS: ↑ ▪ Nocebo VS: $\uparrow_{trend}$ ▪ Nocebo conditioning: n.s. ▪ Placebo conditioning + VS: ↓ ▪ Placebo VS: n.s. ▪ Placebo conditioning: n.s.
e. Social induction						
No studies						
II. Nocebo induction (regular)						
a, By verbal suggestions and hypnosis						
No studies						

Supplementary Table S3 *(continued 6/8)*

Author, year	*Subjects N (sex, age)*	*Study design*	*Category / type of comparison*	*Induction method type*	*Experimental conditions*	*Short summary of study findings*
b, By verbal suggestions only						
Stumpf, 2016 [50]	100 (50.0% male, M_{age}= 24.2)	Randomized controlled between-subjects	Nocebo	4 runs of skin prick tests were given, each with a different substance and suggestion: the substance causes 1) no itch (+ NaCl) (c-1), 2) some itch (+ histamine) (c-2), 3) enormous itch (+ histamine) (e-2), 4) enormous itch (+ NaCl) (e-1).	1) male participant, female investigator 2) male participant, male investigator 3) female participant, female investigator 4) female participant, male investigator	Whole sample: ▪ Wheal response (extent intensity) ↑ following suggestions +NaCl (difference between e1- c1) ▪ Self-rated itch & rated unpleasantness in response to skin prick tests ↑ following suggestions +NaCl (difference between e1- c1) ▪ Flare response (extent intensity) ↑ following suggestions +histamine (difference between e2 - c2) Effects of sex: ▪ Flare response ↑ for female investigators in comparison of histamine control + VS (difference between e2 - c2)
c, By conditioning only						
No studies						
d, By a combination of verbal suggestions and conditioning						
Van de Sand, 2018 [51]	30 (40.0% male, M_{age}= 25.5)	Within-subjects	Nocebo	Verbal suggestions (VS) were given that "subliminal TENS" (Transcutaneous Electrical Nerve Stimulation) aggravates pre-existing itch. Histamine was given in combination with thermal stimulation to generate a slight itch sensation. To convince participants that TENS was used, an initial conditioning procedure was conducted outside the scanner, where subjects experienced an increased itch sensation during "TENS" application. Itch was increased by increasing the amount of histamine provided under cooling conditions.	None	▪ Self-rated itch ↑ during nocebo conditions compared to control fMRI data: ▪ Contralateral (right) rolandic operculum activity ↑ during nocebo conditions compared to control ▪ Functional coupling between the insula and the periaqueductal gray (PAG) ↑ during nocebo conditions compared to control

Supplementary Table S3 *(continued 7/8)*

Author, year	*Subjects N (sex, age)*	*Study design*	*Category / type of comparison*	*Induction method type*	*Experimental conditions*	*Short summary of study findings*
Bartels, 2017 [52] *Part 1*	129 (21.3% male, M_{age}= 20.3 ± 2.5)	Within-subjects	Nocebo *(as preparation to reversal)*	VS about to-be-expected itch levels during presentation of colored lights, and conditioning of itch level (UCS) to colored lights (CS)	1) Nocebo VS + conditioning	Itch intensity during electric stimulation: ▪ Nocebo conditioning + VS: ↑
Bartels, 2017 [52] *Part 2*	129 (21.3% male, M_{age}= 20.3 ± 2.5)	Randomized controlled between-subjects	Placebo *(nocebo reversal)*	VS about to-be-expected itch levels during presentation of colored lights, and counterconditioning of itch level (UCS) to colored lights (CS)	1) Nocebo VS + conditioning 2) Placebo VS + conditioning 3) Extinction	Itch intensity during electric stimulation: ▪ Placebo VS + conditioning ↓
Bartels, 2017 [52] *Part 3*	129 (21.3% male, M_{age}= 20.3 ± 2.5)	Randomized controlled between-subjects	Nocebo & placebo *(carry-over)*	VS about to-be-expected itch levels during presentation of colored lights	1) Nocebo VS + conditioning 2) Placebo VS + conditioning 3) Extinction	Itch intensity during histamine iontophoresis: Placebo VS + conditioning ↓
e. Social induction						
Holle, 2012 [53] *Experiment 1*	51 (33.3% male, M_{age}= 21.1)	Within-subjects	Social induction (contagious itch)	Short videos of people scratching on different body locations were shown and compared with neutral control videos	None	▪ Itch in response to scratching videos: ↑ Scratching in response to videos: ↑
Holle, 2012 [53] *Experiment 2 (fMRI)*	18 (out of 51 participants from experiment 1)	Within-subjects	Social induction (contagious itch)	Short videos of people scratching on different body locations were shown and compared with neutral control videos	None	Brain activation: Activation of major areas of the itch matrix, including the thalamus, primary somatosensory cortex, premotor cortex (BA6), and insula following scratching videos: ↑
Lloyd, 2012 [54]	30 (6.7% male, M_{age}= 19.3)	Within-subjects, 2x3 factorial within-groups design	Social induction (contagious itch)	Pictures were shown to evoke itch sensations and scratching. Neutral, non-itch-related pictures were shown as control. Itch-evoking images were subdivided: 1) 'skin contact', 2) 'skin response', 3) 'context'	None	▪ Main effect of picture type 'itch' compared to 'non-itch' on self-reported itch and scratching: ↑ Picture type effects: ▪ Itch-related skin-contact pictures: ↑ itch, compared to skin-response pictures and context pictures. Itch-related skin-response pictures: ↑ scratching, compared to skin-contact pictures and context pictures.

Supplementary Table S3 *(continued 8/8)*

Author, year	*Subjects N (sex, age)*	*Study design*	*Category / type of comparison*	*Induction method type*	*Experimental conditions*	*Short summary of study findings*
Mitchell, 1995 [55]	63 (42.9% male)	Randomized controlled between-subjects	Nocebo	Suggestions of itch were given either subliminally or supraliminally in an audiotaped music fragment.	1) Music + subliminal suggestions of itch 2) Music only 3) Music + supraliminal suggestions of itch	Itch-associated words scored in an open-ended free-association segment & checklist-segment with itching-type sensations: ▪ reported itching ↑ in supraliminal compared to subliminal & music only groups. ▪ Scratching behavior ↑ in supraliminal vs. subliminal & music only groups.

Note. CS = conditioned stimulus; DCP = dephenylcyclopropenone; DNCB = dinitrochlorobenzene; DTH = delayed-type hypersensitivity response; HR = heart rate; HRV = heart rate variability; LA = left arm; NaCl = natriumchloride; n.s. = non-significant; OXY = oxytocin; PAG = periaqueductal gray; RA = right arm; TENS = transcutaneous electrical nerve stimulation; UCS = unconditioned stimulus; VS = verbal suggestions; VZ = varicella zoster

Supplementary Table S4. Overview of studies with patient samples.

Author, year	*Subjects N (sex, mean age)*	*Condition studied*	*Category / type of comparison*	*Study design*	*Induction method type*	*Experimental conditions*	*Short summary of study findings*
I. Placebo induction (regular)							
a. By verbal suggestions and hypnosis							
Black, 1963a [56]	12	Allergy	Placebo	Within-subjects	Verbal suggestions (VS) not to react to allergen, plus hypnosis	None	Immediate type hypersensitivity response to allergen: ▪ Skin temperature: ↓ ▪ Skinfold-thickness: ↓
Fry, 1964 [57] *Experiment 1*	47	Allergic asthma, hay fever, or pollen or dust mite allergy	Placebo	Between-subjects	VS not to react to test, plus hypnosis	1) controls (no VS, no hypnosis) 2) VS+ hypnosis	▪ Wheal size in response to allergen after VS and hypnosis: ↓ ▪ Flare size in response to allergen after VS and hypnosis: ↓
Fry, 1964 [57] *Experiment 2*	47	Allergic asthma, hay fever, or pollen or dust mite allergy	Placebo	Between-subjects	VS not to react to test, plus hypnosis	1) VS + hypnosis for one arm only 2) VS + hypnosis for both arms 3) Hypnosis, no suggestions	▪ Wheal size after VS and hypnosis: n.s. ▪ Flare after VS and hypnosis: n.s.
Hajek, 1990 [58]	13; 24 healthy controls (HC)	Atopic eczema	Placebo	Within-subjects	VS that the immune system would remove damaged cells and that the disease would be cured, plus hypnosis	1) patients with atopic eczema given suggestions 2) HC given suggestions 3) HC	▪ Cutaneous pain threshold after VS in patients and HC: ↑ ▪ Pain threshold increase correlated with improvement of atopic eczema symptoms
Laidlaw, 1994 [59]	5 (40% male, M_{age}= 22.0)	Asthma	Placebo	Within-subjects	VS of numbness, coolness, non-reactivity and dissociation were given for one arm, and of being alive and reactive for the other arm; plus hypnosis	None	▪ Flare size after positive VS compared to control sessions: ↓ ▪ Wheal sizes after positive VS compared to control sessions: n.s. ▪ Flare and wheal sizes compared to other arm within hypnosis sessions: n.s.

Supplementary Table S4 *(continued 2/10)*

Author, year	*Subjects N (sex, mean age)*	*Condition studied*	*Category / type of comparison*	*Study design*	*Induction method type*	*Experimental conditions*	*Short summary of study findings*
Langewitz, 2005 [60]	79 (51.9% male)	Hay fever	Placebo	Within-subjects counterbalanced	Self-hypnosis was instructed in 2 to 5 sessions: following standard trance induction, patients were instructed to imagine a 'safe place' where breathing was undisturbed, eyes, nose and throat were feeling comfortable and cool. Patients were advised to perform self-hypnosis at the onset of allergic symptoms.	1) 1st season: learning self-hypnosis, 2nd season: continuing self-hypnosis 2) 1st season: standard anti-allergic treatment, 2nd season: learning self-hypnosis	▪ Daily self-reported symptoms and medication use in season 1 following self-hypnosis, compared to standard treatment: n.s. ▪ Retrospective symptoms following self-hypnosis: ↓ ▪ Nasal flow symptoms in season 1 following self-hypnosis compared to control: n.s. ▪ Symptoms reported during nasal flow test following self-hypnosis compared to baseline: ↓ ▪ Time to reach critical dose following self-hypnosis compared to baseline: ↑
Levine, 1966 [61]	Group 1: 10 (70.0% male) Group 2: 10 (20.0% male) Group 3: 10 (40.0% male)	Group 1: allergic or sensitive to ragweed Group 2: urticaria Group 3: healthy controls (HC)	Nocebo vs. placebo	Within-subjects	HC were instructed that the skin tests on one arm would react more compared to previous (baseline) visit, and that the other arm would react less. In patient groups, the instruction was given that one arm would react less and the other the same as previous. Given under hypnosis.	None	▪ Response to histamine after hypnosis and VS (assessed by wheal and flare size to histamine and phosphate dilutions): n.s.
Schertzer, 1987 [62]	15 (26.7% male)	Chronic urticaria	Placebo	Within-subjects, counterbalanced	Participants received two sessions: 1) hypnotic induction and suggestions for symptom relief; and 2) a control session. Suggestions for skin clearing and disappearance of hives were given.	None	Self-rated itch following hypnosis treatment: ↓ ▪ Number of wheals following hypnosis treatment: n.s.

Supplementary Table S4 *(continued 3/10)*

Author, year	*Subjects N (sex, mean age)*	*Condition studied*	*Category / type of comparison*	*Study design*	*Induction method type*	*Experimental conditions*	*Short summary of study findings*
Sinclair-Gieben, 1959 [63]	14	Bilateral and multiple common warts	Placebo	Within-subjects	Suggestions were given that the warts on one side (usually hand) would disappear (other side of the body was control), with hypnosis.	None	▪ Any immediate improvement in number of warts observed was on the treated side.
Surman, 1973 [64]	36	Bilateral warts of common or planar type	Placebo	Between-subjects (comparison with untreated controls)	Suggestions were given that a tingling sensation in the warts on one side of the body (chosen by the patient) would be experienced and that only those warts would subsequently disappear. Suggestions were given with hypnosis.	1) experimental 2) untreated control	▪ Nine patients (53%) experienced improvement in warts following treatment; 0% in untreated controls [A]
Tausk, 1999 [65]	11	Psoriasis	Placebo	Between-subjects	Active suggestions under hypnosis or neutral hypnosis were given. Suggestions were that patients' psoriasis would improve, neutral consisted of suggestions of relaxation and wellbeing inherent to the hypnosis procedure.	1) Active suggestions 2) neutral	▪ Clinical psoriasis severity (PASI) following active suggestions: n.s. *Greater improvement found in highly hypnotizable individuals.*
Ullman, 1960 [66]	15 (good hypnotizable patients) *+ 47 poorly hypnotizable controls*	Multiple vulgar warts (n=9), both multiple vulgar warts and plantar warts (n=4), single vulgar wart (n=1), multiple condyloma acuminate (n=1) *Controls: multiple warts*	Placebo	Within-between-subjects (comparison between good and poorly hypnotizables)	A positive suggestion that the treatment would be successful and that the warts would begin to disappear was given, with hypnosis. The procedure was repeated on subsequent visits if little or no change in the warts was observed.	1) Good hypnotizable 2) Poorly hypnotizable	▪ A significantly higher number of cures for warts was associated with deep hypnosis than with being poorly hypnotizable

Supplementary Table S4 *(continued 4/10)*

Author, year	*Subjects N (sex, mean age)*	*Condition studied*	*Category / type of comparison*	*Study design*	*Induction method type*	*Experimental conditions*	*Short summary of study findings*
West, 1961 [67]	14	Widespread atopic dermatitis	Placebo	Within-subjects	Patients were told under hypnosis their skin was well and would react normally to the tests. The expected normal reactions were described to them as tests were performed.	None	▪ Three (21%) of the 14 patients had no delayed blanch during hypnosis. [A] ▪ Three of the 4, or 75%, who previously had not shown histamine flares, had a definite flare when tested during hypnosis [A] ▪ All 14 patients had a white line response instead of the normal red line as a result of stroking the skin before, during, and after hypnosis [A]
b. By verbal suggestions only							
Schaefer, 2016 [68]	25 (16.0% male, M_{age}= 26.0)	Allergic rhinitis	Open-label (OL) placebo	Randomized controlled, between-subjects	Participants received an explanation about placebos and placebo effects, and were asked to take inert pills twice daily for 14 days.	1) OL placebo + treatment as usual (TAU) 2) TAU only	▪ Allergic symptoms composite score following open-label placebos + TAU ↓ compared to TAU only ▪ Separate allergic symptoms n.s.
Schaefer, 2018 [69]	46 (19.6% male, M_{age}= 24.9)	Allergic rhinitis	Open-label (OL) placebo	Randomized controlled, between-subjects	A 2x2 design was used in which participants were asked to take inert pills twice daily for 14 days or received no inert pills (with and without information on placebo effects) . All patients used medication (treatment as usual, TAU).	1) OL placebos with briefing + TAU 2) OL placebos w/o briefing + TAU 3) TAU with briefing 4) TAU w/o briefing	▪ Allergic symptoms composite score ↓ over time for OL placebos + TAU compared to TAU only. ▪ Briefing effects on symptoms n.s.
c, By conditioning / conditioned dose reduction							
Ader, 2010 [70]	46 (46% male)	Psoriasis	Placebo	Randomized controlled between-subjects	Conditioned dose reduction with partial reinforcement	1) standard therapy 2) partial reinforcement 3) dose control	▪ Psoriasis severity scale (PSS) during partial reinforcement vs. control and standard therapy: ↓ in 1 of 2 sites ▪ Relapse in psoriasis during partial reinforcement vs. control and standard therapy: ↓

Supplementary Table S4 *(continued 5/10)*

Author, year	*Subjects N (sex, mean age)*	*Condition studied*	*Category / type of comparison*	*Study design*	*Induction method type*	*Experimental conditions*	*Short summary of study findings*
Goebel, 2008 [71]	30 (46.7% male, M_{age}= 41.1)	Monovalent allergy to dust mite	Placebo	Randomized controlled between-subjects	Conditioning of antihistamine effects (UCS) to a green-colored milkshake (CS)	1) conditioned-not-evoked (water + placebo) 2) conditioned (drink + placebo) 3) drug (water + desloratadine)	Subjective allergic symptoms: n.s. between groups Wheal size in response to dust mite: n.s. between groups Basophil activation: ↓ in conditioned and drug group vs. conditioned-not-evoked Blood count & flow cytometry: n.s. between groups
Vits, 2013 [72]	63 (39.7% male, M_{age}= 30.3)	Dust mite allergy	Placebo	Randomized controlled between-subjects	Conditioning of antihistamine effects (UCS) to a green-colored milkshake (CS)	1) conditioned 2) sham-conditioned control 3) natural history (NH)	Subjective allergic symptoms in response to nasal provocation test (NPT) ↓ in both conditioned and sham-conditioned compared to NH on 1st and 5th evocation Wheal size in response to dust mite ↓ in both conditioned and sham-conditioned compared to NH on 1st evocation, 5th evocation n.s.
d, By a combination of verbal suggestions and conditioning							
Klinger, 2007 [73]	48 (50.0% male, M_{age}= 27.4) *+ 48 matched healthy controls (HC) that were matched on age and gender*	Atopic dermatitis (AD)	Placebo	Randomized controlled, between-subjects	Conditioning of pain level (UCS) to a stimulus (ointment; CS); with or without positive verbal suggestions (VS) regarding this ointment.	1) AD, no VS, no conditioning 2) AD, no VS, conditioning 3) AD, VS, no conditioning 4) AD, VS, conditioning 5) HC, no VS, no conditioning 6) HC, no VS, conditioning 7) HC, VS, no conditioning 8) HC, VS, conditioning	Main effect of VS on pain (in response to electrical stimulation): ↓ Main effect of conditioning on pain: n.s. VS x conditioning effect on pain: ↓ No differences between HC and AD groups

Supplementary Table S4 *(continued 6/10)*

Author, year	*Subjects N (sex, mean age)*	*Condition studied*	*Category / type of comparison*	*Study design*	*Induction method type*	*Experimental conditions*	*Short summary of study findings*
e. By social induction							
Kamenica, 2013 [74]	340 (37.8% male, M_{age}= 27.7)		Social induction (effects of advertisement)	Randomized controlled between-subjects	A movie was shown to participants, which was interrupted every 5 min with advertisements for antihistamine (ah-)A (the antihistamine used in the study) and for ah-B. The advertisement of ah-B claimed that it works faster than ah-A.	1) Allergic, ah-A advertisement 2) Non-allergic, ah-A advertisement 3) Allergic, ah-B advertisement 4) Non-allergic, ah-B advertisement	Wheal size in non-allergic participants: n.s. at 60 min; ↑ efficacy following ah-A ad. at 120 min, compared to ah-B ad. Wheal size in allergic participants: ↓ efficacy following ah-A ad. at 60 min compared to ah-B ad., n.s. at 120 min.
II. Nocebo induction (regular)							
a, By verbal suggestions and hypnosis							
Hajek, 1992 [75]	8 (M_{age}= 26.4); 6 HC	Atopic eczema	Nocebo	Within-subjects	Verbal suggestions (VS) that pain is experienced in the middle of the upper part of the back, plus hypnosis	1) patients with atopic eczema 2) healthy controls	▪ Skin temperature for patients and HC: ↑ following VS
b. By verbal suggestions only							
Luparello, 1968 [76]	Group 1: 40 (35.0% male, M_{age}= 25.8) Group 2: 15 Group 3: 15 Group 4: 10	Group 1: Asthma due to allergens or irritants Group 2: Sarcoid or tuberculosis (controls) Group 3: Chronic bronchitis (controls) Group 4: healthy controls (HC)	Nocebo *(plus placebo given as nocebo reversal)*	Within-subjects	Participants were told that they would be inhaling five different concentrations of an irritant or allergen which they had previously indicted as being associated with his asthmatic attacks (in progressively increasing amounts). When dyspnea or wheezing was experienced, the inhalations were stopped and a placebo in the form of nebulized physiologic saline solution was administered; participants were told that they were receiving a bronchodilator.	None	<u>After negative suggestions:</u> ▪ conductance—thoracic gas volume ratio (Ga/TGV) ↓ compared to baseline (in 30% of subjects) ▪ Airway resistance (Ra) ↑ compared to baseline (in 30% of subjects) <u>After placebo administration with positive suggestions:</u> ▪ Ga/TGV ↑ compared to measurement after negative suggestions ▪ Ra ↓ compared to measurement after negative suggestions

Supplementary Table S4 *(continued 7/10)*

Author, year	*Subjects N (sex, mean age)*	*Condition studied*	*Category / type of comparison*	*Study design*	*Induction method type*	*Experimental conditions*	*Short summary of study findings*
McFadden, 1969 [77]	29 (34.5% male)	Bronchial asthma	Nocebo *(plus placebo given as nocebo reversal)*	Within-subjects	Each subject was informed that they would be inhaling five concentrations of an irritant or allergen that they associated with their asthma attacks. In the event that dyspnea and wheezing occurred, a placebo in the form of nebulized saline was administered. The subjects were told that they were receiving a bronchodilator. Testing was conducted twice, on separate days.	None	<u>After negative suggestions on day 1 & 2 both:</u> ▪ Conductance—thoracic gas volume ratio (Ga/TGV) ↓ compared to baseline for reactors (51.7%) ▪ Airway resistance (Ra) ↑ compared to baseline for reactors (51.7%) <u>After positive suggestions:</u> ▪ Ga/TGV ↑ compared to measurement after negative suggestions ▪ Ra & TGV ↓ compared to measurement after negative suggestions <u>When bronchodilator given as allergen:</u> ▪ Ga/TGV ↑ in nonreactors. In reactors, Ga/TGV n.s.
Napadow, 2015 [78]	14 (57.1% male, M_{age}= 25.4)	Atopic dermatitis	Nocebo	Within-subjects, counterbalanced	Temperature-modulation fMRI scans were given. A clear, odorless saline solution was pricked into the forearm. For the 'open' saline control run, the solution was a 'simple drop of water, which we are using as a control condition to compare with the drop of allergen you will receive later'. For the 'nocebo' saline fMRI run, subjects were led to expect an allergen solution prick test at this scan, as experienced previously.	None	▪ Itch ↑ in nocebo condition compared to open saline ▪ Expected itch correlated with self-reported itch. <u>fMRI data:</u> ▪ fMRI signal increase during the increasing itch phase in the dorsolateral prefrontal cortex (dlPFC), caudate, and intraparietal sulcus (iPS): ↑ compared to open saline
Weiss, 1970 [79]	16 (62.5% male)	Allergic asthma	Nocebo	Within-subjects	Suggestions were given that patients would be given a bronchial challenge test using a substance to which they were known to be allergic It was told that five inhalations of each of several strengths of extract were given, starting with a highly dilute extract and continuing through higher concentrations (max. 6) until wheezing was experienced. Control (saline) inhalations would be given as well.	None	Suggestion had no significant effects on any of the included measures (Wright-McKerrow Peak Flow meter; maximum expiratory flow rate; respiratory pattern)

Supplementary Table S4 *(continued 8/10)*

Author, year	*Subjects N (sex, mean age)*	*Condition studied*	*Category / type of comparison*	*Study design*	*Induction method type*	*Experimental conditions*	*Short summary of study findings*
c, By conditioning only							
Barrett, 2000 [80] *experiment 1*	60	Seasonal allergic rhinitis	Nocebo	Randomized controlled between-subjects	Conditioning paradigm with single acquisition and 2 evocations	1) Placebo control (No CS, PLA) 2) CS control (CS, PLA) 3) Experimental (CS, UCS -> CS, PLA) 4) UCS control (No CS, UCS -> No CS, PLA)	▪ Subjective symptoms scores (SSS) n.s. between groups ▪ peak nasal inspiratory flow (PNIF) ↑ in conditioned groups on 1st evoc ▪ Histamine levels ↑ in conditioned groups
Barrett, 2000 [80] *experiment 2*	15 (conditionable participants of exp. 1)	Seasonal allergic rhinitis	Nocebo	Randomized controlled between-subjects	Conditioning paradigm with 1, 2, or 3 acquisitions and 2 evocations	1) 1 acq trial group 2) 2 acq trials group 3) 3 acq trials group	▪ SSS n.s. between groups ▪ PNIF n.s. between groups ▪ Histamine levels: ↑ on 1st evoc in 3 acq. group exclusively
Booth, 1995 [81]	15 (33.3% male, M_{age}= 21.1)	Allergic to dust mite, gras pollen, animals	Nocebo	Within-subjects	Conditioning paradigm with 8 acquisitions and 2 evocations	None	▪ Wheal size to sham allergen compared to real allergen response: n.s.
Gauci, 1994 [82]	22 (22.7% male)	Allergic to dust mite	Nocebo	Randomized controlled between-subjects	Conditioning of an allergic response to dust mite (UCS) to a blue colored drink (CS)	1) Conditioned group 2) CS control group 3) UCS control group	▪ Subjective allergic symptoms after conditioning: n.s. ▪ Nasal tryptase level after conditioning: ↑
Jordan, 1972 [83]	18 (33.3% male, M_{age}= 25.3) *+ 18 matched controls with non-dermatological medical conditions*	Atopic dermatitis (AD)	Nocebo	Within-subjects	Conditioning of an itch stimulus (UCS) with a modified bell of constant loudness (CS), with 75% intermittent reinforcement	1) AD patients 2) Other non-dermatology (control) patients	Effects on itch (as measured by scratching; patients were instructed to scratch lightly when an itch stimulus was perceived) and on galvanic skin response (GSR): ▪ No. of trials to habituate to CS: ↑ in AD ▪ No. of conditioned scratch & GSR responses: ↑ ▪ in AD

Supplementary Table S4 *(continued 9/10)*

Author, year	*Subjects N (sex, mean age)*	*Condition studied*	*Category / type of comparison*	*Study design*	*Induction method type*	*Experimental conditions*	*Short summary of study findings*
Robertson, 1975 [84]	11 (45.5% male, M_{age}= 50.5) *+ 11 matched controls*	Lichen simplex *Controls were treated for: solar keratoses (n=8), tinea cruris (healing, n=1), pityriasis rosea (healed, n=1), chronic psoriasis (n=1)*	Nocebo	Within-subjects *Effects were compared across patients and HC*	Scratch responses were conditioned; an electrical itch stimulus (UCS) was paired with a tone (CS). Patients were instructed to scratch if they experienced itch.	None	▪ No. of conditioned scratch responses in lichen simplex compared to controls: ↑
d, By a combination of verbal suggestions and conditioning							
No studies							
d, By social induction							
Niemeier, 2000 [85]	14 (M_{age}= 36.4) *+ 11 (M_{age}= 43.1) who stated to be free of skin diseases*	Skin disease	Nocebo (contagious itch)	Within-subjects ABA design	A public lecture was organized: 'Itching – what's behind it?'. The first part of the lecture included slides that induce itching (pictures of fleas, mites, scratch marks on the skin, allergic reactions etc.), while the second part showed slides that induce relaxation and sense of well-being.	None	In both participants with and without skin disease: ▪ itch ↑ when itch slides were presented compared to relaxation part. ▪ scratching ↑ when itch slides were presented compared to relaxation part.
Papoiu, 2011 [86]	11 (27.3% male, M_{age}= 32.7) *+ 14 healthy volunteers (57.1% male, M_{age}=27.1)*	Mild to moderate atopic dermatitis (AD)	Nocebo *(visual transmission of itch)*	Within-subjects	Participants watched short 5 min clips of people scratching their forearm, or of neutral content as control. The order of videos was randomized. Participants received either a mock or itch stimulus during the video (iontophoresis with either an isotonic aqueous (saline) solution or 1% histamine dihydrochloride).	None	▪ In AD: itch ↑ for itch video + itch stimulus compared to neutral video + itch stimulus. ▪ In healthy: itch n.s. ▪ Atopics and in AD: scratching behaviour ↑ for itch video+saline and itch video+itch stimulus.

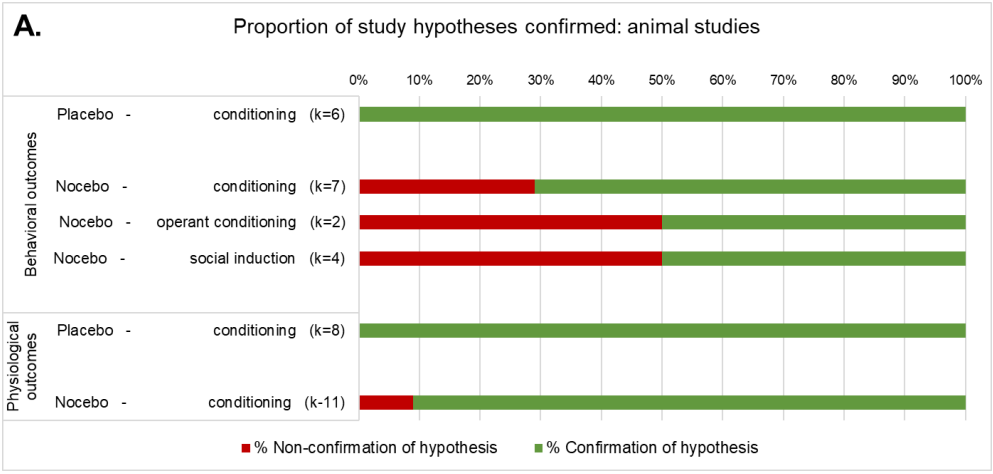
Supplementary Table S4 *(continued 10/10)*

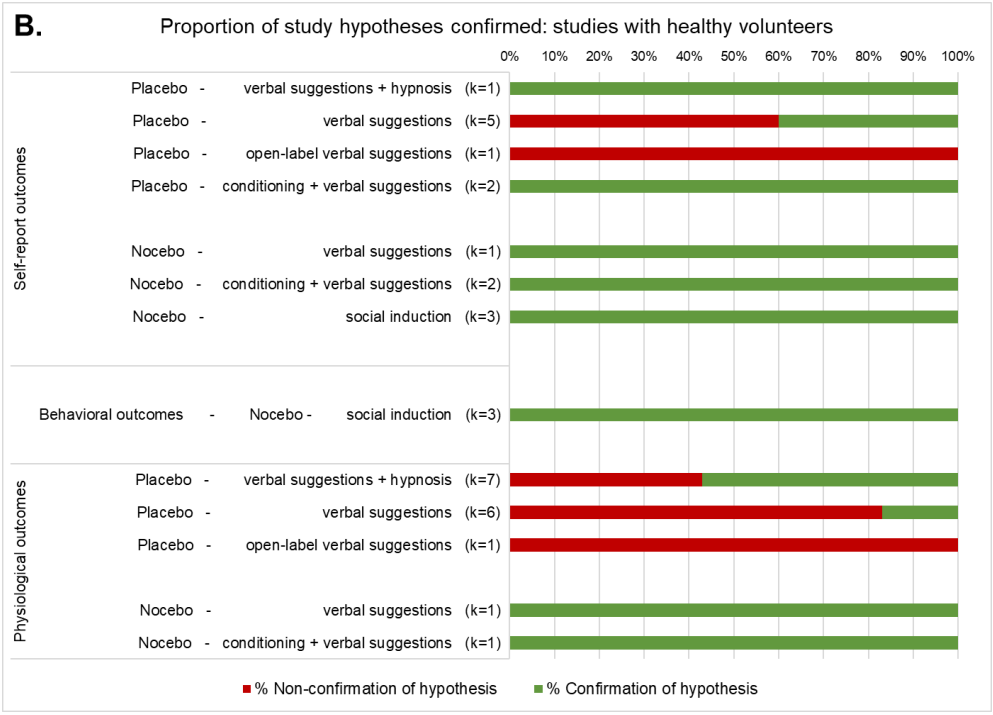
Author, year	*Subjects N (sex, mean age)*	*Condition studied*	*Category*	*Study design*	*Induction method type*	*Experimental conditions*	*Short summary of study findings*
Schut, 2014 [87]	27 (44.4% male, M_{age}= 23.6) *+ 28 healthy volunteers (35.7% male, M_{age}= 23.3)*	Atopic dermatitis	Nocebo (contagious itch)	Within-between-subjects (comparison between patients and controls)	Itch-inducing and neutral videos were presented (order was counterbalanced). An experimental video (EV) on "Itch – what is behind it?" was used to induce itch, while a video on "Skin – the communication organ" served as a control video (CV). Pictures were selected according to a former study (Niemeier, 2000)	None	▪ Self-rated itch ↑ after EV compared to CV ▪ Number of scratch movements ↑ after EV compared to CV
Schut, 2017 [88]	11 (45.5% male, M_{age}= 32.8) *Participants were selected on being responsive to visual itch cues*	Atopic dermatitis	Nocebo (contagious itch)	Within-subjects, counterbalanced	Itch was induced by a video showing people scratching (EV). A video showing the same people sitting idle was used as a control (CV).	None	Self-rated itch ↑ after EV compared to CV Number of scratch movements ↑ after EV compared to CV <u>fMRI data:</u> SMA, the left ventral striatum and the right OFC activation ↑ after EV compared to CV.
Von Leupoldt, 2012 [89]	19 (26.3% male, M_{age}= 32.2) *+ 19 matched healthy controls (31.6% male, M_{age}= 31.7)*	Allergic asthma	Nocebo (visually induced)	Within-between-subjects	2 series of 30 pictures were shown. The Allergy series depicted house dust, cat fur, and various plants with their pollens. The Neutral series depicted emotionally neutral scenes (e.g., household objects and neutral faces).	1) Allergic asthma 2) HC	Allergic symptoms ↑ for Neutral to Allergy series in patients compared to HC <u>Respiratory parameters:</u> Breathing frequency ↑ for Neutral to Allergy series in patients compared to HC. Other respiratory parameters (airflow, V'; tidal volume, VT; inspiratory time, TI; and respiratory resistance, ROS) n.s.

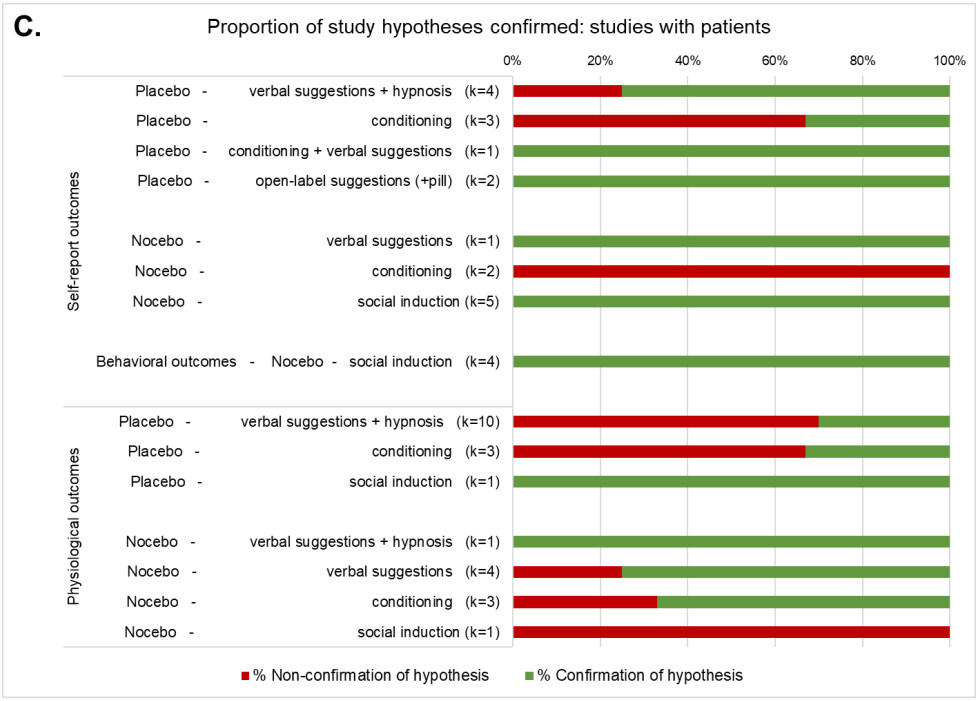
Note. acq = acquisition; AD = atopic dermatitis; ad. = advertisement; ah = antihistamine; CS = conditioned stimulus; CV = control video; dlPFC = dorsolateral prefrontal cortex; EV = experimental video; evoc = evocation; Ga/TGV = conductance—thoracic gas volume ratio; GSR = galvanic skin response; HC = healthy controls; iPS = intraparietal sulcus; NH = natural history; NPT = nasal provocation test; n.s. = non-significant; OL = open label; PLA = placebo; PNIF = peak nasal inspiratory flow; PSS = psoriasis severity scale; Ra = airway resistance; ROS = respiratory resistance; SSS = subjective symptom scores; TAU = treatment as usual; TI = inspiratory time; UCS = unconditioned stimulus; V' = airflow; VS = verbal suggestions; VT = tidal volume.

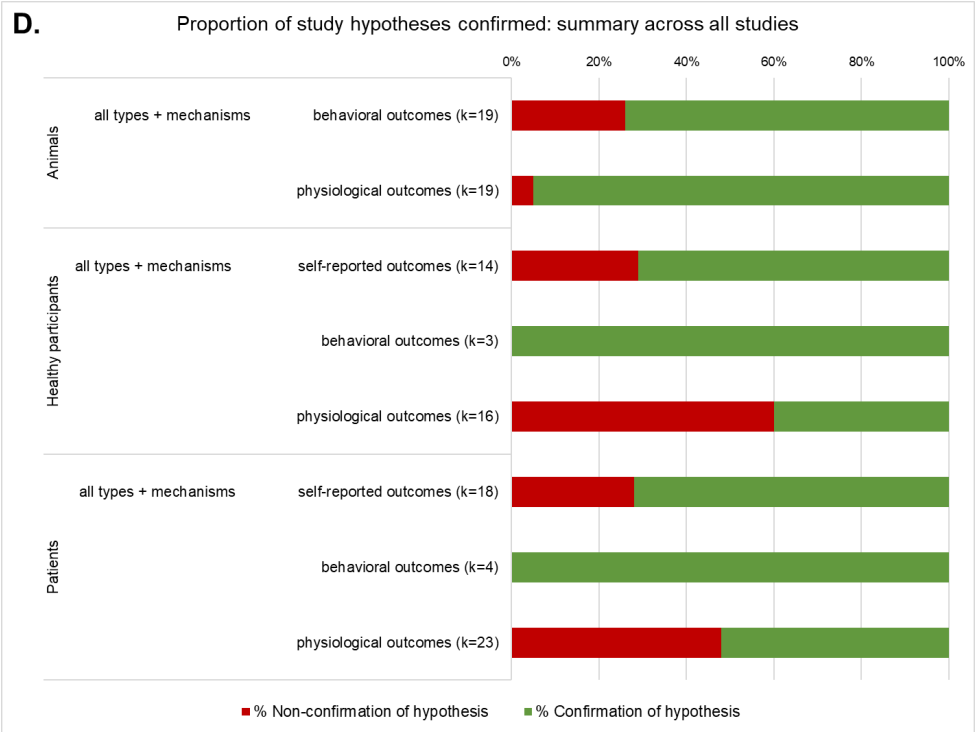
[A] Studies did not indicate statistical significance of findings. As such, percentages are given in this table.

Supplementary Figure S1. Proportion of hypotheses confirmed by different placebo and nocebo effect induction methods for (A) animals studies, (B) studies with healthy volunteers, and (C) studies with patients, with a summary of results presented in (D). Percentages were derived from the results described in Supplementary Table S1.

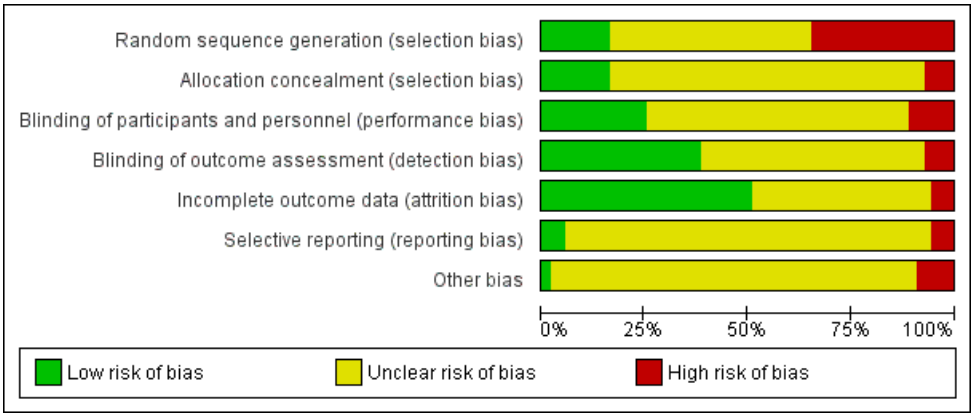




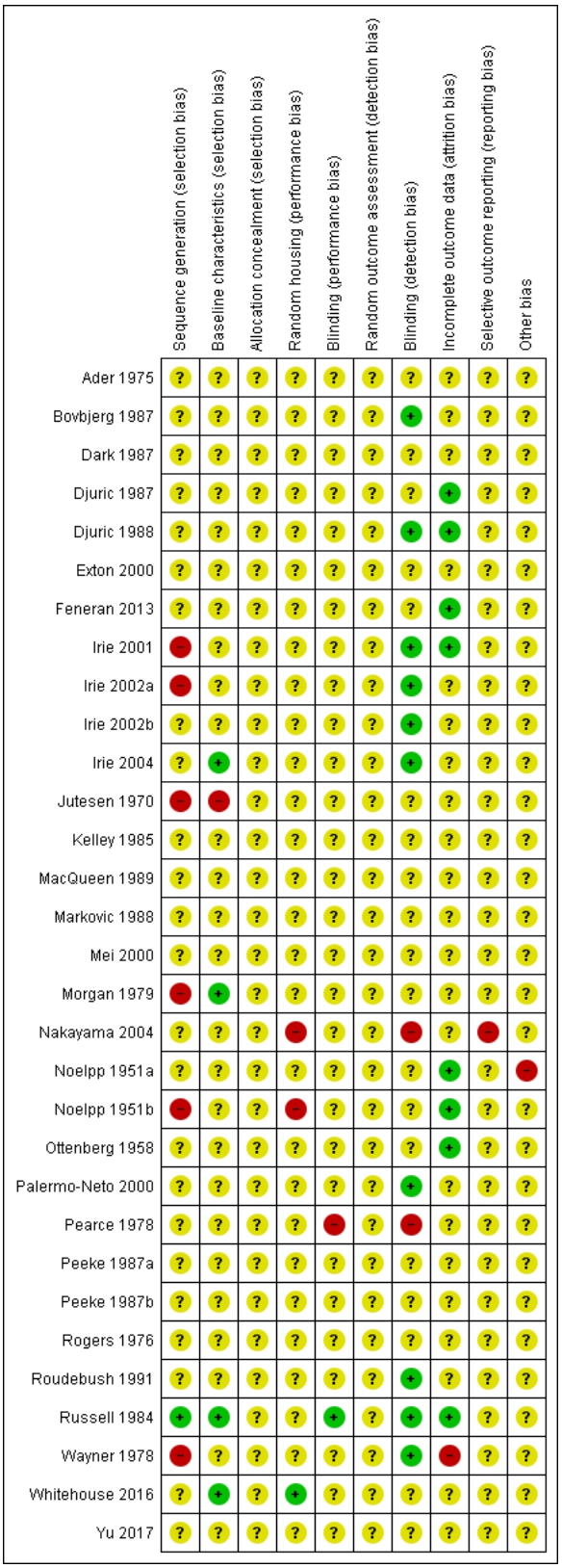

C.
Proportion of study hypotheses confirmed: studies with patients
0%
20%
40%
60%
80%
100%
Self-report outcomes
Placebo - verbal suggestions + hypnosis (k=4)
Placebo - conditioning (k=3)
Placebo - conditioning + verbal suggestions (k=1)
Placebo - open-label suggestions (+pill) (k=2)
Nocebo - verbal suggestions (k=1)
Nocebo - conditioning (k=2)
Nocebo - social induction (k=5)
Behavioral outcomes - Nocebo - social induction (k=4)
Physiological outcomes
Placebo - verbal suggestions + hypnosis (k=10)
Placebo - conditioning (k=3)
Placebo - social induction (k=1)
Nocebo - verbal suggestions + hypnosis (k=1)
Nocebo - verbal suggestions (k=4)
Nocebo - conditioning (k=3)
Nocebo - social induction (k=1)
% Non-confirmation of hypothesis
% Confirmation of hypothesis


D.
Proportion of study hypotheses confirmed: summary across all studies
0%
20%
40%
60%
80%
100%
Animals
all types + mechanisms
behavioral outcomes (k=19)
physiological outcomes (k=19)
Healthy participants
all types + mechanisms
self-reported outcomes (k=14)
behavioral outcomes (k=3)
physiological outcomes (k=16)
Patients
all types + mechanisms
self-reported outcomes (k=18)
behavioral outcomes (k=4)
physiological outcomes (k=23)
% Non-confirmation of hypothesis
% Confirmation of hypothesis

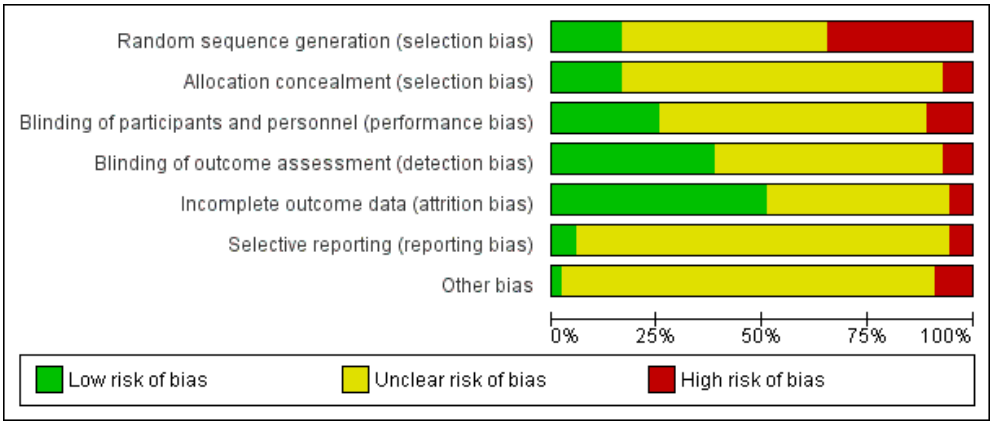
Supplementary Figure S2. General summary of the results for the Risk of Bias analysis for animal trials.



Supplementary Figure S3. Overview of the Risk of Bias for each article: animal trials.



Supplementary Figure S4. General summary of the results for the Risk of Bias analysis for human trials.



Supplementary Figure S5. Overview of the Risk of Bias for each article: human trials

Supplementary Figure S6. Search strategies for PubMed, Embase, and PsycInfo databases

PubMed search

(„nocebo“ [tiab] OR „inert“ [tiab] OR “sham” [tiab] OR “dummy” [tiab] OR “aversive conditioning” [tiab] OR “configural learning” [tiab] OR “associative learning” [tiab] OR “mediated learning” [tiab] OR “animal learning” [tiab] OR “verbal reinforcement” [tiab] OR “attribution” [tiab] OR “conditioning” [tiab] OR “pavlov*” [tiab] OR “expecta*” [tiab] OR “expectation*” [tiab] OR “social learning” [tiab] OR “suggestibility” [tiab] OR “placebo response*” [tiab] OR “placebo effect*” [tiab] OR “placebo induced” [tiab] OR “suggestion*” [tiab] OR “placebo effect” [mesh] OR “nocebo effect” [mesh] OR “conditioning (psychology)” [mesh] OR “association learning” [mesh] OR “anticipatory learning” [tiab] OR “contagious” [tiab]) AND (“quantitative sensory testing” [tiab] OR “QST” [tiab] OR “histamin*” [tiab] OR “capsaicin” [tiab] OR “cowhage” [tiab] OR “cowage” [tiab] OR “scratch*” [tiab] OR “rash” [tiab] OR “pruri*” [tiab] OR “Pruritus” [tiab] OR “Pruritic” [tiab] OR “Prurigo” [tiab] OR “itch*” [tiab] OR “wheal*” [tiab] OR “weal*” [tiab] OR “extravasation” [tiab] OR “flare” [tiab] OR “neurogenic inflammation” [tiab] OR “skin” [tiab] OR “cutaneous” [tiab] OR “inflamm*” [tiab] OR “allerg*” [tiab] OR “hypersens*” [tiab] OR “anaphyla*” [tiab] OR “antigenic” [tiab] OR “pruritus” [mesh] OR “psoriasis” [tiab] OR “dermatitis” [tiab] OR “eczema” [tiab] OR “lichen planus” [tiab] OR “prurigo nodularis” [tiab] OR “neurodermatitis” [tiab] OR “lichen simplex chronicus” [tiab] OR “neurofibroma*” [tiab])

Embase search

((nocebo OR inert OR sham OR dummy OR aversive conditioning OR configural learning OR associative learning OR mediated learning OR animal learning OR verbal reinforcement OR attribution OR conditioning OR pavlov* OR expecta* OR expectation* OR social learning OR suggestibility OR placebo response* OR placebo effect* OR placebo-induced OR suggestion* OR anticipatory learning OR contagious).ti,ab. OR (placebo effect OR nocebo effect OR conditioning psychology OR association learning).sh.) AND ((quantitative sensory testing OR QST OR histamin* OR capsaicin OR cowhage OR cowage).ti,ab. OR (Scratch* OR Rash OR Pruri* OR pruritus OR prurigo OR pruritic OR Itch* OR Wheal* OR Weal* OR Extravasation OR Flare OR neurogenic inflammation OR Skin OR Cutaneous Inflamm* OR Allerg* OR Hypersens* OR Anaphyla* OR Antigenic OR psoriasis OR dermatitis OR eczema OR lichen planus OR prurigo nodularis OR neurodermitis OR lichen simplex chronicus OR neurofibroma*).ti,ab. OR (Pruritus).sh.)

PsycInfo search

((TI nocebo) OR (TI inert) OR (TI sham) OR (TI dummy) OR (TI “aversive conditioning”) OR (TI “configural learning”) OR (TI “associative learning”) OR (TI “mediated learning”) OR (TI “animal learning”) OR (TI “verbal reinforcement”) OR (TI attribution) OR (TI conditioning) OR (TI pavlov*) OR (TI expecta*) OR (TI expectation*) OR (TI “social learning”) OR (TI suggestibility) OR (TI “placebo response*) OR (TI “placebo effect*) OR (TI “placebo-induced”) OR (TI suggestion*) OR (MA “placebo effect”) OR (MA “nocebo effect” OR (MA “conditioning (psychology)” OR (MA “association learning”)) OR ((AB nocebo) OR (AB inert) OR (AB sham) OR (AB dummy) OR (AB “aversive conditioning”) OR (AB “configural learning”) OR (AB “associative learning”) OR (AB “mediated learning”) OR AB “animal learning”) OR (AB “verbal reinforcement”) OR (AB attribution) OR (AB conditioning) OR (AB pavlov*) OR (AB expecta*) OR (AB expectation*) OR (AB “social learning”) OR (AB suggestibility) OR (AB “placebo response*) OR (AB “placebo effect*) OR (AB “placebo-induced”) OR (AB suggestion*) OR (TI anticipatory learning) OR (TI contagious) OR (AB anticipatory learning) OR (AB contagious))

AND

((TI “quantitative sensory testing”) OR (TI QST) OR (TI histamin*) OR (TI “capsaicin”) OR (TI “cowhage”) OR (TI “cowage”)) OR ((AB “quantitative sensory testing”) OR (AB QST) OR (AB histamin*) OR (AB “capsaicin”) OR (AB “cowhage”) OR (AB “cowage”)) OR ((TI Scratch*) OR (TI Rash) OR (TI Pruri*) OR (TI Pruritus) OR (TI pruritic) OR (TI prurigo) OR (TI Itch*) OR (TI Wheal*) OR (TI Weal*) OR (TI Extravasation) OR (TI Flare) OR (TI “neurogenic inflammation”) OR (TI Skin) OR (TI “Cutaneous Inflamm*”) OR (TI Allerg*) OR (TI Hypersens*) OR (TI Anaphyla*) OR (MA “Antigenic Pruritus”)) OR ((AB Scratch*) OR (AB Rash) OR (AB Pruri*) OR (AB Pruritus) OR (AB pruritic) OR (AB prurigo) OR (AB Itch*) OR (AB Wheal*) OR (AB Weal*) OR (AB Extravasation) OR (AB Flare) OR (AB “neurogenic inflammation”) OR (AB Skin) OR (AB “Cutaneous Inflamm*”) OR (AB Allerg*) OR (AB Hypersens*) OR (AB Anaphyla*) OR (TI psoriasis) OR (TI dermatitis) OR (TI eczema) OR (TI lichen planus) OR (TI prurigo nodularis) OR (TI neurodermatitis) OR (TI lichen simplex chronicus) OR (TI neurofibroma*) OR (AB psoriasis) OR (AB dermatitis) OR (AB eczema) OR (AB lichen planus) OR (AB prurigo nodularis) OR (AB neurodermatitis) OR (AB lichen simplex chronicus) OR (AB neurofibroma*))

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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.

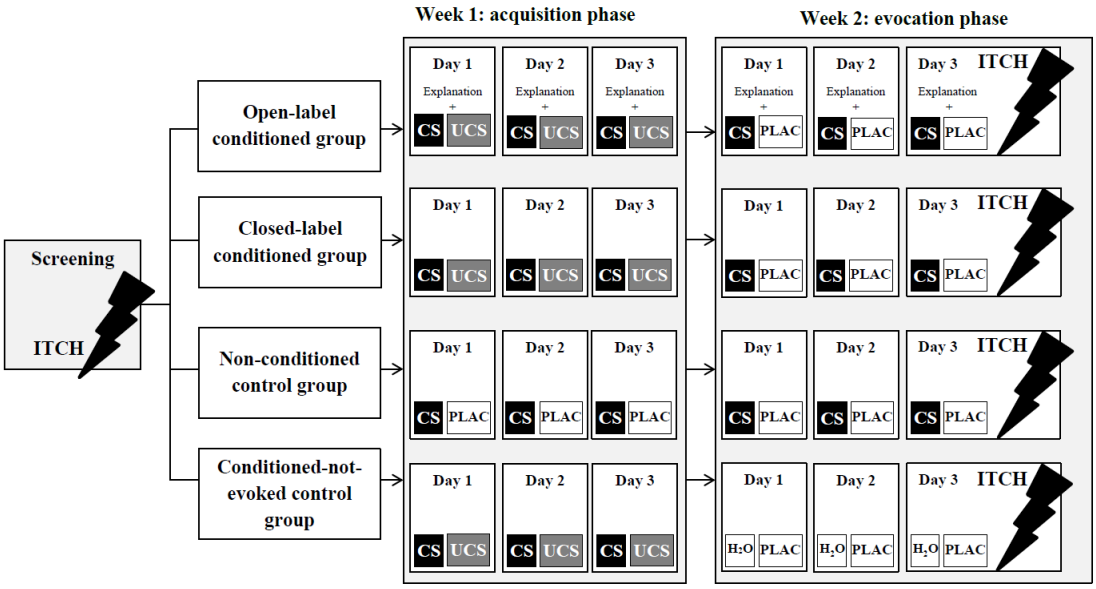


Figure 1. Overview of the study protocol. A conditioned stimulus (CS; distinctively tasting drink) was combined with an unconditioned stimulus (UCS; levocetrizine) or placebo capsule (PLAC) during acquisition. During evocation, the CS was combined with PLAC, and for the conditioned-not-evoked group,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

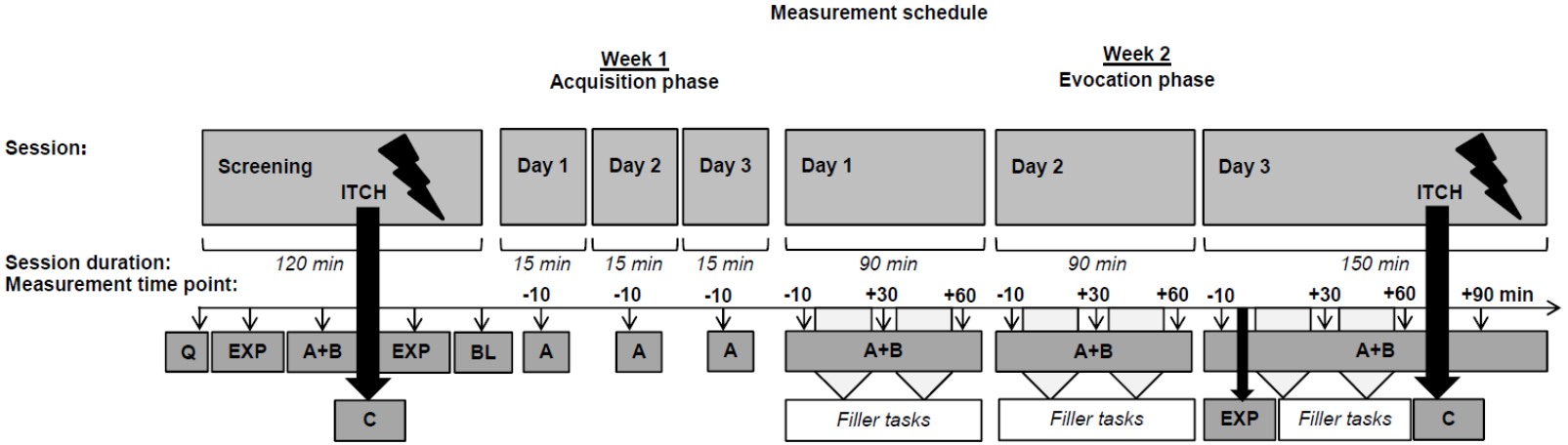


Figure 2. Overview of the measurement schedule. Numbers on the timeline are deducted from CS administration, with −10 representing pre-CS, and +30, +60, and +90 representing post-CS measurements. Personality questionnaires (Q); expected itch (EXP); measurement sets for well-being (A); spirometry, heart 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.

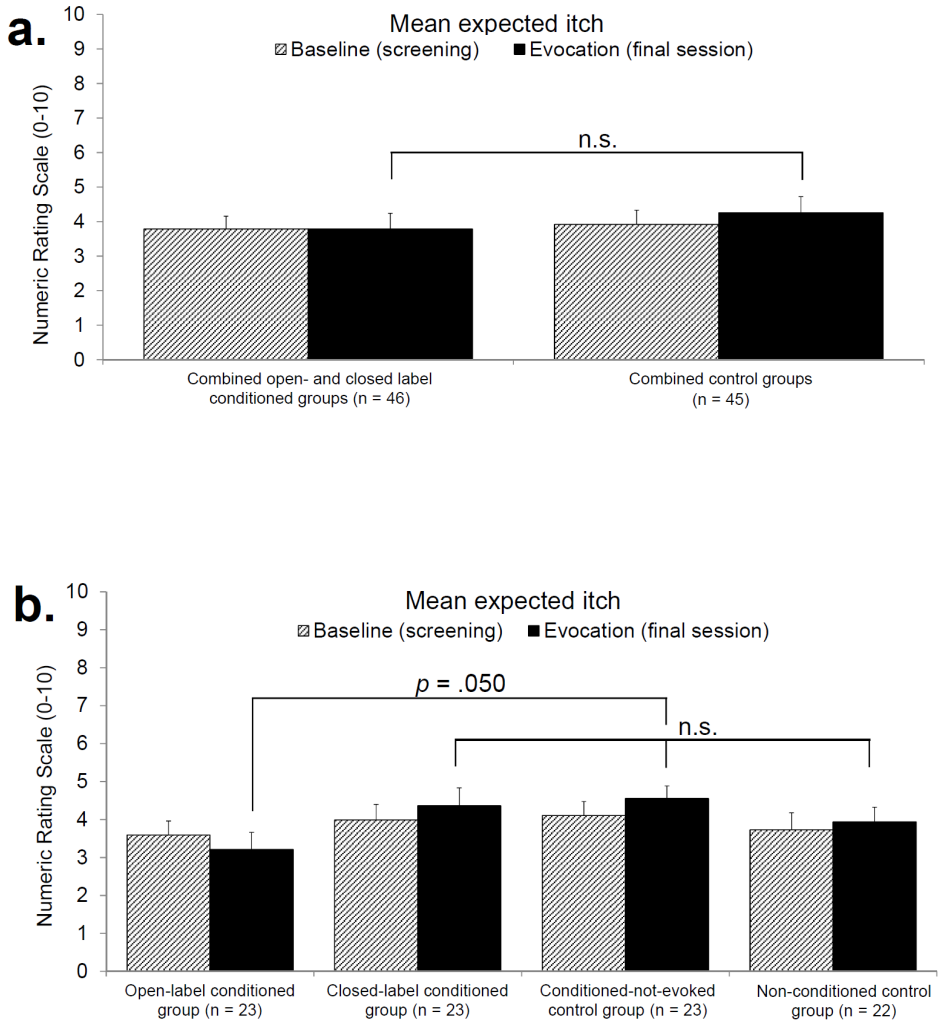
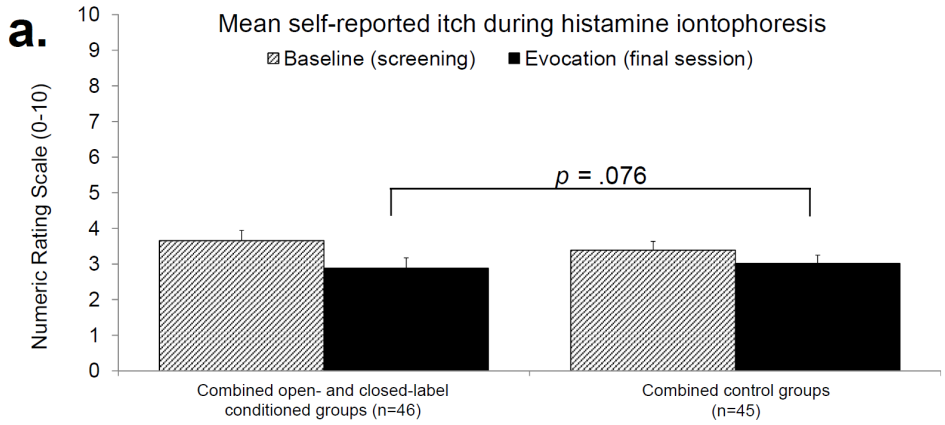


Figure 3. Means and standard errors of expected itch, with (A) the effects of the combined conditioned groups and the combined control groups on expected itch, controlled for baseline expected itch as measured post-iontophoresis during the screening, and (B) the effects of the separate groups on expected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



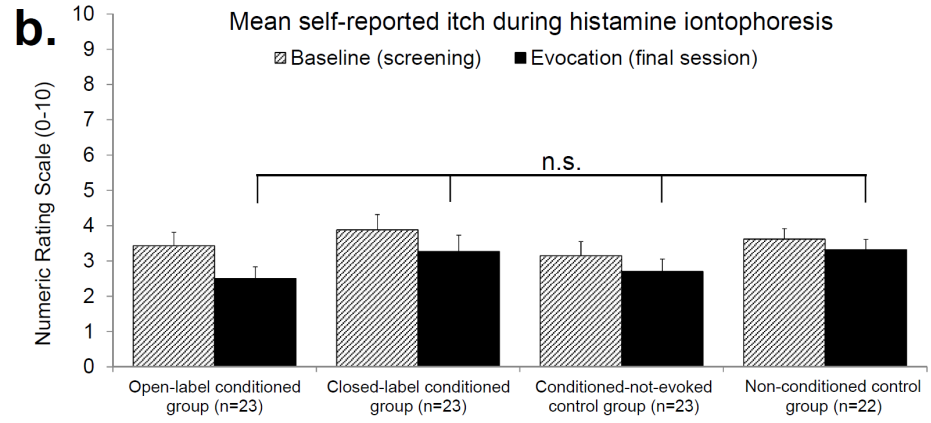


Figure 4. Means and standard errors of the mean for itch during iontophoresis in the final evocation session, with (A) mean itch for the combined conditioned and the combined control groups, and (B) mean itch for the separate groups.

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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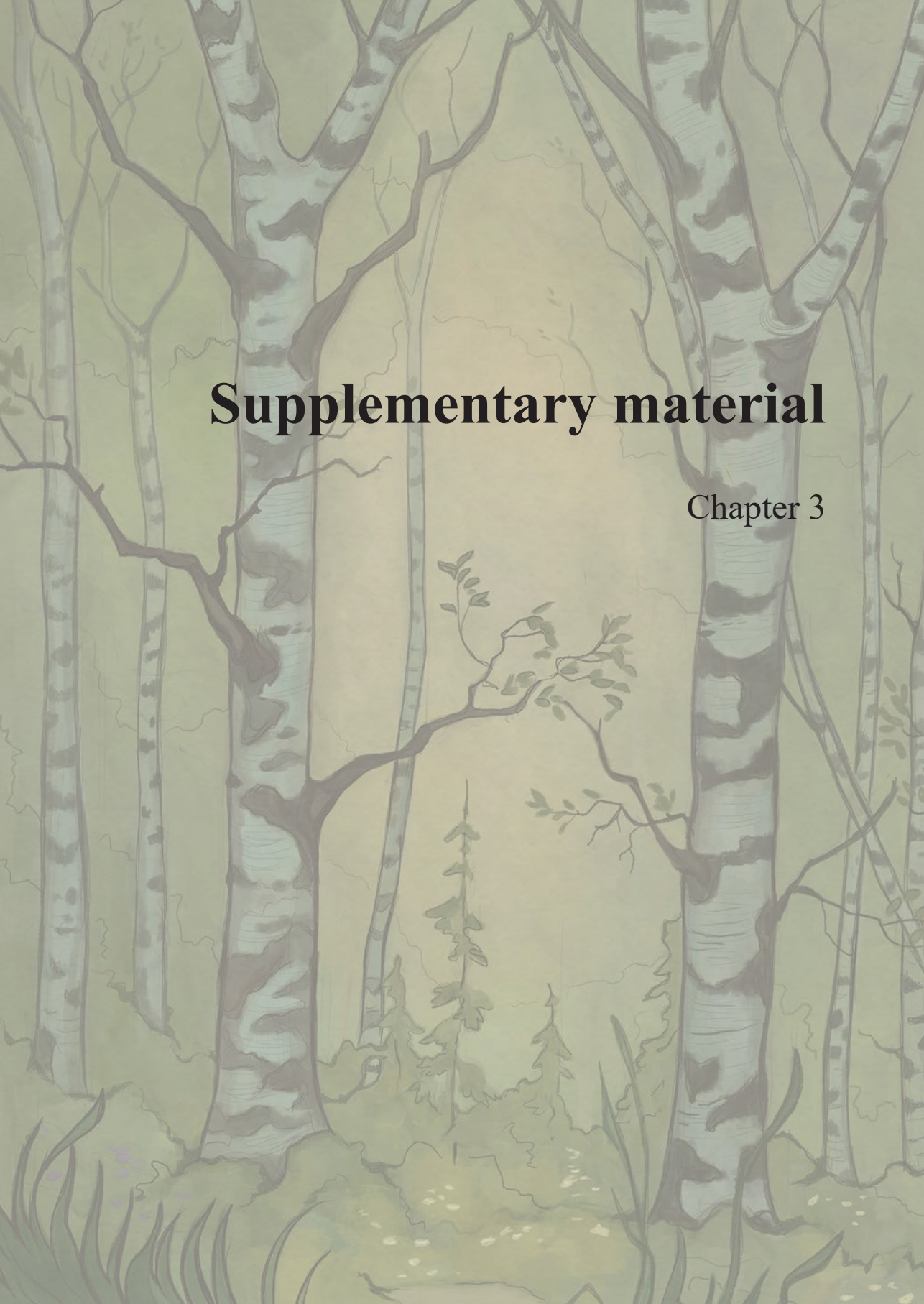


Supplementary material

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

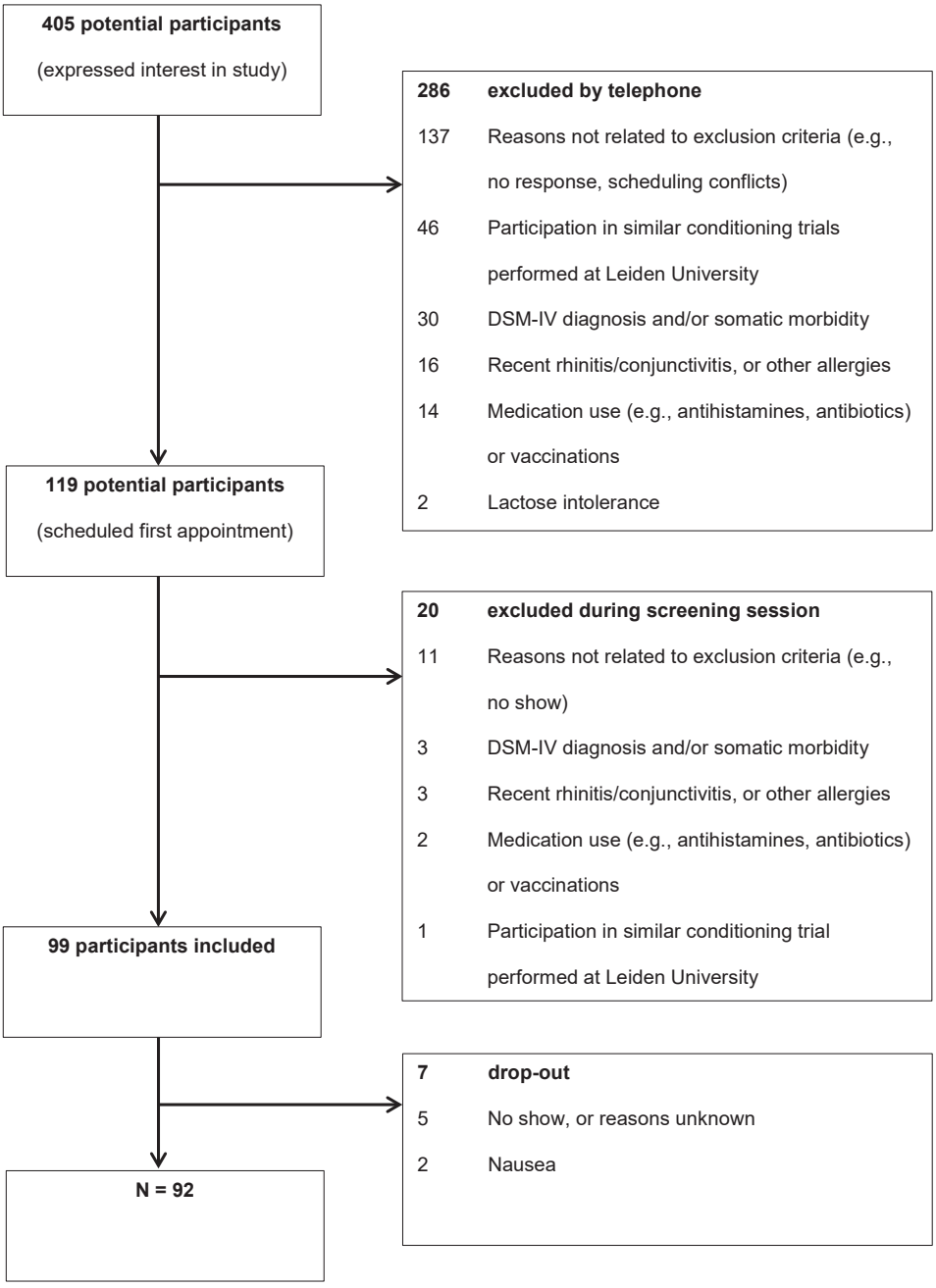
Optimism was found to moderate the effects of closed-label conditioning on mean itch in response to iontophoresis during evocation, compared to the other groups (closed-label conditioning dummy variable x optimism interaction: p=.021; see **Supplementary Table S8**). Higher levels of optimism were related 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).

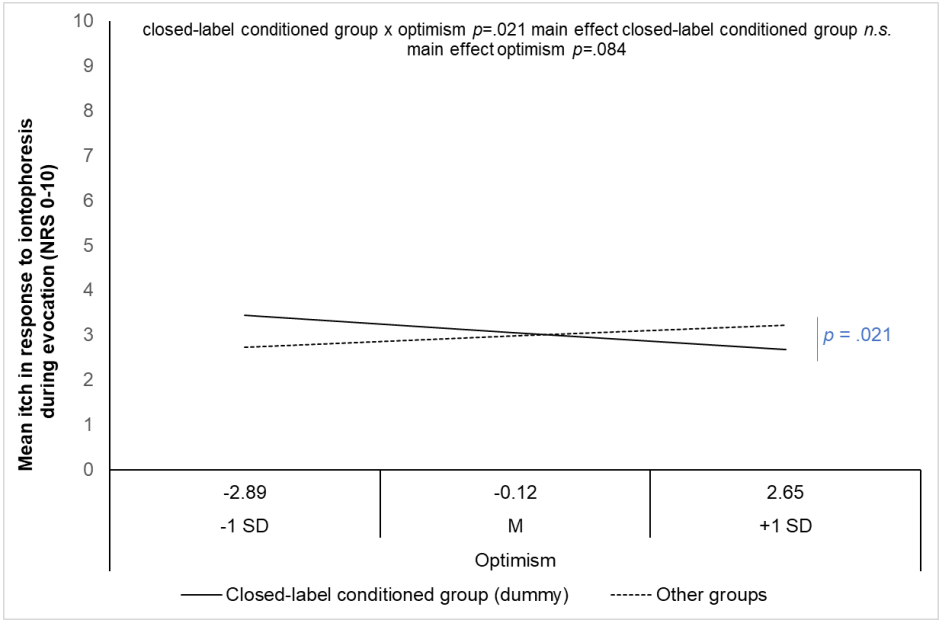
BAS reward responsiveness was found to significantly moderate the effect of the conditioned-not-evoked group on mean itch in response to iontophoresis during evocation, compared to the other groups (conditioned-not-evoked dummy variable x BAS reward responsiveness: p=.020). Higher levels of reward responsiveness 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

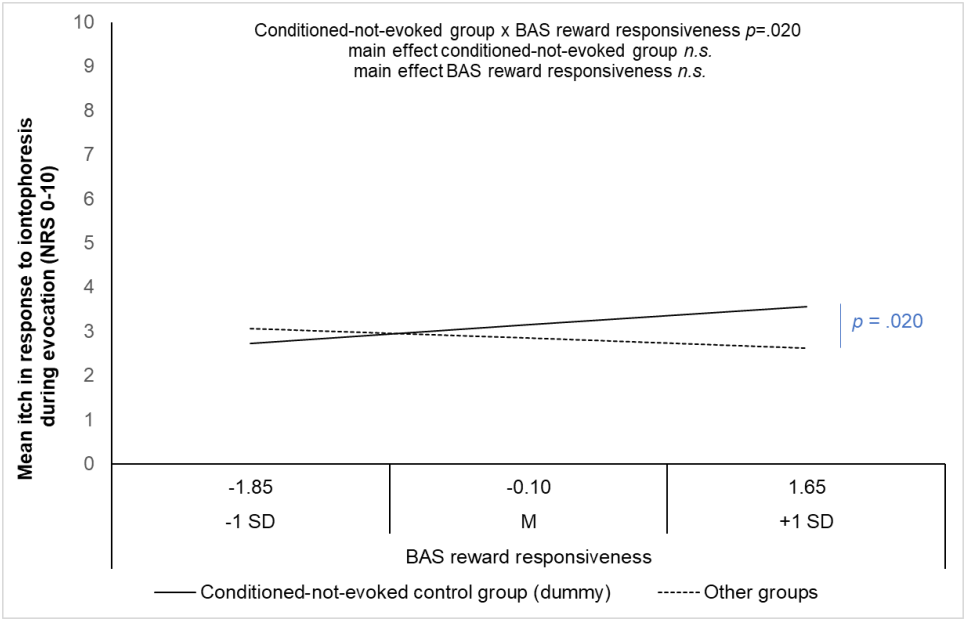
Some evidence was found for a moderating role for optimism in the closed-label conditioned group compared to others, however, post-hoc conditional effects at various levels of optimism were not significant, illustrating that such an effect may be limited. These results need to be interpreted very cautiously, 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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Chapter 4

Placebo effects of open-label verbal suggestions on itch

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ABSTRACT

Placebo effects are positive outcomes that are not due to active treatment components, which may be elicited even when patients are aware of receiving an inert substance (open-label). This proof-of-principle study investigated for the first time whether open-label placebo effects on itch can be induced by verbal suggestions alone. Ninety-two healthy volunteers were randomized to experimental (open-label suggestions) or control (no suggestions) groups. Self-reported itch evoked by histamine iontophoresis was the primary study outcome. In addition, itch expectations, skin condition and affect were assessed. The experimental group expected lower itch than the control group, which was, in turn, related to less experienced itch in this group only, although no significantly different itch levels were reported between groups. The results illustrate a potential role for open-label placebo effects in itch, and suggest that further study of verbal suggestions through an extensive explanation of placebo effects might be promising for clinical practice.

INTRODUCTION

Itch is the most common somatosensory symptom in skin conditions such as psoriasis and atopic dermatitis, and can cause significant impairment in patients [1]. For example, itch has previously been associated with impaired quality of life, a reduction in social activities, reduced quality of sleep, concentration problems, and depression [2]. Current treatments are often aimed at reducing the severity of the skin condition through pharmacological interventions with, for example, (topical) antihistamines or corticosteroids. However, these interventions have usually shown limited effects and are often accompanied by side-effects [3,4]. Over recent years, researchers have aimed to identify other factors involved in the experience of itch that might be used to improve treatment effectiveness [5]. A promising factor influencing the experience of itch without requiring medication is the placebo effect [6–8].

Placebo effects are beneficial effects of pharmacologically inert treatment components [6,8]. A recent meta-analysis indicated that itch may be especially prone to such effects, and that up to 30% of improvement in itch may be attributed to the occurrence of placebo effects rather than pharmacological intervention [9]. Experimental studies further demonstrate that placebo effects can be induced in itch by providing suggestions that a treatment is able to alleviate itch, or by suggesting that a test that generally provokes itch will elicit no itch [10,11]. In addition, there is evidence that the opposite instructions (e.g. suggesting that a treatment will sensitize a person to itch) can increase itch, a phenomenon known as the nocebo effect [10,12–14]. In addition to studies investigating the effects of verbal suggestions on self-report measures such as itch, a few studies have investigated whether verbal suggestions can influence physical skin conditions, for example wheal and flare size in response to histamine [11,12,14,15]. It has been demonstrated recently that negative outcome expectations, or nocebo, can result in a greater physical skin response, as demonstrated by larger flare size in response to histamine and wheal size in response to natrium chloride following negative verbal suggestions [14]. Placebo (and nocebo) effects can be established by a patient's belief in treatment effectiveness and outcomes [6,8,16–18]. The main working mechanisms of placebo effects include associative learning processes, such as conditioning, and expectations, such as positive information regarding treatment outcomes provided by means of verbal suggestions [6,8,16,18].

Most studies on placebo effects have used an experimental approach eliciting placebo effects by providing uncertainty or deception about the specific treatment provided (e.g., actual medicine or placebo). It is assumed that the benefits that patients experience from inert substances stem from the covert belief that a pharmacologically effective treatment is being given [19]. This uncertainty or deceptive component complicates the potential utilization of placebo effects in clinical practice, considering that omission of treatment information and provision of deceptive information are unethical [18,20]. Studies have, however, indicated that a placebo treatment can still be effective when patients are aware of receiving an inert treatment [21–28]. Most of these studies on open-label placebo treatment have reported medium-to-large effect sizes [21,22,25], comparable to the effect sizes found by studies in which patients were not informed about receiving an inert substance (closed-label placebo [29]). A recent pilot study furthermore demonstrated that such an open-label placebo treatment may also be effective for symptoms of allergic rhinitis, including itch [28]. Within this pilot study, symptom improvement by open-label placebo treatment was furthermore associated with higher subjective well-being [28].

It is not yet clear by which mechanisms open-label placebo effects may be elicited. Previous open-label studies have combined different components, namely the administration of an inert pill and a rationale concerning placebo effectiveness and its mechanisms [21–28]. This complicates the understanding of which of these components contribute to open-label placebo effects, or the extent to which they contribute. It is not yet known whether providing a positive rationale (e.g. verbal suggestions) exclusively might be sufficient to induce open-label placebo effects by changing expectations regarding itch and affecting the response to an itch stimulus. If proven possible, this would facilitate clinical applications; for example, by optimizing existing treatment methods for chronic itch by improving doctor–patient communication.

The aim of the current proof-of-principle study was to investigate for the first time in an open-label design whether positive verbal suggestions about itch in response to an itch-provoking test without combining it with an inert treatment can induce positive outcome expectancies and reduce self-reported itch in response to a short-term itch-provoking histamine test. Physical and self-reported skin condition and positive and negative affect were secondary outcomes. In addition, the specific role of expectations in the effects of open-label verbal suggestions on itch was examined. It was expected that open-label positive verbal suggestions about itch would reduce the level of itch that participants expected to experience during the histamine test as well as the mean level of itch

experienced during the test, compared with a control condition that received no verbal suggestions. In addition, it was expected that open-label positive verbal suggestions compared with the control group would reduce the severity of the participants' skin condition, and that verbal suggestions would diminish changes in positive and negative affect as a consequence of itch induction by histamine iontophoresis.

METHODS

The study was approved by the Medical Ethical Committee at the Leiden University Medical Center, The Netherlands (protocol number NL54570.058.15) and performed in accordance with the Declaration of Helsinki. All participants provided written informed consent.

Participants

Healthy male and female volunteers were recruited primarily through advertisements and flyers at various sites of Leiden University, The Netherlands, and through online media. Participants were included if they were between 18 and 35 years old and had a sufficient understanding of written and spoken Dutch. Exclusion criteria consisted of severe somatic or psychological morbidity (e.g. heart and lung diseases, histamine intolerance, or the Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition, Text Revision (DSM-IV-TR) psychiatric diagnoses) that might interfere with the study protocol, current chronic itch or pain complaints, current medication use (analgesics, anti-inflammatory drugs, antihistamines, or antibiotics), or pregnancy. In order to prevent potential influences on the response to the itch stimulus, participants were asked to refrain from the consumption of food, caffeine, and smoking 2 h prior to the laboratory session. In addition, they were asked to avoid heavy exercise 12 h, and alcohol and drugs 24 h prior to the session. This was verified at the beginning of the laboratory session, including a brief check of participant eligibility.

Study design

A between-subjects experimental randomized controlled trial design was used. Subjects considered eligible to participate in the study were invited to a 1-h laboratory session and randomized to either the experimental (i.e. open-label positive verbal suggestions) group or the control (no verbal suggestions) group. The randomization sequence was acquired through the use of an online random number generator (www.random.org, Dublin, Ireland). The laboratory session was conducted by 2 experimenters; only one of whom was aware of group allocation and provided the verbal suggestions. Participants and the experimenter who conducted the outcome assessments were unaware of group allocation during the laboratory session.

Materials and measures

1. Histamine iontophoresis.

Histamine was applied to the skin through transdermal iontophoresis (Chattanooga Group, Hixson, TN, USA). This method has been used previously to experimentally induce itch in healthy volunteers [10,30,31]. A 0.6% diphosphate histamine solution was prepared in distilled water with propylene glycol and hypromellose 4000 mPa. An electrode (Iogel, Iomed, DJO Global, Hannover, Germany) was prepared that contained 2.5 ml of the histamine solution. The electrode had an active area of 11.7 cm^2 and was placed on the volar side of the non-dominant forearm, and the reference electrode was placed on the volar surface of the upper arm. Current level was set at 0.4 mA. Histamine iontophoresis was applied for 2.5 min, after which the electrodes were removed and a follow-up period of 4 min was started.

2. Verbal suggestions.

Participants were informed prior to the session that the study aimed to investigate individual differences in the experience of itch. Upon arrival at the laboratory session, the following general instructions were given to all participants: *"During the test, histamine will be applied on your skin by means of a small electrical current. This elicits a response that is similar to a mosquito bite. Your skin may become red and may swell up."* In the experimental group exclusively, participants were given the following verbal suggestion,

followed by an open-label instruction: *"Previous research indicates that the test elicits little or no itch in most healthy people, meaning in 95% of cases. We would also like to give you some extra information. From research we know that expectations play a large role in how itch is experienced, for example through giving information about what to expect from a test such as this one. I just told you that the test that you are about to do elicits little or no itch in most healthy people. From research we know that this suggestion will really cause people to experience little itch, even when they are aware of receiving this suggestion. Thus, the suggestion alone that the test causes little or no itch will already cause you to experience little itch."*

3. Process measure: expectations about itch.

Participants were asked to indicate on the computer the level of itch they expected to experience, using a numerical rating scale (NRS) ranging from 0 ("no itch at all") to 10 ("worst itch ever experienced"). Expected itch was assessed at 2 time-points during the laboratory session: once during baseline assessments and once after the verbal suggestions but before the histamine iontophoresis.

4. Primary outcome measure: self-reported itch.

The level of experienced itch during histamine iontophoresis was assessed verbally every 30 s during iontophoresis and during a 4-min follow-up period, using the same NRS as described in the previous paragraph. Directly following histamine iontophoresis, the mean experienced itch during iontophoresis was assessed verbally using the same NRS. As part of a series of online questionnaires that assessed baseline measures (see Procedure), the level of itch experienced prior to iontophoresis was measured graphically by dragging a slider over a bar slide using the same NRS, with a 2-decimal score depicted next to the bar slide.

5. Secondary outcome measure: physical skin condition.

Wheal size and flare size in response to histamine iontophoresis were traced on a 1 cm^2 gridded, transparent sheet following histamine application using a 0.4-mm black permanent

marker (Staedtler, Germany). Scans of the sheets were then uploaded and analysed using ImageJ [32]. Images were calibrated to the 1 cm^2 grid in ImageJ, after which wheal and flare area were estimated in cm^2 through tracking the outer edges of the drawn wheal and flare areas. In addition, skin temperature was assessed following histamine iontophoresis on the application site on the arm using a hand-held infrared digital thermometer (accuracy ±2.0°C, resolution 0.1°C, BaseTech, Conrad Electronic Benelux B.V., Hirschau, Germany). The thermometer was held vertically approximately 1 cm above the area. To control for individual differences in skin temperature, a second measurement was taken of a similar skin area of the contralateral arm and used as a covariate in the analysis.

6. Secondary outcome measure: self-reported skin condition.

The Sensitive Scale-10 (SS-10) was used to assess self-reported skin condition [33]. This scale assesses the severity of skin sensitivity over the past 3 days through evaluation of 9 skin condition items (e.g. burning, tautness, itch, and redness of the skin) on a 0 ("zero intensity") to 10 ("intolerable intensity") scale. In addition, the scale assesses skin irritation on a visual analogue scale (VAS), ranging from 0 to 10 [33]. The SS-10 total score was calculated by summing all items, with a higher score indicating more intense skin sensations (range 0–100). Upon arrival in the laboratory, participants completed the SS-10 for a baseline measurement. A slightly adjusted version of the SS-10 was used to assess self-reported skin condition following histamine iontophoresis, with participants having to indicate the symptoms experienced during histamine iontophoresis rather than symptoms experienced during the past 3 days. In the current study, Cronbach's alpha was 0.83 for baseline SS-10 and 0.89 for the adjusted post-test SS-10.

7. Secondary outcome measure: positive and negative affect.

The Positive and Negative Affect Schedule (PANAS) was used to assess positive (PA) and negative affect (NA; [34]). In this 20-item questionnaire, participants indicated the extent to which they experience specific emotions (e.g. interested, excited, or nervous) at that moment on a 1 ("very slightly or not at all") to 5 ("extremely") scale. PA total score was calculated by summing the 10 positive items of the scale and NA total score by summing the 10 negative items (total score range 10–50, with higher scores indicating higher PA or NA). Baseline affect was measured upon arrival in the laboratory (baseline PA and NA)

and following histamine iontophoresis (post-test PA and NA). Cronbach's alpha was 0.83 for baseline PA and 0.79 for post-test PA. For NA, Cronbach's alpha was 0.82 and 0.91, respectively. To examine group differences in the changes over time in positive and negative affect in response to the histamine iontophoresis, PA and NA change scores were calculated for each scale by subtracting baseline scores from post-test scores. For both positive and negative affect change scores, positive scores indicated an increase in that particular affect.

Procedure

Prior to being invited to a laboratory session, participants were provided with written information about the study. Participants subsequently completed a series of online questionnaires assessing the screening criteria and several personality characteristics, which are not described here since they are unrelated to the aim of the current study. Prior to this, participants provided written informed consent for the online questionnaires. At the start of the laboratory session, the study procedures were explained to all participants and written informed consent for the entire study was provided, following which participants were given instructions regarding the histamine test by the first experimenter. Next, baseline measurements were taken of itch, self-reported skin condition, positive and negative affect, and itch expectations before verbal suggestions. Positive expectations were then induced through open-label verbal suggestions in the experimental group. Participants then again indicated the level of itch they expected to experience. In the meantime, the first experimenter left the room and was replaced with the second experimenter. Histamine iontophoresis was then conducted by this second experimenter. During histamine iontophoresis as well as during the 4-min follow-up period, the level of itch participants experienced was assessed verbally on the NRS every 30 s. Directly following iontophoresis, mean experienced itch during iontophoresis was assessed, again using the same NRS. Subsequently, measurements of physical skin condition (e.g. wheal, flare) were taken and post-test questionnaires SS-10 and PANAS were administered. The second experimenter was then replaced by the first experimenter and participants were debriefed about group allocation and the true purpose of the study.

Statistical analyses

A power calculation for an analysis of covariance (ANCOVA) using G*Power 3.1 [35] indicated that a total of 92 participants was needed to achieve a power of β=0.80 at a 2-sided significance level of α=0.05 to detect a Cohen's d effect size of at least 0.30 on the primary outcome measure of mean itch. Analyses were performed using SPSS 21.0 for Windows (IBM SPSS Inc., Chicago, Illinois, US). Variables were checked for normal distribution. In order to achieve normal distributions, square root transformations were applied for baseline itch and self-reported skin condition, as well as the physical skin condition parameters wheal size and flare response, and a log10 transformation was applied to baseline skin temperature. For PANAS negative affect change scores, transformations failed to achieve a normal distribution; therefore, a non-parametric Kruskal–Wallis test was used to examine group differences on this outcome measure. χ^2 tests and general linear model (GLM) ANOVAs were used to detect differences between groups on demographic factors and baseline measurements of itch, self-reported skin condition (SS-10), and positive and negative affect. The primary outcome measure of mean experienced itch, as rated by participants following histamine iontophoresis (mean ± standard deviation (SD) 3.3 ± 1.6) was correlated very highly with the mean score of itch ratings during the histamine iontophoresis (mean ± SD 2.9 ± 1.4; r = 0.93, $p < 0.001$), supporting the reliability of the itch measure used. To examine whether the groups differed on the process measure of itch expectation (post-verbal suggestions) and on the primary outcome of mean itch experienced during the histamine test, 2 GLM ANCOVAs were conducted, with baseline expectations and baseline itch level included as covariates in the analyses, respectively. Similar ANCOVAs were conducted for the secondary outcome measures of the SS-10 and skin temperature. For wheal and flare size in response to histamine iontophoresis, as well as for PANAS positive affect change scores, GLM ANOVAs were performed. As an effect size measure, Cohen's *d* was calculated for analyses on the primary and secondary outcome parameters, with $d = 0.2$, 0.5 and 0.8 being interpreted as a small, medium and large effects, respectively [36]. In order to explore whether itch expectation after the verbal suggestions was related to the level of mean experienced itch during histamine iontophoresis, and whether this relationship differed between groups, separate effects, as well as an interaction effect of post-verbal suggestions itch expectation and group, were examined in a multiple regression analysis, with baseline itch and pre-verbal suggestions itch expectation as covariates. Post-verbal suggestions itch expectation was centred prior to the analysis. All analyses were conducted 2-sided with a significance level of $\alpha < 0.05$.

RESULTS

Group characteristics

A total of 139 potential participants expressed interest in the study, of whom 24 were excluded due to medical morbidity (e.g. migraine, asthma, presence of a skin disorder) and 9 due to psychological morbidity (e.g. depression, anxiety). In addition, 14 persons refrained from participation for other reasons (e.g. no response after first postal contact). In total, the sample consisted of 92 healthy male ($n = 17$) and female ($n = 75$) participants between 18 and 26 years of age (mean ± SD 21.3 ± 1.9 years). Participants were of Dutch (98%), Dutch–Turkish (1%), or German (1%) nationality. Of all participants, 41% had a partner, of whom 7% were living with a partner or were married. Of the female participants, 69% used oral contraceptives. Randomization resulted in a total of 46 participants in the experimental group and 46 participants in the control group. Data for one participant in the experimental group were excluded from analysis, due to technical issues during histamine iontophoresis. χ^2 tests and ANCOVAs revealed no differences between groups with regard to age, sex, education, or nationality (all p-values ≥ 0.19). **Table I** displays the means ± SD for the baseline measurements, and the primary and secondary study outcomes for the 2 groups.

Effects of verbal suggestions on the process measure of post-suggestion expected itch

As shown in **Fig. 1**, in the experimental group significantly lower itch expectations were reported following verbal suggestions (mean ± SD 2.66 ± 2.04) than in the control group (mean ± SD 5.73 ± 1.51). A statistically significant large-sized effect of verbal suggestions on post-verbal suggestions itch expectation, controlled for pre-verbal suggestions itch expectation, was demonstrated in the ANCOVA; $F(1, 89)=59.57$, $p < 0.001$, Cohen's $d = 1.62$.

Effects of open-label verbal suggestions on the mean level of itch during histamine iontophoresis

No statistically significant difference between groups in mean self-reported itch in response to histamine iontophoresis, controlled for baseline itch, was found in the ANCOVA; F(1,

90)=1.40, $p = 0.24$, Cohen's $d = 0.21$. Multiple regression analysis to test for interaction effects between post-verbal suggestions itch expectation and group on self-reported mean itch during histamine iontophoresis did not show significant main effects for group ($\beta = 0.06$, $p = 0.69$) or post-verbal suggestions expected itch ($\beta = 0.36$, $p = 0.10$). As expected, baseline itch and pre-verbal suggestions expected itch were not predictive of self-reported mean itch during histamine iontophoresis ($p \geq 0.36$). However, a statistically significant interaction effect for group × post-verbal suggestions itch expectation was found ($p = 0.04$), indicating that in the experimental group only, lower expected itch was associated with lower self-reported mean itch during the histamine iontophoresis, whereas no association between expected and experienced itch was found in the control group (full model: R^2=0.11, F(5,89)=2.04, $p = 0.08$; see also **Fig. 2**).

Effects of open-label verbal suggestions on skin condition

Self-reported skin condition (SS10) scores following histamine iontophoresis were marginally significantly lower, indicating better self-reported skin condition, in the experimental group (25.88 ± 13.55) than in the control group (29.88 ± 14.48; F(1, 90)=3.67, $p = 0.059$, Cohen's $d = 0.29$). No statistically significant group differences were found for the physical parameters wheal and flare size, and skin temperature in response to histamine iontophoresis ($p \geq 0.14$).

Table 1. Means ± standard deviations for baseline and outcome measures of study parameters per group.

	***Experimental* group (n = 45)**	***Control* group (n = 46)**	***p* value**
Process measure			
Pre-VS itch expectation [a]	4.84 ± 1.61	5.19 ± 1.83	.28
Post-VS itch expectation [a]	2.66 ± 2.04	5.73 ± 1.72	< .001
Primary outcome measure			
Baseline itch	0.47 ± 0.84	0.39 ± 0.89	.27
Self-reported average itch	3.14 ± 1.61	3.46 ± 1.51	.24
Secondary outcome measures			
Subjective skin response			
Baseline self-reported skin condition	6.94 ± 9.12	5.02 ± 5.50	.16
Self-reported skin condition [b]	25.88 ± 13.55	29.88 ± 14.48	.059
Physical skin response			
Wheal size (cm^2)	10.40 ± 2.67	11.23 ± 2.67	.16
Flare response (cm^2)	33.81 ± 7.85	35.54 ±10.19	.44
Skin temperature (°C)	33.35 ± 1.21	33.54 ± 1.38	.26
Change in Positive and Negative Affect			
Baseline positive affect	24.74 ± 6.34	25.54 ± 6.21	.54
Post-histamine positive affect	24.09 ± 6.58	22.61 ± 4.69	.22
Baseline negative affect [d]	12.00 ± 4.00	12.00 ± 4.00	.67
Post-histamine negative affect [d]	11.00 ± 3.00	11.00 ± 2.00	.53
Change in positive affect [c]	-0.64 ± 3.22	-2.93 ± 3.69	.002
Change in negative affect [c d]	0.00 ± 2.00	0.00 ± 3.00	.98

Note. [a] VS = open-label verbal suggestions; [b] As measured by an adjusted version of the Sensitive Scale-10 [33]; [c] Change in mood parameters measured by the Positive and Negative Affect scales [34] over time, as calculated by subtracting the baseline scores from the post-histamine scores. More positive scores indicate an increase over time, whereas zero indicates no change and more negative scores indicate a decrease over time; [d] Median ± interquartile range is presented, with p value calculated by a non-parametric Kruskal-Wallis test

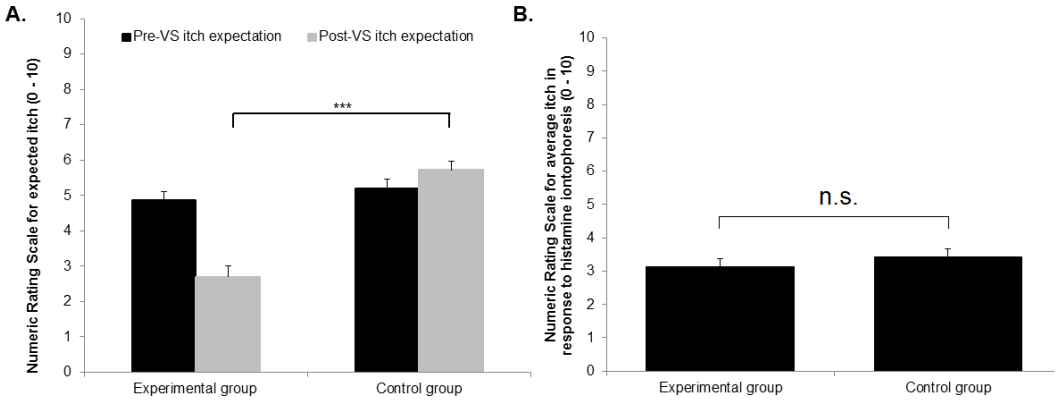

Figure 1. Mean Numeric Rating Scale (NRS) scores for the experimental (open-label positive verbal suggestions; n=45) and control (no suggestions; n=46) group for the process measure and primary study outcome measure: expected itch from before to after instructions on the histamine iontophoresis test (A), average itch in response to histamine iontophoresis (B). Error bars represent standard error of the mean. *** $p < .001$; n.s. non-significant

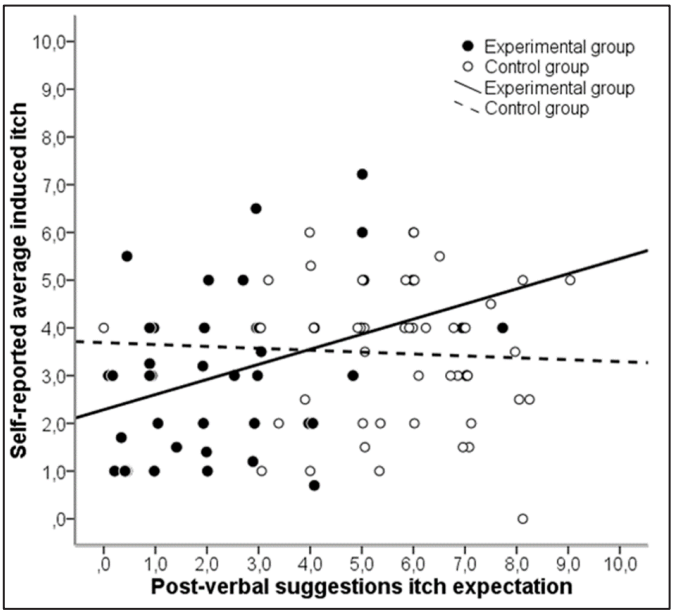

Figure 2. Scatter plot of the interaction effect between expected itch following verbal suggestions, controlled for pre-verbal suggestions expected itch, and group (experimental, n = 45 and control, n = 46) on self-reported mean itch during histamine iontophoresis controlled for baseline itch.

Effects of open-label verbal suggestions on change in positive and negative affect

A statistically significant and medium-sized effect of verbal suggestions on PA change scores was demonstrated compared with the control group; $F(1, 90)=9.93$, $p = 0.002$, Cohen's $d = 0.66$. Participants in the control group showed a stronger decline in positive affect from before to after the histamine iontophoresis (mean ± SD PA change score –2.93 ± 3.69, range –15 to 5), whereas participants in the experimental group remained more stable over time (mean ± SD PA change score –0.64 ± 3.22, range –8 to 8). The Kruskal–Wallis test revealed no statistically significant difference in NA change scores between the experimental and control groups ($p = 0.98$).

DISCUSSION

This proof-of principle study investigated for the first time whether open-label positive verbal suggestions alone can induce outcome expectations and reduce the level of itch experienced during histamine iontophoresis. It was demonstrated that the open-label positive verbal suggestions were successful in reducing the level of itch participants expected to experience during histamine iontophoresis, but not in reducing itch experienced in response to the histamine test. The relevance of expectations was demonstrated further by showing that a decrease in expected itch in response to verbal suggestions was significantly associated with lower experienced itch in response to the histamine test in the experimental group, but not in the control group. Moreover, a tendency was found for patients to rate the severity of the self-reported skin condition as lower following open-label suggestions, compared with the control group, but no effects on physical skin condition were found. Furthermore, a significantly smaller decrease in positive, but not negative, affect was found in response to the histamine test in the verbal suggestions group, compared with the control group.

These findings, that open-label positive verbal suggestions did not affect actual experienced level of itch, are not in line with the findings of previous studies on open-label placebo in, for example, allergic rhinitis, low-back pain and irritable bowel syndrome [21–26,28]. This may be due to the current study using verbal suggestions exclusively without an inert treatment. In previous studies, open-label placebo effects have been induced by asking participants to ingest pharmacologically inert pills combined with providing a suggestive positive rationale to participants, in which it was explained how placebo effects induce

symptom improvement and in which the effectiveness of placebo treatment was emphasized [21,22,28]. It is possible that the mere act of taking medicine could have elicited stronger placebo effects. Influential conditioning theories of placebo effects state that the placebo effect is a conditioned response that is a result of previously learned associations [16]. As pointed out previously by Carvalho et al. [21], the rituals surrounding administration of medication may have activated previously learned associations between symptom alleviation and capsule or pill ingestion. Considering that all previous open-label studies have been conducted in patient populations in which medication administration is common, it seems likely that these effects were further strengthened by associative learning pathways and heightened relevance of symptom improvement for patients in these studies. The current study, on the contrary, sought to examine the ability to elicit placebo effects by provision of positive verbal suggestions without coupling with an inert substance in healthy participants. Investigating these effects not only provides information regarding the mechanisms of open-label placebo, but if proven possible would also allow for easier implementation in clinical practice, for example by optimizing the information provided to patients in existing treatment in order to maximize placebo effects. The placebo effects induced in the current study may have had some impact, as evidenced by the successful expectation induction, which was in turn related to lower itch level, but speculatively may not have been strong enough to significantly alter the way in which itch was actually perceived in response to the itch stimulus.

Next to effects on itch expectancy, there was also a tendency for participants in the experimental group to indicate better self-reported skin condition following the histamine test than controls. That positive affect in the open-label suggestions group also decreased significantly less than in the control group upon itch induction also indicates that some effects may have occurred. This may be compared with the previous finding that improvement in allergic rhinitis by open-label placebo treatment is related to higher wellbeing [28]. However, no effects of open-label positive verbal suggestions on physical skin condition (i.e., wheal and flare size, skin temperature) were found, which is in line with previous findings for closed-label placebo effects on physical skin responses [11,15], but not with findings for closed-label nocebo effects on physical skin response [14]. Although indications were found that the verbal suggestions may have mostly influenced subjective measures, they were potentially not strong enough to significantly alter the symptom and physical outcomes between groups.

In this study, a potential predictive role of a change in itch expectations due to open-label verbal suggestions was observed for the resultant itch level that was reported in response to the histamine test, showing that a larger decrease in itch expectations was associated with lower experienced itch. This is in accordance with the idea that placebo effects can impact symptoms by means of changing people's expectations and is in line with the provided rationale explaining that expectations can alter the way in which itch can be perceived. Whereas in closed-label placebo studies the expectation that a certain treatment or medicine results in symptom improvement is attributed to the provision of uncertainty or deceptive information by the doctor or experimenter [27,37], in open-label studies this might be attributed to the provision of a rationale on how placebo effects could instead lead to beneficial treatment outcomes. For both, the actual expectation of symptom improvement of the patient is suggested to be present and to have an impact. Previous studies, however, have always combined open-label placebo pill administration with the rationale that explained that "the placebo effect can be powerful" and that "the body may respond automatically to placebo treatment" [21,22,28]. Furthermore, as in most of these trials, open-label placebo treatment was given along with treatment as usual [21,22,28] and the effects that patients might experience were not specified, placebo effects may have been enlarged by simultaneously occurring pharmacologically-induced reduction in symptoms. As such, it is difficult to determine the exact underlying cause of placebo effects in these studies. Demonstrating that placebo effects could be exclusively due to a positive rationale, on the other hand, would facilitate easier implementation in clinical practice, as no additional inert pills would need to be given. Instead, a positive framework for patients in which placebo mechanisms are illustrated could then potentially suffice to strengthen existing treatment methods for itch.

Some strengths and limitations of this study need to be taken into account. This is the first study to examine the ability of positive verbal suggestions to induce placebo effects in itch in an experimental, open-label design without combining it with an inert treatment. The relatively large sample size and use of a homogenous participant group allowed for a robust indication of effect sizes. Assessment of outcome variables was conducted by an experimenter who was blinded to group allocation in order to minimalize reporting bias. However, as in all research in which placebo effects are induced by verbal suggestions, reporting bias cannot be ruled out, as participants may still have adjusted their answers due to the explicit instructions on expectations. Fourthly, participants underwent histamine iontophoresis only once and most were unfamiliar with histamine iontophoresis. Prior to the

test, participants were told that the response to histamine iontophoresis could feel like a mosquito bite. However, since the participants did not know exactly what to expect during the test, this lack of a reference frame for itch may have complicated changing these expectations and, consequently, the induction of placebo effects. Future studies could examine whether providing a more familiar stimulus, for example by providing a baseline test prior to placebo induction, would lead to clearer expectation effects [13]. Finally, while in the open-label verbal suggestions a distinction was made between the suggestion (i.e., "research indicates that the tests elicits little to no itch in most healthy people, meaning in 95% of cases") and the open-label rationale, both were given to one group only. As such, we cannot distinguish between effects of the suggestion itself and the open-label rationale that followed. For future research, it may also be useful to compare open-label with closed-label placebo induction, in order to better distinguish between open- and closed-label placebo effects in itch.

In conclusion, this proof-of-principle study indicates for the first time that open-label positive verbal suggestions were able to reduce itch expectations prior to a histamine test. Also, open-label suggestions were associated with a smaller impact on positive affect and indications were found for a more positive self-perceived, but not physical, skin condition in response to the validated histamine test. However, the suggestions did not significantly impact actual itch levels between groups, although within the experimental group an association was found between expected and experienced itch after verbal suggestions. Future research should aim to strengthen the open-label verbal suggestions, for example by providing a more explicit explanation of placebo mechanisms and effectiveness, in order to investigate whether open-label placebo effects can be induced in itch without the need to administer a substance.

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Chapter 5

Effects of open- and closed-label nocebo and placebo suggestions on itch and itch expectations

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ABSTRACT

Placebo and nocebo effects have been shown to influence subjective symptoms such as itch. These effects can be induced by influencing outcome expectations through, for example, combining the application of an inert substance (e.g., a cream) with verbal suggestions on the anticipated effects of this substance. Interestingly, placebo effects also occur when it is known that a treatment is inert (i.e., open-label placebo). However, no study to date has examined the efficacy of negative and positive verbal suggestions under similar open-label and closed-label (i.e., concealed placebo/nocebo) conditions in itch. A randomized controlled between-subjects study design was applied in which healthy volunteers (n = 92) were randomized to 1) an open-label positive verbal suggestion group, 2) a closed-label positive verbal suggestion group, 3) an open-label negative verbal suggestion group, or 4) a closed-label negative verbal suggestion group. Verbal suggestions were made regarding the topical application of an inert substance. Itch was evoked experimentally by histamine iontophoresis at baseline and again following suggestions. Itch expectations, self-reported itch during and following iontophoresis, and skin response parameters were measured. Positive suggestions were found to result in significantly lower expected itch than were negative suggestions in both open- and closed-label conditions. No effects of the suggestions on itch during iontophoresis were found, but significantly lower itch was reported in the 4 min following iontophoresis in the (combined open- and closed-label) positive compared with negative verbal suggestion groups. In addition, a smaller increase in skin temperature was found in the positive compared with negative suggestion groups. The findings illustrate a potential role of (open- and closed-label) placebo for optimizing expectations and treatment effects for itch in clinical practice.

Clinical Trial Registration: Netherlands Trial Register, trial number: NTR6530.

INTRODUCTION

Itch is the most common somatosensory symptom in dermatological conditions. It is a hallmark symptom of atopic eczema [1], and its prevalence in psoriasis is high [2]. Moreover, itch is a common symptom of various other disorders, including kidney failure, liver disease, cancer, allergy, and diabetes mellitus [3-5]. Due to its high prevalence—approximately 8% of the general population and over 50% of dermatological patients—the burden of itch and its impact on society are high [6,7]. Often, patients report significantly lowered quality of life, increased depressive and anxious symptoms, and sleep disturbances due to chronic itch [8]. While current treatments aim to suppress itch through pharmacological interventions, oftentimes, limited effects and significant side effects are reported [3,9]. As such, it is important to identify factors that contribute to treatment efficacy.

One of the factors that may be especially relevant for treatment outcomes is the placebo effect. Placebo effects are defined as beneficial effects of otherwise pharmacologically inert substances [10,11] and have been studied in a variety of medical conditions and symptoms, including itch and pain [12-14]. Multiple pathways through which these effects can be elicited have been identified, including associative learning processes, social learning, or instructional learning [12,15-17]. Within these pathways, expectancy is a key component. To illustrate, a positive expectation may be elicited through past experiences with the beneficial effects of a certain type of medication (associative learning), through observation of treatment efficacy in others (social learning), or through receiving positive (verbal) information about a treatment (instructional learning) [17]. In turn, this positive expectation can then result in psychoneurobiological changes and symptom reduction [18,19]. On the other hand, when expectations regarding a treatment outcome are low or negative outcomes are expected, symptoms may worsen or the occurrence of treatment side effects may increase, known as the nocebo effect [12,20].

The current literature indicates that at least 30% of itch reduction in clinical practice might be attributable to placebo effects [21]. Placebo and nocebo effects can be experimentally induced for itch by changing expectations through verbal suggestions regarding inert treatments or through the use of associative learning mechanisms [22-28]. However, not all studies confirm these findings [28,29]. In addition, there is some evidence that it may be necessary to combine multiple placebo induction methods (e.g., associative learning and positive suggestions) and that a single induction method may not be sufficient to elicit

significant placebo effects [22]. Hence, further study of the circumstances under which placebo effects may be elicited for itch is relevant.

Most studies on placebo effects take on a traditional approach, in which patients or healthy individuals are told that a pharmacologically effective substance (e.g., a pill or cream) is given, whereas in reality, the substance is pharmacologically inert [30,31]. While this concealing or deceptive approach is useful for studying the underlying mechanisms of placebo effects in general, it may become problematic when it comes to utilizing these effects in clinical practice, where concealment or deception regarding the treatment provided brings along ethical issues. For a long time, it was believed that this would prevent strategic use of the placebo effect in clinical practice [30]. In the past years, however, studies have demonstrated that placebo effects can also occur when it is explicitly told that, although a given substance is inert, placebo effects may still help in alleviating symptoms. These so-called open-label placebo effects have been found to significantly reduce symptoms of irritable bowel syndrome, depression, attention deficit hyperactivity disorder, chronic low back pain, and allergic rhinitis [31-39]. Most of these studies induce open-label placebo effects through a combination of an attribute (e.g., an inert pill) that may trigger previously learned associations between medicine and symptom reduction in general, and a scripted briefing in which the positive effects of placebo pills are emphasized (a suggestive framework) [31-34,36-28]. Findings on whether these effects can be attributed to the provided pill or the provided explanation alone are contradictory [35,39,40].

In view of the previous findings, further research is needed to demonstrate the efficacy of both open-label and closed-label (i.e., concealed) placebo effects for itch. It is not yet known whether effect sizes of open-label and closed-label placebo effects are comparable. Moreover, no study to date has investigated whether nocebo effects can be induced under both closed-label and open-label conditions for itch. To this end, we investigated in the current study whether negative or positive outcome expectations, induced by a suggestive framework (negative and positive verbal suggestions, provided in an open-label and closed-label context) combined with an attribute (an inert tonic), can influence self-reported itch during an experimental itch induction by histamine in healthy volunteers. We primarily tested the effects of the positive and negative suggestions on itch by combining open- and closed-label groups. Secondarily, we tested these effects for open-label and closed-label contexts separately to see whether these effects were comparable, and we investigated the effects of suggestions on other markers of the response to this test, for example, the

physical skin response to histamine. We expected a decrease in itch following positive verbal suggestions compared with an increase in itch following negative verbal suggestions for both the open-label and closed-label conditions.

MATERIALS AND METHODS

The study was approved by the Medical Ethical Committee at the Leiden University Medical Center, the Netherlands (NL58792.058.16), and registered in the Dutch Trial Register (NTR6530). The study was performed in accordance with the Declaration of Helsinki [41]. All participants provided written informed consent.

Participants

Healthy male and female participants were recruited through advertisements at Leiden University and through social media (e.g., Facebook). Inclusion criteria consisted of an age between 18 and 35 years and a good understanding of the Dutch written and spoken language. Interested volunteers were excluded in case of self-reported severe somatic or psychological morbidity that could interfere with the participant's safety or study protocol [e.g., heart or lung diseases, histamine intolerance, or Diagnostic and Statistical Manual of Mental Disorders - Fourth Edition Text Revised (DSM-IV-TR) psychiatric diagnoses]; current chronic itch or pain complaints; current use of analgesics, anti-inflammatory medication, antihistamines, or antibiotics; and (suspected) pregnancy. Participants were asked to refrain from the consumption of heavy meals, caffeine, and smoking 2 h, exercise 12 h, and alcohol as well as drugs 24 h prior to the sessions to prevent potential influences on study outcomes. Adherence to these lifestyle guidelines, as well as the exclusion criteria, was verified at the start of each session by means of interviewing.

Study design

A between-subjects, single-blinded, randomized controlled experimental trial design was applied. Eligible participants were randomized to (I) an open-label positive verbal suggestion (VS) group, (II) a closed-label positive VS group, (III) an open-label negative VS group, or (IV) a closed-label negative VS group. Randomization sequence was acquired

using an online random number generator (www.random.org, Dublin, Ireland). Allocation was not concealed for experimenters. Participants were invited for a baseline and an experimental session, which were timed 1 week apart. An overview of the study design and measurement schedule is provided in **Figure 1**.

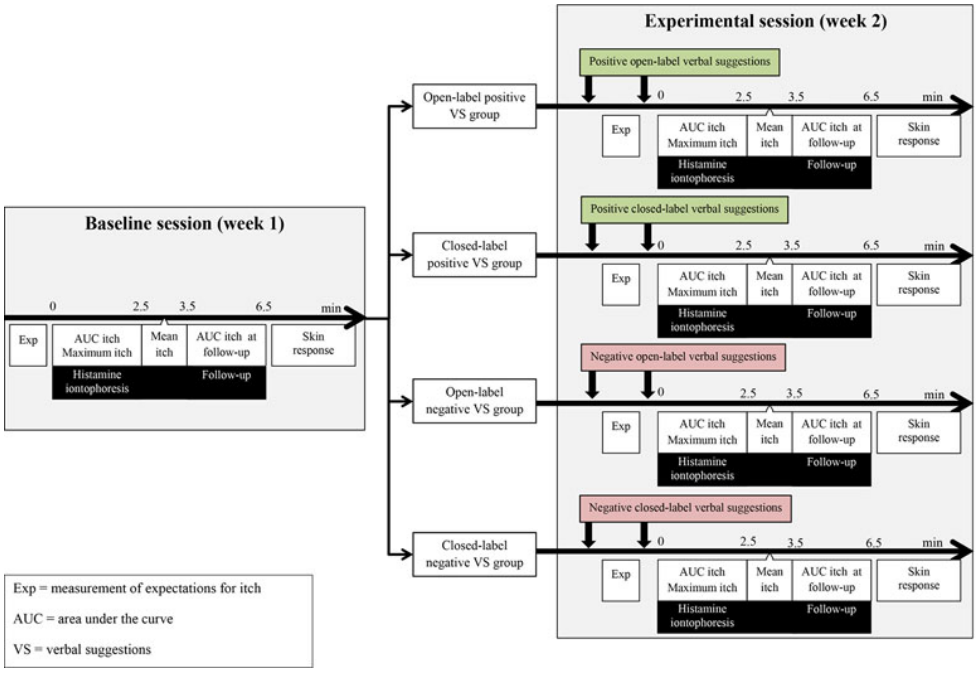

Figure 1 Overview of the design of the study and the measurement schedule for the different verbal suggestions (VS) groups.

Measures and Materials

1. Verbal suggestions.

Before the study, participants were informed that the study aimed to investigate individual differences in the sensitivity to itch and the role of psychological factors in explaining these differences. They were informed that the itch induction method would elicit a response similar to a mosquito bite (e.g., that their skin may become red and swollen). During the experimental session, participants were told that, prior to itch induction, a tonic would be applied that influences sensitivity to itch. In reality, this tonic was a pink-colored skin disinfectant (Orphi Pharma B.V., Dordrecht, the Netherlands). The itch induction during the baseline session was used as a reference point for the suggestions. In the positive VS groups (I and II), the following suggestion was given: "*This tonic has an itch-reducing effect and will make the skin less sensitive to itch. From previous research we know that the application of this tonic will reduce itch for most people, meaning around 95 percent of the studied people. As such, we expect that you will experience less itch, compared to the previous test*." Participants in the negative VS groups (III and IV) were given the same information, but negative words were used instead of positive words (e.g., "itch-increasing" rather than "itch-reducing").

When participants were allocated to one of the two open-label groups, additional instructions were given. For the positive VS group, these were: "*I just told you that the tonic reduces itch. In fact, the tonic is a placebo. From research we know that the expectation that a remedy reduces itch will really cause people to experience less itch. This is caused by different processes, for example itch-reducing substances that are released in the brain. These substances are also released when people know that they receive a placebo. So, even though I told you this, you will likely experience less itch during the test*." For the open-label negative VS groups, negative words were again used in the instructions instead of positive words. During application of the tonic, the provided suggestions and, if applicable, open-label instructions were briefly repeated in a single sentence.

2. Expected itch.

Expected itch in response to histamine iontophoresis was assessed on a Numeric Rating Scale (NRS) ranging from 0 ("no itch") to 10 ("worst itch imaginable"). Participants rated the amount of itch they expected to experience during iontophoresis twice: once at the start

of the baseline session and once during the experimental session, following the verbal suggestions but prior to histamine iontophoresis.

3. Histamine iontophoresis.

Histamine was applied to the skin by transdermal iontophoresis (Chattanooga Group, Hixson, Tennessee, US). This method has been previously validated and reliably induces itch in healthy populations [22,28,29,35]. A 0.6% diphosphate (equivalent to 1% histamine dihydrochloride) histamine solution was prepared in distilled water with propylene glycol and hypromellose 4,000 mPa by the local pharmacy. In preparation of iontophoresis, the skin was cleaned with either a transparent disinfectant (alcohol 70%; baseline session) or a pink-colored disinfectant (0.5% chlorhexidine in alcohol 70%, with rhodamine; experimental session) suggested to be itch-reducing or itch-increasing, depending on placebo or nocebo condition. A 2.5-cc electrode (Iogel, Iomed, DJO Global, Hannover, Germany; active surface: 11.7 cm^2) was treated with the histamine solution and applied to the volar side of the non-dominant forearm. A reference electrode was placed on the volar side of the upper arm. The electrode nodes were spaced approximately 10 cm apart. Histamine was applied to the skin by iontophoresis with a current level set at 0.4 mA for 2.5 min, following which the electrodes and any residual histamine were removed from the skin.

4. Self-reported itch.

Self-reported itch in response to histamine iontophoresis was assessed using the same 0–10 NRS as described under 'Expected itch'. During iontophoresis, participants continuously rated itch using a vertical bar slide depicting the NRS. Scores were sampled at a 10-Hz rate using E-Prime 2.0 [42]. Directly following iontophoresis, mean itch was verbally assessed by asking participants how much itch (on a 0–10 scale) they experienced in general during the test. From 1 to 4 min after iontophoresis, participants were asked to rate self-reported itch every 30 s on the bar slide as a follow-up period. The primary study outcome was the area under the curve (AUC) of itch during the 2.5 min of iontophoresis. Secondary outcomes were maximum itch reported during the 2.5 min of iontophoresis, verbally assessed mean itch, and AUC itch during the 4-min follow-up. AUC of itch and maximum

itch during iontophoresis were computed using MATLAB Release 2012b (The MathWorks, Inc., Natick, Massachusetts, US).

5. Subjective skin response.

Participants filled in the Sensitive Scale-10 (SS-10 [43]) to measure their subjective skin response. The SS-10 contains 10 items, of which 9 items assessed specific skin symptoms (e.g., itch, pain, general discomfort, and heat sensations). Participants were asked to rate in what intensity they had experienced these symptoms over the past 3 days as a baseline measurement, as well as during histamine iontophoresis. Symptoms were rated on NRS ranging from 0 ("zero intensity") to 10 ("intolerable intensity"). An additional symptom ("redness of the skin") was assessed on a 0–10 NRS [43]. Total scores were calculated by summing all items and ranged from 0 to 100. Cronbach's alpha ranged from .83 to .87 in the current study for baseline and post-iontophoresis assessments of the SS-10.

6. Physical skin response.

Wheal size and flare areas following histamine application were measured after the 4-min follow-up period after the iontophoresis test. The size of the skin response was measured by drawing the outline of the redness and thickening of the skin onto a 1-cm^2 gridded transparent sheet with a 0.4-mm black permanent marker (Staedtler, Germany). The sheets were scanned and then retraced using ImageJ software [44], after which the wheal and flare area (in cm^2) were calculated. In addition, skin temperature was measured following iontophoresis, using a handheld infrared digital thermometer (accuracy ± 2.0 °C, resolution 0.1 °C; BaseTech, Conrad Electronic Benelux B.V.). Measurements were taken with the thermometer held vertically and approximately 1 cm above the center of the histamine application area. To control for individual differences in skin temperature, a baseline measurement was taken prior to iontophoresis, with change from baseline temperature being used as the outcome measure.

Procedures

Prior to participation, written information regarding the study was provided, and volunteers were asked to fill in an online questionnaire assessing the study's inclusion and exclusion criteria. When volunteers were considered eligible for participation, they were invited to the lab for a 30-min baseline session and a 45-min experimental session, which were timed 1 week apart. At the start of the baseline session, the study procedures were explained, and written informed consent was provided. Next, personality questionnaires were administered, which are not further described here as they are unrelated to the aim of the current study. Baseline measurements of itch expectation and subjective skin responses in the past 3 days were taken. Next, the skin of the non-dominant forearm was disinfected, and electrodes were placed on the arm, after which the histamine test was conducted. Measurements of itch and physical skin responses were taken, followed by an assessment of subjective skin responses. After 1 week, the experimental session took place. First, the general procedure of the second session was explained, and verbal suggestions were given (the content of which depended on group allocation). Measurements of post-VS expected itch and of subjective skin responses in the past 3 days were taken. Next, the skin was cleaned using the pink disinfectant, during which the verbal suggestions were briefly repeated. Histamine iontophoresis was conducted; and measurements of itch, physical skin response, and subjective skin response were taken. At the end of the session, participants were asked to fill in a final questionnaire assessing the general amount of itch experienced during both baseline and post-VS iontophoresis and, for the open-label groups only, how believable and convincing participants thought the open-label rationale was (on a 0–10 NRS). Upon completion, they were debriefed on the true purpose of the study. For each session, participants received a compensation of €7.50.

Statistical Analyses

As input for the power calculation, we used the effect size of Cohen's d = 1.10, that was found by Napadow et al. [25], who investigated nocebo effects induced by an inert substance (i.e., a sham allergen solution) on itch. As the current study investigated not only nocebo effects following application of an inert substance but also placebo effects, and both were investigated under closed-label and open-label conditions, a more conservative effect size of d = 0.90 was used for computation of sample sizes for the separate open-label and closed-label analyses. A power calculation for an analysis of covariance (ANCOVA) using

G*Power 3.1 [45] indicated that 21 participants per group would be needed at a power of $\beta = .80$ and a significance level of $\alpha = .05$ for the primary outcome of AUC itch during iontophoresis in the experimental session between the (separate closed-label or open-label) positive verbal suggestion group and the negative verbal suggestion group while controlling for AUC itch at baseline. A missing data rate of 10% was taken into account, resulting in a sample size of 23 participants in each group.

Analyses were performed using SPSS 21.0 for Windows (IBM SPSS Inc., Chicago, Illinois, US). Normal distribution of the variables and the assumptions of each analysis were checked prior to analysis. To test for group differences in demographics and baseline variables, chi-square tests and one-way analyses of variance (ANOVAs) were conducted. As *a priori* determined primary analysis, differences between the combined negative VS groups and positive VS groups in AUC itch during iontophoresis were assessed by a general linear model (GLM) ANCOVA, controlled for AUC itch during baseline iontophoresis. Similar analyses were conducted for the secondary outcome parameters, maximum itch during iontophoresis, mean itch (assessed verbally following iontophoresis), AUC itch during the 4-min follow-up, subjective skin response, and the physical skin response parameters.

Due to technical difficulties with the NRS bar slide and E-Prime, data of some participants ($n = 6$) were missing for the analyses of AUC itch and maximum itch during iontophoresis. Data of one participant were missing for the skin temperature measurements. For those variables that were non-normally distributed (i.e., AUC itch during follow-up), a change score was calculated by subtracting baseline scores from those measured post-VS (with zero indicating no change, negative scores indicating a decrease, and positive scores indicating an increase from baseline to post-VS). A GLM ANOVA was then performed to detect differences in change scores between groups. For expected itch following suggestions, an ANOVA was also conducted. For each AN(C)OVA, Cohen's *d* was calculated, and the following interpretations were used: small effect size 0.20, medium effect size 0.50, and large effect size 0.80 [46]. When appropriate, covariate-adjusted means were used for calculation of Cohen's *d*. In addition, paired sample *t*-tests were conducted within each group to assess changes in each outcome parameter from the baseline to post-VS measurements. In order to examine whether the effects of verbal suggestions were similar regardless of participants knowing about the expectation induction, all analyses were repeated for the separate open-label and closed-label conditions. As the effects of suggestions were expected to be similar under open-label and closed-label contexts,

differences between open- and closed-label groups were not tested statistically. Rather, effect sizes generated by the separate open-label and closed-label analyses were used for indirect comparisons.

To explore potential group differences in the strength of associations between the process measure of post-VS itch expectation and the outcome measures of itch and skin response, Pearson's *r* correlations were calculated within each group, and Cohen's *q* was computed as an effect size for the difference in strength of association, with the following categories of interpretation: no effect < 0.10, small effect size 0.10 < 0.30, medium effect size 0.30 < 0.50, and large effect size ≥ 0.50 [46]. For AUC itch during follow-up, Spearman's rho was calculated. The open-label groups were compared on how believable and convincing participants thought the open-label rationale was by independent-samples *t*-tests. All analyses were conducted two sided with $\alpha = .05$. For the secondary analyses (i.e., AN(C)OVAs and paired-sample *t*-tests for separate open-label and closed-label analyses), Bonferroni's correction for multiple comparisons was used, thus resulting in a significance level of $\alpha/2 = .025$. To correct for alpha inflation due to multiple itch outcomes, an additional Bonferroni's correction was applied for the secondary itch outcomes, resulting in a significance level of $\alpha/3 = .017$ for the combined-group analyses and $(\alpha/3)/2 = .008$ for the separate-group analyses of the secondary itch outcomes. All values described in the Results section represent mean ± SD, unless stated otherwise.

RESULTS

Participants

A total of 138 potential participants expressed interest in the study, of whom 44 were not included (18 had somatic or psychological morbidity, 4 were non-proficient in the Dutch language, and 22 gave no response following screening). Two participants dropped out after the baseline session and were replaced. This resulted in the intended sample size of 92 participants (16 males, 17.4%; 76 females, 82.6%), whose age ranged from 18 to 30 (M = 21.8 ± 2.7). Participants were randomized into 1) the open-label positive VS group (n = 22), 2) the closed-label positive VS group (n = 23), 3) the open-label negative VS group (n = 23), or 4) the closed-label negative VS group (n = 24). The groups did not differ in demographic factors (all $p \geq .42$), baseline itch expectation prior to iontophoresis ($p = .13$), baseline self-reported itch parameters (all $p \geq .58$), and baseline subjective and physical

skin condition (all $p \geq .12$). An overview of the means and standard deviations of the baseline and outcome measures is presented in **Table 1** (combined open-label and closed-label groups) and in **Supplementary Table S1** (separate open-label and closed-label groups).

Expected itch

A large-sized effect of verbal suggestions on expected itch was found; $F(1,90) = 20.94$, $p < .001$, Cohen's $d = 0.96$. As illustrated in **Figure 2**, expected itch following suggestions was significantly lower in the combined positive VS groups ($M = 2.62 \pm 1.82$) compared with the combined negative VS groups ($M = 4.41 \pm 1.93$). A secondary analysis showed a large-sized effect of suggestions in the open-label groups [$F(1,43) = 15.84$, $p < .001$, Cohen's $d = 1.21$] and a medium-sized effect in the closed-label groups [$F(1,45) = 6.15$, $p = .017$, Cohen's $d = 0.74$], both indicating significantly lower expected itch in the positive VS group (open label: $M = 2.35 \pm 1.88$; closed label: $M = 2.88 \pm 1.77$) than in the negative VS group (open label: $M = 4.59 \pm 1.91$; closed label: $M = 4.24 \pm 1.99$).

Primary itch measure: area under the curve (AUC) of itch during histamine iontophoresis

For the primary outcome AUC itch, a small-sized non-significant difference between the combined positive VS groups and the combined negative VS groups was found; $F(1,83) = 1.75$, $p = .19$, Cohen's $d = 0.29$. Secondary analyses for the separate open- and closed-label groups revealed similar findings (both $p \geq .31$; see **Figure 3**). Within-group analyses of baseline to post-VS changes indicated that AUC itch decreased marginally in the combined positive VS groups [$t(39) = 1.98$, $p = .055$] but did not change in the combined negative VS groups [$t(45) = -0.19$, $p = .85$]. No within-group changes in AUC itch from baseline to post-VS were detected for the separate open- and closed-label groups (all $p \geq .12$). An overview of within-group comparisons for AUC itch and other outcome measures is presented in **Table 2** (combined open-label and closed-label groups) and **Supplementary Table S2** (separate open-label and closed-label groups).

Table 1. Means ± standard deviations for the combined open- and closed-label positive and the combined open- and closed-label negative verbal suggestion groups.

	Combined open- and closed-label				
				AN(C)OVA	
	n	**Positive VS (*n*=45)**	**Negative VS (*n*=47)**	***p*-value**	**Cohen's *d***
Process measure					
Pre-iontophoresis itch expectation	92	5.15 ± 1.95	4.82 ± 1.75	.40	
Post-VS itch expectation [A]	92	2.62 ± 1.82	4.41 ± 1.93	<.001	0.96
Baseline histamine iontophoresis					
AUC itch [B]	88	369.79 ± 241.69	361.35 ± 230.24	.87	
Maximum itch	88	3.95 ± 2.44	3.78 ± 2.26	.73	
Mean itch [C]	92	3.10 ± 1.90	2.93 ± 1.75	.66	
Post-VS histamine iontophoresis					
AUC itch [B,D]	86	314.61 ± 237.34	367.54 ± 266.63	.19	0.29
Maximum itch [D]	86	3.44 ± 2.54	3.81 ± 2.45	.24	0.26
Mean itch [C,D]	92	2.83 ± 1.93	3.19 ± 2.09	.076	0.38
Change from baseline to post-VS scores					
AUC itch during follow-up [B,E]	90	-3.38 ± 6.37	0.02 ± 6.88	.017	0.52
Baseline skin response to iontophoresis					
Subjective skin response [F]	92	24.37 ± 11.77	22.78 ± 12.25	.53	
Wheal area [cm^2]	92	10.52 ± 3.47	11.09 ± 3.00	.40	
Flare area [cm^2]	92	47.74 ± 11.05	48.16 ± 12.45	.86	
Change in skin temperature [°C] [G]	91	1.70 ± 1.01	1.58 ± 1.22	.61	
Post-VS skin response to iontophoresis					
Subjective skin response [D,F]	91	21.08 ± 12.31	20.79 ± 12.21	.58	0.12
Wheal area [cm^2] [D]	92	10.12 ± 3.80	10.68 ± 3.69	.78	0.06
Flare area [cm^2] [D]	92	45.54 ± 13.11	47.17 ± 11.75	.54	0.13
Change in skin temperature [°C] [D,G]	90	1.83 ± 1.15	2.34 ± 1.62	.018	0.52

Note. [A] VS = verbal suggestions. [B] AUC = Area under the Curve. [C] Assessed verbally on a Numeric Rating Scale ranging from 0-10. [D] Group differences assessed by ANCOVA, controlled for baseline. Cohen's d was calculated with the estimated marginal means (controlled for baseline). [E] Calculated as post-VS measure – baseline measure (session 2 – session 1) and corrected for significant outliers. [F] As measured by an adjusted version of the Sensitive Scale 10 (Misery et al., 2014). [G] Calculated as post-iontophoresis temperature – pre-iontophoresis temperature.

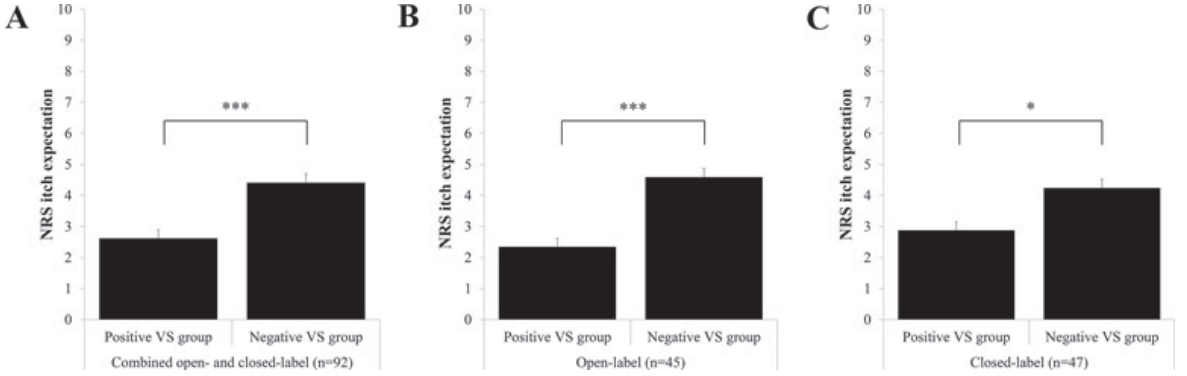

Figure 2. Mean Numeric Rating Scale (NRS) score for post-verbal suggestions (VS) itch expectation with the standard error of the mean (SEM) for **(A)** the combined open- and closed-label positive VS group (n = 45) and negative VS group (n = 47); **(B)** the open-label positive VS group (n = 22) and open-label negative VS group (n = 23); and **(C)** the closed-label positive VS group (n = 23) and closed-label negative VS group (n = 24). ***p < .001, *p < .05.

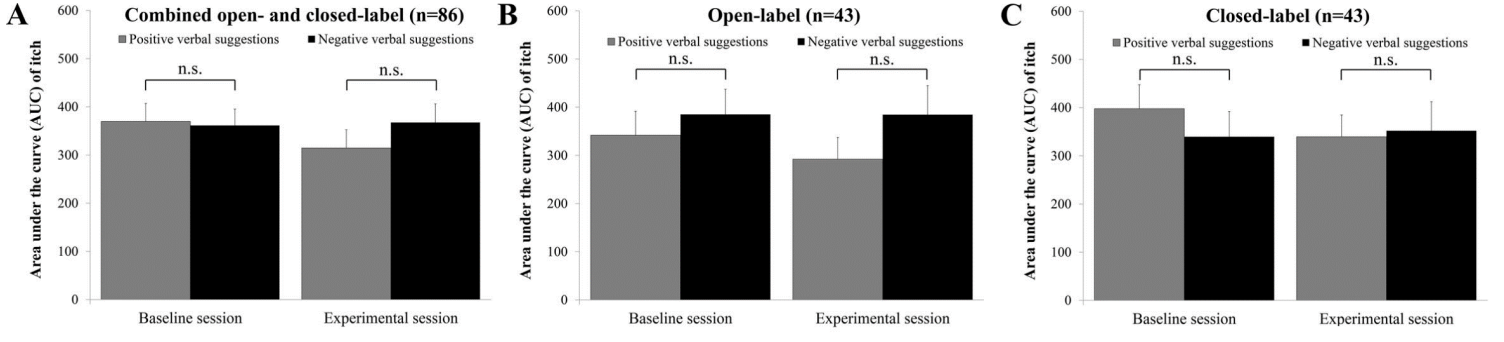

Figure 3. Mean area under the curve (AUC) of self-rated itch during histamine iontophoresis in the baseline and experimental session, with the standard error of the mean (SEM) for **(A)** the combined open- and closed-label positive VS group (n = 40) and negative VS group (n = 46); **(B)** the open-label positive VS group (n = 21) and open-label negative VS group (n = 22); and **(C)** the closed-label positive VS group (n = 19) and closed-label negative VS group (n = 24). n.s. = not significant (p > .05).

Table 2. Within-group mean changes from baseline and separate paired sample t-test results for the combined open- and closed-label positive verbal suggestion groups and combined negative verbal suggestion groups.

	Combined open- and closed- label positive VS groups (*n*=45)				Combined open- and closed-label negative VS groups (*n*=47)			
	n	Mean change	*t*	*p*	*n*	Mean change	*t*	*p*
Histamine iontophoresis								
AUC itch [A]	40	-46.91	1.98	.055	46	6.19	-0.19	.85
Maximum itch	40	-0.44	2.00	.053	46	0.02	-0.07	.94
Mean itch [B]	45	-0.26	1.34	.19	47	0.26	-1.30	.20
Post-iontophoresis follow-up								
AUC itch [A]	43	-3.73	3.24	.002	47	0.02	-0.02	.98
Skin response to iontophoresis								
Subjective skin response [C]	44	-3.30	2.59	.013	47	-2.00	1.61	.12
Wheal area [cm^2]	45	-0.40	0.79	.43	47	-0.41	0.87	.39
Flare area [cm^2]	45	-2.20	1.31	.20	47	-0.99	0.50	.62
Change in skin temperature [°C] [D]	44	0.14	-0.88	.38	46	0.76	-3.88	<.001

Note**.** Mean change was calculated as post-verbal suggestions score – baseline score, with negative values indicating a decrease from baseline, and positive scores indicating an increase from baseline. [A] AUC = Area under the Curve. [B] Assessed verbally on a Numeric Rating Scale ranging from 0-10. [C] As measured by an adjusted version of the Sensitive Scale 10 (Misery et al., 2014). [D] Calculated as post-iontophoresis temperature – pre-iontophoresis temperature.

Secondary itch measures during and following histamine iontophoresis

1. Maximum itch and mean itch during iontophoresis.

Findings for maximum itch during iontophoresis were similar to those of AUC itch, with no effects of suggestions for the combined as well as separate groups (all $p \geq .24$) and a marginal decrease from baseline to post-VS exclusively for the combined positive VS groups [$t(39) = 2.00$, $p = .053$]. The combined positive VS groups showed a small-sized tendency to report lower (post-iontophoresis-assessed) mean itch ($M = 2.83 \pm 1.93$) than did the combined negative VS groups ($M = 3.19 \pm 2.09$); $F(1,89) = 3.22$, $p = .076$, Cohen's $d = 0.38$. No effects of verbal suggestions were found when open- and closed-label groups were separated, nor were changes from baseline to post-VS scores detected for any of the groups (all $p \geq .19$).

2. AUC of itch during follow-up after iontophoresis

A significant and medium-sized difference in the change scores of AUC for itch during the 4-min follow-up was found when open- and closed-label groups were combined [$F(1,88) = 6.09$, $p = .016$, Cohen's $d = 0.52$], with AUC itch during follow-up decreasing significantly in the combined positive VS groups ($M = -3.73 \pm 7.55$) compared with the combined negative VS groups ($M = 0.02 \pm 6.88$). A small-sized non-significant effect of verbal suggestions was found in the open-label groups; $F(1,43) = 2.11$, $p = .15$, Cohen's $d = 0.43$, and a marginal and medium-sized effect in the closed-label groups, in the same direction as for the combined groups; $F(1,43) = 4.94$, $p = .032$, Cohen's $d = 0.67$. A significant change from baseline to post-VS in AUC itch during follow-up was demonstrated for the combined positive VS groups [$t(42) = 3.24$, $p = .002$]. In the combined negative VS groups, however, no change was detected [$t(46) = -0.02$, $p = .98$]. Separating open- and closed-label groups revealed a non-significant change within the open-label positive VS group [$t(21) = 1.87$, $p = .075$] and a significant change within the closed-label positive VS group [$t(20) = 3.14$, $p = .005$].

Skin response

1. Subjective skin response (SS-10)

For subjective skin response following the histamine test, no significant difference was found between the combined positive and negative VS groups, nor between the separate open- and closed-label positive and negative VS groups (all $p \geq .12$). A significant decrease in subjective skin response from baseline to post-VS was demonstrated in the combined positive VS groups [$t(43) = 2.59$, $p = .013$], but not in the negative VS groups [$t(46) = 1.61$, $p = .12$]. When analyses were conducted for separate open- and closed-label groups, a significant decrease was demonstrated only for the closed-label positive VS group; $t(22) = 3.75$, $p < .001$.

2. Physical skin response

No effects of verbal suggestions on wheal or flare areas were found for either the combined or separate open- and closed-label groups (all $p \geq .23$). Regarding skin temperature, the combined positive VS groups showed a medium-sized lower increase in skin temperature

from before to after iontophoresis (M = 1.83 ± 1.15) than did the combined negative VS groups (M = 2.34 ± 1.62); $F(1,87) = 5.84$, $p = .018$, Cohen's $d = 0.52$. In the same direction, marginally significant medium-sized effects of verbal suggestions on skin temperature increase were found in the open-label [$F(1,41) = 3.01$, $p = .090$, Cohen's $d = 0.54$] and closed-label groups [$F(1,43) = 2.93$, $p = .094$, Cohen's $d = 0.52$], respectively. Within-group comparisons for both combined and separate open- and closed-label positive and negative VS groups showed that skin temperature increased significantly from baseline to post-VS for the negative VS groups (all $p \leq .048$), but not for the positive VS groups (all $p \geq .12$).

Associations between expected itch and the outcome measures of itch

In the combined open- and closed-label groups, expected itch following suggestions was significantly and positively associated with all itch measures during and following iontophoresis (all $r \geq .43$, all $p \leq .01$). Comparisons of the strength of the association between expected itch and the itch outcome measures showed small-sized to no differences in associative strength between the combined positive and combined negative VS groups (all Cohen's $q \leq 0.15$). In the separate open-label and closed-label groups, findings were similar, with one exception: in the open-label positive VS group exclusively, itch expectations were not associated with mean itch and AUC of itch during follow-up (both $p \geq .11$). An overview of Pearson's r and Spearman's ρ correlation coefficients can be found in **Table 3** (combined open- and closed-label groups) and **Supplementary Table S3** (separate open- and closed-label groups).

Open-label instruction believability

Overall, participants in the open-label conditions rated the instructions as very clear (M = 7.90 ± 2.32). Ratings on how convincing the instructions had been were more ambiguous (M = 5.37 ± 2.46). In general, participants in the open-label groups believed that expectations are able to influence itch (M = 6.49 ± 1.97) but rated the extent in which their own itch experience was influenced by the application of the tonic as low (M = 3.81 ± 2.43). Groups did not differ in their ratings of the instructions (all $p \geq .21$).

Table 3 Within-group Pearson's r and Spearman's rho correlations for the process measure of post-VS itch expectation and outcome measures of self-reported itch and skin response for the combined open- and closed-label group comparisons, with Cohen's q as estimate of the difference in effect size between groups.

	Combined open- and closed-label groups		
	Positive VS (*n*=45)	**Negative VS (*n*=47)**	**Cohen's *q***
Post-VS histamine iontophoresis			
AUC itch [A]	.67 ***	.58 ***	0.15
Maximum itch	.63 ***	.59 ***	0.06
Mean itch [B]	.52 ***	.60 ***	0.12
Post-VS follow-up on iontophoresis			
AUC itch during follow-up [A, C]	.43 **	.49 ***	0.08
Post-VS skin response to iontophoresis			
Subjective skin response [D]	.50 ***	.59 ***	0.13
Wheal area [cm^2]	-.09	-.01	0.08
Flare area [cm^2]	.03	-.23	0.26
Change in skin temperature [°C] [E]	.04	-.19	0.23

Note. [A] AUC = Area under the Curve. [B] Assessed verbally on a Numeric Rating Scale ranging from 0-10. [C] Calculated using the non-parametric Spearman's rho. [D] As measured by an adjusted version of the Sensitive Scale 10 (Misery et al., 2014). [E] Calculated as post-iontophoresis temperature – pre-iontophoresis temperature. † p<.10; * p<.05; ** p<.01; *** p<.001

DISCUSSION

The current study investigated whether positive and negative outcome expectations, induced by open-label and closed-label positive and negative verbal suggestions regarding an inert tonic, could influence self-reported itch in response to a histamine test. For the first time, open- and closed-label placebo effects for itch were investigated within a single study, including a comparison with open- and closed-label nocebo effects. It was demonstrated that both open-label and closed-label verbal suggestions were able to influence itch expectations. For the primary outcome of area under the curve for itch during histamine iontophoresis, a small-sized but non-significant effect of verbal suggestions was found. Participants in the combined open- and closed-label positive VS groups reported lower itch during an immediate follow-up period after iontophoresis compared to the negative VS groups. *Post hoc* tests indicated that this was mostly due to differences between positive and negative VS groups under closed-label conditions. In addition, a significantly smaller increase in skin temperature was observed in the combined positive VS groups compared with the negative VS groups, but no effects on other markers of the physical skin response to histamine were found. Overall, the current study shows that verbal suggestions regarding

a topical application of a substance can influence expectations for itch, regardless of whether or not participants know about receiving suggestions, and provides limited evidence that these suggestions may influence itch and skin response in response to histamine.

The findings that verbal suggestions were able to influence itch in the follow-up period after histamine iontophoresis are in line with a previous study that found medium-to-large-sized effects of positive suggestions on histamine-induced itch [24]. While that particular study made use of a cream to help induce placebo effects, the current study used a pink-colored tonic. Potentially, the use of this particular attribute may have led towards smaller effects in the current study, since a cream could be perceived as a common treatment for itch by some participants, could trigger previously learned associations, and could thus potentially elicit stronger effects overall [47]. Moreover, negative verbal suggestions did not elicit negative expectations for itch in the current study and did not increase itch either during or following the histamine test, which is not in line with previous evidence for verbal suggestion-induced nocebo effects in itch [25,26,28]. It should be noted though that these previous studies have induced nocebo effects through negative suggestions regarding the experimental itch induction method that was used, whereas the current study provided suggestions regarding the topical application of an attribute prior to itch induction. While this did allow for a direct comparison of positive and negative expectation induction, potentially, it may have influenced the credibility of the negative verbal suggestions as well. Topical application of, for example, a cream or tonic in a laboratory setting might be associated more easily with symptom reduction rather than worsening of symptoms. In comparison, information regarding an experimental itch induction method, though less clinically relevant, may provide a more neutral basis for induction of nocebo effects through suggestions. Alternatively, although the baseline histamine application was valuable for participants as a comparison point for the second application, nocebo effects induced through negative verbal suggestions could have been influenced by participants being less anxious about the second histamine test, in comparison with the first test (since participants were generally unfamiliar with histamine iontophoresis prior to participating in the study). Future research may utilize a counterbalanced design to examine this more in detail. Likewise, more research is needed to investigate under which circumstances and through which attributes placebo and nocebo effects may be elicited for itch.

An effect of negative verbal suggestions on change in skin temperature due to histamine application was demonstrated. This finding is similar to previous work on placebo effects in

autonomically controlled parameters and wheal responses [26,48], a meta-analysis of clinical trials demonstrating placebo effects on physical outcome parameters controlled by the autonomic nervous system [49], and early studies on suggestions and hypnosis [50-52]. Considering that either the outcome measure differed from these previous studies (i.e., skin temperature change rather than wheal size) or the expectation induction method was different (i.e., verbal suggestions given without hypnosis), caution is needed in interpreting these results. Moreover, the verbal suggestions in the current study did not influence wheal and flare areas to histamine, which is in line with most recent studies [24,29,35,53,54].

Our design allowed for the first time comparisons of effect sizes of positive and negative verbal suggestions under open- and closed-label conditions for itch. The findings demonstrate that positive verbal suggestions are able to significantly reduce expectations of itch under both open-label and closed-label conditions, with open-label verbal suggestions seemingly inducing larger expectancy effects. Overall, the effects of positive and negative verbal suggestions on itch were approximately similar sized under open-label and closed-label conditions. However, some differences between the conditions could be seen when examining the within-group changes from baseline. Closed-label suggestions appeared slightly more effective for itch, as illustrated by the significant within-group changes in itch during follow-up from baseline to post-suggestions under closed-label conditions. That open-label placebo treatment can significantly influence expectations and, potentially, symptoms of itch is in line with previous findings on other outcome parameters [31,32,34-39]. It also provides further preliminary support for the notion that concealment of treatment is not necessary to elicit placebo responses, and that placebo mechanisms can potentially be utilized in clinical practice. Small differences between the open-label instructions of the current study and previous work need to be noted. Previous studies [e.g., refs. 31-34, 40] began their open-label placebo instructions by indicating that the pill that was used was a placebo, prior to indicating the efficacy and mechanisms of these effects. The current study on the other hand began by introducing the tonic as an effective tool for itch reduction and explaining that it was a placebo afterwards, together with a rationale on why it would still be effective. Differences in the order in which this type of information is presented may impact the strength of open-label placebo and nocebo effects. In addition, previous work has incorporated the concept of learning in the open-label instructions (i.e., by giving the example of Pavlov's dog). This aspect has been omitted here, as the current study investigates placebo responses evoked by conscious expectancy (i.e., verbal information) rather than associative learning mechanisms. Potentially, this may have

influenced the efficacy of the open-label rationale. Some caution needs to be taken in interpreting the effects of negative verbal suggestions under the separate open-label and closed-label conditions, since neither type of negative verbal suggestions was able to increase expectations of itch.

Some strengths and limitations need to be taken into account. This is the first study that compares open- and closed-label positive and negative verbal suggestions to elicit placebo and nocebo effects in itch and other responses to histamine. Since the study was conducted single blinded, a reporting bias cannot be ruled out, as participants may have adjusted their answers to the experimenters' expectations. To minimize influences of response bias on assessments of expectations and itch, participants used a (computerized) bar slide to indicate these parameters. Future research might, however, consider using a double-blinded approach. The effect sizes found in the current study are considerably small, which may be due to the itch stimulus being perceived as low by participants. As such, the study may have been underpowered to find small effects, which seems to be supported by finding more significant effects of the combined open- and closed-label groups than for the separate groups. Moreover, the design of the current study did not include a no-treatment group. This prevents an estimation of a true placebo or nocebo response, as itch may reduce from the first to second histamine test regardless of group allocation. Though habituation to the itch stimulus cannot be ruled out, its role is likely small, since the itch stimuli were relatively short and presented with 1 week in between. Alternatively, anxiety may have resulted in higher itch ratings during baseline. Including a no-treatment group to control for these reductions or utilizing a counterbalanced design could provide better estimates of a true placebo and nocebo response. Lastly, verbal suggestions were given regarding an inert tonic. While this approach may have worked for placebo induction, potentially, it may have been harder to elicit nocebo effects in this manner, as negative consequences regarding such a treatment method may be counterintuitive. To compare open-label and closed-label nocebo effects for itch, a different approach could be needed. For example, future research could investigate whether nocebo effects can be induced when the effects of an inert substance on itch are introduced as side effects of this substance, as changing to such an introduction of negative effects may be more closely related to how negative effects would be experienced in clinical practice.

In conclusion, this study provides evidence for the first time that positive verbal suggestions can induce expectations for itch reduction under both open-label and closed-label conditions. Suggestions are able to reduce the amount of itch experienced after

histamine iontophoresis under both open-label and closed-label conditions, with closed-label suggestions appearing more effective in reducing itch during follow-up. However, experienced itch during histamine iontophoresis was not influenced by suggestions. Future research may aim to investigate under which circumstances and with which type of attribute these suggestions could elicit effects for itch. Further demonstrating the efficacy of open-label placebo effects may help facilitate the application of these effects in clinical practice.

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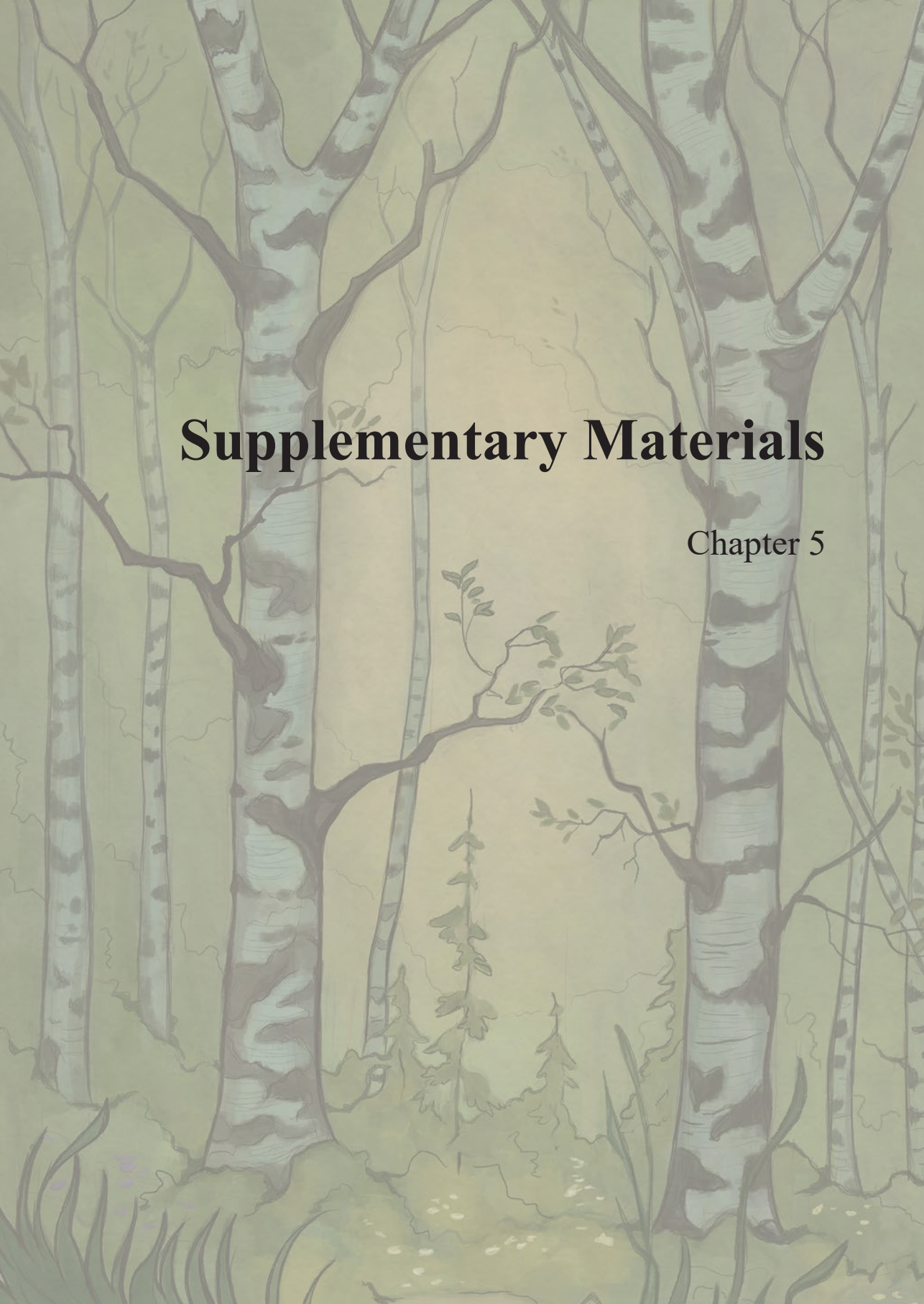

Supplementary Materials

Chapter 5

Supplementary Table S1. Means ± standard deviations for the separate open- and closed-label positive and the negative verbal suggestion groups.

	Open-label groups					Closed-label groups				
				AN(C)OVA					AN(C)OVA	
	n	**Positive VS (*n*=22)**	**Negative VS (*n*=23)**	***p*-value**	**Cohen's *d***	***n***	**Positive VS (*n*=23)**	**Negative VS (*n*=24)**	***p*-value**	**Cohen's *d***
Process measure										
Pre-iontophoresis itch expectation	45	4.87 ± 1.93	5.37 ± 1.40	.32		47	5.41 ± 1.97	4.29 ± 1.92	.055	
Post-VS itch expectation [A]	45	2.35 ± 1.88	4.59 ± 1.91	<.001	1.21	47	2.88 ± 1.77	4.24 ± 1.99	.017	0.74
Baseline histamine iontophoresis										
AUC itch [B]	43	341.78 ± 228.13	384.96 ± 245.59	.55		45	397.79 ± 257.03	339.70 ± 218.22	.42	
Maximum itch	43	3.75 ± 2.47	4.00 ± 2.31	.73		45	4.16 ± 2.45	3.58 ± 2.24	.42	
Mean itch [C]	45	2.84 ± 1.77	3.11 ± 1.89	.62		47	3.34 ± 2.03	2.75 ± 1.63	.27	
Post-VS histamine iontophoresis										
AUC itch [B,D]	43	292.07 ± 217.57	384.44 ± 296.16	.31	0.32	43	339.52 ± 261.15	352.05 ± 241.83	.47	0.23
Maximum itch [D]	43	3.27 ± 2.65	3.91 ± 2.70	.45	0.24	43	3.64 ± 2.47	3.71 ± 2.24	.42	0.26
Mean itch [C,D]	45	2.59 ± 1.70	3.29 ± 2.11	.20	0.40	47	3.06 ± 2.14	3.10 ± 2.12	.22	0.37
Change from baseline to post-VS scores										
AUC itch during follow-up [B,E]	45	-2.98 ± 7.19	0.05 ± 7.97	.19	0.41	45	-3.79 ± 5.54	-0.01 ± 5.83	.032	0.68
Baseline skin response to iontophoresis										
Subjective skin response [F]	44	24.65 ± 11.17	24.31 ± 12.98	.93		47	24.12 ± 12.53	21.32 ± 11.59	.43	
Wheal area [cm^2]	45	11.30 ± 3.01	11.31 ± 3.17	.997		47	9.76 ± 3.78	10.89 ± 2.89	.26	
Flare area [cm^2]	45	47.02 ± 11.34	50.94 ± 11.53	.26		47	48.44 ± 10.97	45.51 ± 12.96	.41	
Change in skin temperature [°C] [G]	45	1.57 ± 1.03	1.60 ± 0.96	.93		46	1.83 ± 1.00	1.57 ± 1.46	.48	
Post-VS skin response to iontophoresis										
Subjective skin response [D,F]	44	23.60 ± 14.19	21.33 ± 11.54	.43	0.24	47	18.77 ± 10.07	20.27 ± 13.06	.12	0.47
Wheal area [cm^2] [D]	45	10.17 ± 3.40	11.27 ± 3.04	.23	0.37	47	10.07 ± 4.22	10.11 ± 4.22	.32	0.30
Flare area [cm^2] [E,H]	45	43.00 ± 10.88	50.93 ± 11.88	.054	0.33	47	47.97 ± 14.76	43.58 ± 10.66	.25	0.12
Change in skin temperature [°C] [D,G]	44	1.91 ± 0.88	2.51 ± 1.50	.090	0.54	46	1.76 ± 1.36	2.16 ± 1.74	.094	0.52

Note. VS = verbal suggestions. [B] AUC = Area under the Curve. [C] Assessed verbally on a Numeric Rating Scale ranging from 0-10. [D] Group differences assessed by ANCOVA, controlled for baseline. Cohen's d was calculated with the estimated marginal means (controlled for baseline). [E] Calculated as post-VS measure – baseline measure (session 2 – session 1) and corrected for significant outliers. [F] As measured by an adjusted version of the Sensitive Scale 10 (Misery et al., 2014). [G] Calculated as post-iontophoresis temperature – pre-iontophoresis temperature. [H] For flare area, an ANOVA was conducted as homogeneity of regression slopes was unequal.

Supplementary Table S2. Within-group mean changes from baseline and separate paired sample t-test results for the open- and closed-label positive verbal suggestion groups and negative verbal suggestion groups.

	Open-label groups								Closed-label groups							
	Positive VS (*n*=22)				Negative VS (*n*=23)				Positive VS (*n*=23)				Negative VS (*n*=24)			
	n	Mean change	*t*	*p*	*n*	Mean change	*t*	*p*	*n*	Mean change	*t*	*p*	*n*	Mean change	*t*	*p*
Histamine iontophoresis																
AUC itch [A]	21	-49.71	1.61	.12	22	-0.52	0.01	.99	19	-43.81	1.17	.26	24	12.35	-0.26	.80
Maximum itch	21	-0.48	1.52	.15	22	-0.09	0.20	.84	19	-0.39	1.26	.22	24	0.13	-0.33	.75
Mean itch [B]	22	-0.25	0.87	.39	23	0.18	-0.71	.48	23	-0.28	1.00	.33	24	0.35	-1.07	.30
Post-iontophoresis follow-up																
AUC itch [A]	22	-3.68	1.87	.075	23	0.05	-0.03	.98	21	-3.79	3.14	.005	24	-0.01	0.01	.99
Skin response to iontophoresis																
Subjective skin response [C]	21	-1.05	0.50	.62	23	-2.98	1.91	.07	23	-5.35	3.75	.001	24	-1.05	0.55	.59
Wheal area [cm^2]	22	-1.13	1.60	.13	23	-0.04	0.05	.96	23	0.31	-0.45	.66	24	-0.77	1.31	.20
Flare area [cm^2]	22	-4.01	1.72	.10	23	-0.01	<0.01	>.99	23	-0.46	0.19	.85	24	-1.93	0.68	.50
Change in skin temperature [°C] [D]	21	0.36	-1.65	.12	23	0.92	-3.42	.002	23	-0.07	0.30	.77	23	0.60	-2.09	.048

Note. Mean change was calculated as post-verbal suggestions score – baseline score, with negative values indicating a decrease from baseline, and positive scores indicating an increase from baseline. [A] AUC = Area under the Curve. [B] Assessed verbally on a Numeric Rating Scale ranging from 0-10. [C] As measured by an adjusted version of the Sensitive Scale 10 (Misery et al., 2014). [D] Calculated as post-iontophoresis temperature – pre-iontophoresis temperature.

Supplementary Table S3. Within-group Pearson's r and Spearman's rho correlations for the process measure of post-VS itch expectation and outcome measures of self-reported itch and skin response for the separate open- and closed-label group comparisons separately.

	Open-label groups			Closed-label groups		
	Positive VS (*n*=22)	**Negative VS (*n*=23)**	**Cohen's *q***	**Positive VS (*n*=23)**	**Negative VS (*n*=24)**	**Cohen's *q***
Post-VS histamine iontophoresis						
AUC itch [A]	.68 ***	.59 **	0.15	.67 ***	.57 **	0.16
Maximum itch	.56 **	.59 **	0.05	.68 ***	.59 **	0.15
Mean itch [B]	.33	.68 ***	0.49	.68 ***	.53 **	0.24
Post-VS follow-up on iontophoresis						
AUC itch during follow-up [A, C]	.35	.53 **	0.23	.57 **	.50 *	0.10
Post-VS skin response to iontophoresis						
Subjective skin response [D]	.51 *	.52 *	0.01	.60 **	.64 ***	0.07
Wheal area [cm^2]	-.27	.12	0.40	.06	-.12	0.18
Flare area [cm^2]	-.21	-.40 †	0.21	.17	-.15	0.32
Change in skin temperature [°C] [E]	-.43 †	-.32	0.13	.35	-.12	0.49

Note. [A] AUC = Area under the Curve. [B] Assessed verbally on a Numeric Rating Scale ranging from 0-10. [C] Calculated using the non-parametric Spearman's rho. [D] As measured by an adjusted version of the Sensitive Scale 10 (Misery et al., 2014). [E] Calculated as post-iontophoresis temperature – pre-iontophoresis temperature. † p<.10; * p<.05; ** p<.01; *** p<.001

Chapter 6

Open- and closed-label placebo and nocebo suggestions about a sham transdermal patch

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ABSTRACT

Objective. Accumulating evidence indicates that placebo effects may also occur when it is known that a placebo is given. It is not yet clear whether these open-label placebo effects are similar to those of concealed (i.e. closed-label) placebo effects for somatic symptoms such as itch or whether nocebo effects can be induced under open-label conditions.

Methods. Healthy volunteers (n=112) were randomized to I) an open-label positive suggestions group, II) a closed-label positive suggestions group, III) an open-label negative suggestions group, or IV) a closed-label negative suggestions group. Participants were told, as cover story, that a transdermal caffeine patch would be applied that positively influences cognitive abilities and, as a side effect, positively or negatively (depending on group allocation) influences itch. Participants in the open-label groups were given a rationale explaining placebo and nocebo effect mechanisms. Itch was induced at baseline and post-suggestions by histamine iontophoresis.

Results. In the positive suggestions groups, significantly lower itch was reported than in the negative suggestions groups for both open- and closed-label contexts (all $p \leq .008$, Cohen's $d \geq 0.47$). Self-rated skin response was rated as less severe following positive versus negative suggestions (all $p \leq .017$, Cohen's $d \geq 0.33$), but no effects on physical skin responses to histamine were found (all $p \geq .23$, Cohen's $d \leq 0.30$).

Conclusion. Itch can be reduced by positive compared to negative suggestions under both open- and closed-label conditions. These findings indicate that open-label suggestions may potentially be a tool to utilize placebo effects for self-reported outcomes in clinical practice, for example by explaining role of expectancy in treatment. It needs to be investigated further under which circumstances an open-label rationale may impact placebo and nocebo effects.

Trial registration. www.trialregister.nl; NTR7174

INTRODUCTION

Placebo effects are beneficial effects that are not attributable to active treatment components such as pharmacological substances [1, 2]. Instead, these effects emerge through expectations about treatment outcomes, that are shaped by information that is provided about a treatment, learning, and environmental and social cues such as a positive patient-clinician interaction [1, 3-5]. Nocebo effects (i.e. adverse treatment outcomes such as side effects, that can be attributed to negative outcome expectations) can be similarly shaped by these pathways [1,6]. Experimental studies have demonstrated that placebo and nocebo effects can be induced in itch [7-9], although some studies show mixed or limited evidence [10-14]. In fact, meta-analyses show that over 30% of symptom improvement in clinical trials for itch and allergic symptoms can be explained by the placebo effect [15,16]. Itch ranks as one of the 50 most common interdisciplinary symptoms which affects an estimated one-fifth of the population, and that it has a debilitating impact on quality of life while existing treatments show limited effects [17-19]. Therefore finding ways to enhance existing treatments for itch becomes increasingly important. Potentially, placebo and nocebo effects may be used to facilitate improvement of existing treatments for itch.

Most studies on placebo and nocebo effects for physical symptoms such as pain or itch have investigated concealed placebo or nocebo induction, in which participants were unaware of receiving a placebo (sham) treatment. Such an approach does not allow for an easy translation towards clinical practice, mostly due to ethical considerations [20]. In the past decade, accumulating evidence shows that placebo effects can also occur when patients are fully informed about receiving placebos. Studies have shown that providing an inert pill in combination with a rationale on how placebo effects can impact medical conditions can reduce symptoms of a variety of medical conditions, amongst which irritable bowel syndrome, low back pain, and symptoms of allergic rhinitis [21-30].

There is limited literature available on whether a non-deceptive (open-label) approach can induce placebo effects for itch specifically, or how these effects relate to concealed (closed-label) placebo effects. Likewise, while we do know that nocebo effects often present as side effects to active treatments (e.g., induced by reading the leaflet of a pharmacological substance) [31-33], not much is known about whether these effects can also be induced using an open-label approach. A single study investigated open-label and closed-label placebo and nocebo effects induced by verbal suggestions about sham cutaneous treatment, and found that both open-label and closed-label suggestions influenced itch after, but not

during, histamine application on the skin [34]. The current study builds on these previous findings and investigates whether positive and negative outcome expectations, induced by a novel suggestive framework (verbal suggestions regarding a transdermal caffeine patch, where positive or negative effects on itch were purported as side effects, provided with either an open-label context or a closed-label context), could influence self-reported itch during an experimental itch induction test using histamine. Secondary outcomes include self-rated and clinical (physical) skin responses to histamine as well as psychological outcomes such as wellbeing. We first examine differences between the combined positive and the combined negative suggestions groups, and next assess effects for open-label and closed-label contexts separately. We expect low itch following positive verbal suggestions compared to high itch following negative verbal suggestions for both open-label and closed-label contexts.

METHODS

The study was approved by the Medical Ethics Committee at the Leiden University Medical Center, The Netherlands (NL64502.058.17) and pre-registered in the Dutch Trial Register on May 6th 2018 (trial ID: NTR7174). The study was conducted in accordance with the Declaration of Helsinki. All participants provided written informed consent. Data for the study were collected between April 2018 and January 2019.

Participants

Healthy male and female volunteers were recruited through advertisements on sites of Leiden University and social media. Participants between 18 and 35 years old that had a good understanding of written and spoken Dutch were included. Exclusion criteria consisted of severe somatic or psychological morbidity (e.g., heart and lung diseases, Diagnostic and Statistical Manual fifth edition (DSM-V) psychiatric disorders); current chronic itch or pain; current use of analgesics, anti-inflammatory drugs, antihistamines, or antibiotics; recent vaccinations; pregnancy; and colour blindness. Participants were asked to refrain from caffeine or nicotine consumption and heavy meals 2h, exercising 12h, and alcohol and drugs 24h prior to participation in the study, which was verified at the start of their appointment.

Study design

A between-subjects, single-blinded, randomized controlled design was applied. Participants were allocated (by block-randomization (n=8/block), online random number generator: www.random.org, Dublin, Ireland) to I) an open-label positive verbal suggestions (VS), II) closed-label positive VS, III) open-label negative VS, or IV) closed-label negative VS group. Allocation was not concealed from the experimenter. Participants were invited to a single laboratory session at the faculty of Social and Behavioural Sciences, Leiden University, The Netherlands. Itch was induced at baseline and post-VS by histamine iontophoresis (see also **Figure 1**).

Materials and Measures

1. Verbal suggestions

The study was advertised as a study that investigated the effects of a transdermal caffeine patch on cognitive abilities and sensitivity to physical stimuli. As part of this cover story, cognitive tasks[3] were conducted before and following suggestions. Following baseline measurements, participants were told that (1) a caffeine-containing patch would be placed on their shoulder, (2) caffeine, like nicotine, can be delivered by this method, and (3) this would influence both cognitive abilities and sensitivity to physical stimuli such as itch. In the positive VS groups, the following suggestion was then given: "*Previous research has shown that itch decreases strongly after applying this patch for most people, i.e. about 95% of people. The caffeine makes your skin less sensitive to physical stimuli. As such, we expect that you will experience less itch, compared to the first test*". In the open-label groups, an additional explanation of the placebo effect was given that stressed the following points: (1) the patch actually did not contain caffeine, (2) the purpose of the study was to test the effects of such positive suggestions, (3) previous research has shown that suggestions can reduce itch, (4) these effects are due to bodily processes, as the brain responds to information about a treatment in the same manner as to the actual treatment, and (5) this may also work when people know that they receive a placebo. For the negative VS groups, positive words were replaced by negative words (i.e. 'more itch' instead of 'less itch', and

[3] Considering that the verbal suggestions were not directly aimed at manipulating the outcomes of the cognitive tasks (but that these were rather included as part of the cover story), the detailed methodology for these tasks, their outcome measures (including related outcomes, e.g., expectations) and their results can be found in the Supplementary Material.

'nocebo' instead of 'placebo'). A 10x10 cm hydrocolloid patch (Medeco B.V., Oud-Beijerland, the Netherlands) was then placed on the non-dominant shoulder.

2. Itch induction: histamine iontophoresis

Itch was induced experimentally by histamine iontophoresis (see Meeuwis, Van Middendorp [13] for detailed methodology). Briefly, itch was induced for 2.5 minutes on the volar side of the forearm. After 2.5 minutes, iontophoresis electrodes were removed, after which a 3-minutes follow-up period commenced. Baseline iontophoresis was conducted on the dominant forearm, and post-VS iontophoresis on the non-dominant forearm.

3. Outcome measures

3.1. Expected itch and expected patch efficacy for skin sensitivity

Prior to each itch induction, participants rated expected itch on a Numeric Rating Scale (NRS) from 0 ('no itch') to 10 ('worst imaginable itch'). In addition, participants rated (post-VS, but prior to iontophoresis) the extent to which they believed the patch would influence skin sensitivity during the itch induction test on a NRS (0 'no effect', 10 'very effective').

3.2. Self-rated itch

Self-rated itch was assessed every 30 seconds during both iontophoresis tests and their follow-up period, using the same NRS as described in **section 3.1.** Participants were asked to rate mean itch experienced during iontophoresis (the primary study outcome) immediately upon removal of the iontophoresis electrodes. Correlations between mean itch assessed following iontophoresis and itch scores assessed every 30 seconds during iontophoresis were calculated to assess reliability of the primary outcome: self-rated mean itch (as assessed directly following the test) was significantly associated with all other itch measurements during iontophoresis for both baseline and post-VS measurements (all $r \geq .35$, all $p < .001$).

3.3. Self-rated and clinical skin response to histamine

As a measure of self-rated skin response, participants were asked to fill in a version of the Sensitive Scale-10 (SS-10) questionnaire [35] that was adjusted for use with histamine iontophoresis (see also [13]). In the current study, Cronbach's alphas for the post-iontophoresis SS-10 were .85 and .86, respectively. Wheal size and flare response to histamine were assessed following both iontophoresis tests by tracing the outer edges on a transparent, 1 cm^2-gridded sheet. Images were uploaded and retraced in ImageJ [36], and wheal and flare areas were calculated (in cm^2). In addition, skin temperature measurements were taken with a handheld infrared digital thermometer pre- and post-iontophoresis. Rise in skin temperature due to iontophoresis (Δ-temperature) was calculated as an outcome measure by subtracting the pre- from post-iontophoresis measurements.

3.4. Wellbeing: the Positive and Negative Affect Schedule

To assess the effects of suggestions on wellbeing, participants filled out the Positive and Negative Affect Schedule (PANAS [37]) at four moments during the laboratory session (see **Figure 1**). In the current study, Cronbach's alpha ranged .88 – .91 for the PANAS positive affect (PA) scale. Considering the scores on negative affect were very low at all measurement points (M_{range} = 11.49–12.32; with variances between 4.09 – 9.60, while the scale ranges from 0–50), group differences for this scale were not analysed. Two additional scales for wellbeing were assessed at the same moments as the PANAS, and are discussed in the **Supplementary Material**.

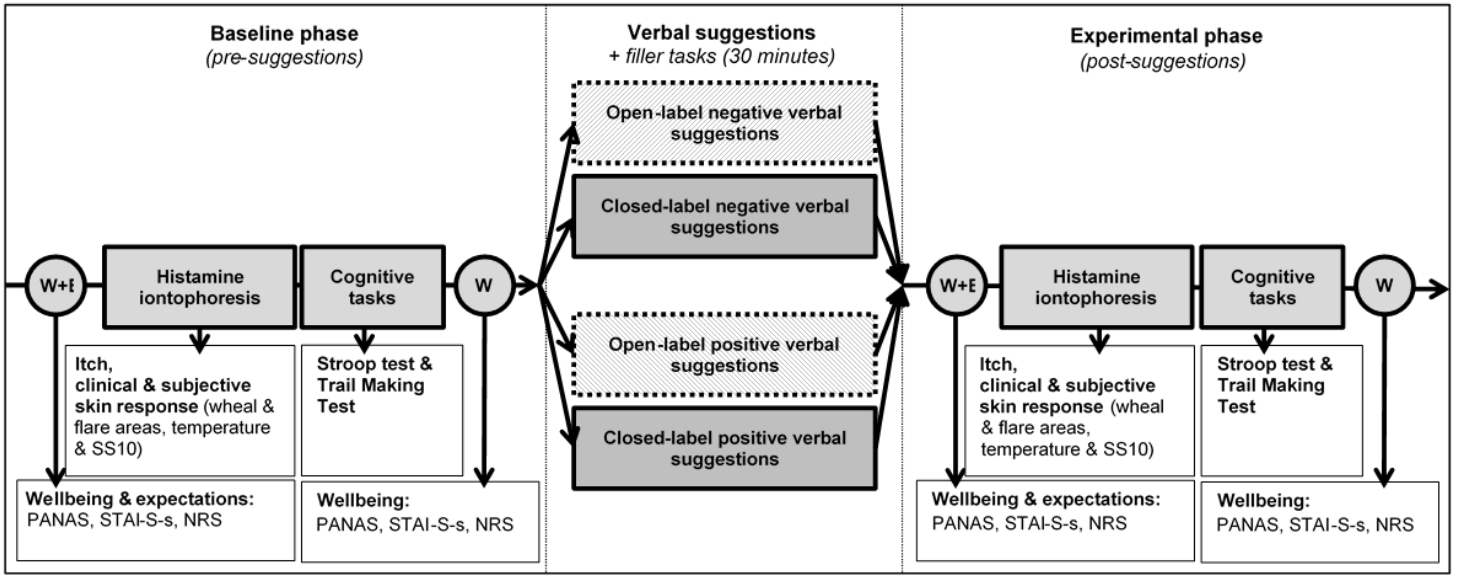


Figure 1. Overview of study design and measurement schedule for the laboratory session. W = wellbeing; E = expectations; PANAS = Positive and Negative Affect Schedule [37]; STAI-S-s = Spielberger State Trait Anxiety Inventory, State anxiety short scale [48]; NRS = Numeric Rating Scale; SS10 = Sensitive Scale-10 [35]; Stroop test, [49]; Trail Making Test [50,51].

Procedure

Prior to participation, volunteers filled out an online screening questionnaire. Eligible volunteers were invited for a single 2-hours laboratory session at the research site of the Social and Behavioural Sciences Department, Leiden University, The Netherlands. Upon arrival, the general procedures were explained and participants provided written informed consent (for the online screening questionnaire, separate online consent was given). Briefly, the in- and exclusion criteria were checked and adherence to lifestyle rules was verified. Next, the baseline phase started and participants filled out questionnaires for wellbeing and expectations. Demographics and personality factors were assessed (the latter were not related to the current study purpose and will be reported elsewhere). Histamine iontophoresis was conducted on the dominant arm, during and following which participants rated itch. Clinical and self-rated skin responses were assessed, followed by cognitive tests, and assessment of wellbeing. Verbal suggestions were given (depending on group allocation) and the inert patch was placed on the participant's shoulder. Participants were asked to perform some neutral filler tasks (i.e., Sudoku's, word & picture search puzzles) while the experimenter left the room, with a twofold purpose: 1) so that carry-over effects in itch could be minimized, an 2) so that the cover story of testing effects of the patch on cognitive tasks could be further reinforced. Thirty minutes after the baseline phase ended, the experimenter returned, and wellbeing and expectations were assessed. Histamine iontophoresis was conducted on the non-dominant forearm, followed by the cognitive tests and wellbeing questionnaires. Finally, participants filled out a closing questionnaire. They were debriefed on the true purpose of the study (in the open-label groups, the study purpose was reconfirmed) by the experimenter. Participants received a compensation of €20,- for the laboratory session.

Statistical analysis

Power analysis was conducted in G*Power [38] to determine the optimal sample size for detecting between-group differences in mean itch, controlled for baseline. The estimated effect size was based on a meta-analysis of open-label placebo [39], which found an average effect of d=0.88 for open-label placebo effect induction in patient samples, compared to a no-treatment control group. As the current study investigated effects in healthy volunteers rather than patients, a more conservative effect size of d=0.78 was used. An a priori power analysis for analysis of covariance (ANCOVA), with α=.05 and β=.80, indicated that, taking into account an additional 5% missing data rate, 28 participants per

group were needed to detect differences between the positive and negative verbal suggestion groups (for separate analysis of open-label and closed-label contexts).
All analyses were conducted in SPSS 23.0 for Windows (IBM SPSS Inc., Chicago, Illinois, US) with an alpha level of α=.05. Normal distribution of the variables, baseline differences, and assumptions were checked prior to data analysis. As was a priori determined, open-label and closed-label groups were first combined to detect differences between the effects of positive verbal suggestions and negative verbal suggestions and to increase power for these analyses. General linear model (GLM) analyses of covariance (ANCOVAs) were conducted for each outcome measure of itch and self-rated and clinical skin response, in which baseline measures were controlled. Within-group baseline-to-post-VS change was explored for each group by paired-sample t-tests (Bonferroni corrected: α/2=.025) to assess impact of each type of verbal suggestions on itch, and self-rated and clinical skin response. Effects of group on wellbeing were explored by mixed between-within repeated measures ANOVA. For itch expectations, GLM ANOVA was used. As an effect size, Cohen's d was calculated from (covariate adjusted) group means and SD's, with the following categories for interpretations: 0.2 small effect, 0.5 medium effect, 0.8 large effect [40]. All analyses were repeated for the separate open-label groups, and the separate closed-label groups. For these secondary analyses, a Bonferroni correction for multiple comparisons was applied (α/2=.025 for ANCOVA and (α/2)/2=.0125 for further within-group t-tests). Data of one participant was excluded from the analyses, as technical issues with the iontophoresis device prevented a baseline measurement of itch. Group means are described as Mean±SD, unless stated otherwise.

RESULTS

Participants

In total, 236 potential participants expressed interest in the study, of whom 79 volunteers refrained from participating for reasons unknown (e.g., no response following invitation), and of whom 43 were excluded (30 for somatic and/or psychological conditions, 7 for medication use, and 6 for having trouble understanding Dutch). Two participants dropped out during the laboratory session, resulting in a final sample of 112 participants (16.1% male) aged between 18 and 31 years old (M_{age}=21.88±2.77). No group differences were found for demographic factors, baseline itch expectation and baseline iontophoresis

outcome parameters for either the combined open- and closed-label groups (see **Table 1**, all $p \geq .16$) or separate groups (see **Supplementary Table E1**; all $p \geq .13$).

Expected itch and expected patch efficacy for skin sensitivity

Expected itch during iontophoresis was significantly lower following suggestions in the combined positive VS groups (M = 4.00±1.87) compared to the combined negative VS groups (M = 5.69±2.16); F(1,109)=19.23, $p<.001$, Cohen's d=0.84. When analyses were repeated for open-label and closed-label contexts separately, group differences in the same direction as for the combined groups were found, with larger effect sizes found for the open-label rather than closed-label context (open-label: F(1,53)=15.00, $p<.001$, Cohen's d=1.04; closed-label: F(1,54)=6.67, $p=.013$, Cohen's d=0.69; see **Figure 2A and B**). Expected patch efficacy for skin sensitivity was somewhat lower in the combined positive VS groups (M=3.43±2.11) compared to the combined negative VS groups (M=4.28±2.55), however, effects were marginal and small; F(1,109)=3.64, $p=.059$, Cohen's d=0.36. When groups were separated for open-label and closed-label context, no differences were found (both $p \geq .13$; see **Figure 2C and D**).

Self-rated mean itch

Self-rated mean itch during iontophoresis was significantly lower in the combined positive VS groups (M=3.29±1.53) compared to the combined negative VS groups (M=4.21±1.96); $F(1,108)=17.14$, $p<.001$, Cohen's d=0.51. Similar group differences were found when analyses were repeated for open-label and closed-label contexts separately, with medium-sized differences for the closed-label context (F(1,53)=9.02, $p=.004$, Cohen's d=0.54), and small-to-medium-sized differences for the open-label context (F(1,52)=7.62, $p=.008$, Cohen's d=0.47; see **Figure 3A and B)**. Within-group analysis of baseline-to-post-VS-change for itch indicated that mean itch reduced significantly following positive VS (both combined and separate groups: all $p \leq .007$), while it did not change in the negative VS groups (all $p \geq .22$) (see **Table 2** for the combined-groups analyses, and **Supplementary Table E2** for the separate-groups analyses).

Table 1. Means ± standard deviations, and analysis of (co)variance (AN(C)OVA) outcomes for the combined open- and closed-label positive verbal suggestions (VS) groups and the combined open- and closed-label negative VS groups.

	Combined open- and closed-label contexts			
			AN(C)OVA	
	Positive VS (*n*=55)	**Negative VS (*n*=56)**	***p*-value**	**Cohen's d**
Demographics				
Sex [male: n (%)]	8 (14.55)	10 (17.86)	.64	
Age	21.89 ± 2.49	21.93 ± 3.02	.94	0.01
Baseline histamine iontophoresis				
Mean itch	3.98 ± 1.43	4.00 ± 1.73	.94	0.01
Self-rated skin response (SS-10) [a]	30.92 ± 13.26	29.79 ± 12.90	.65	0.09
Wheal area [cm^2]	8.92 ± 3.38	9.28 ± 3.87	.61	0.10
Flare area [cm^2]	43.36 ± 15.70	42.17 ± 13.01	.19	0.08
Change in skin temperature [°C] [b, c]	1.39 ± 1.15	1.68 ± 1.00	.16	0.27
Post-VS expectation outcomes for itch				
Expected itch	4.00 ± 1.87	5.69 ± 2.16	< .001	0.84
Expected patch effectiveness for skin sensitivity	3.43 ± 2.11	4.28 ± 2.55	.059	0.36
Post-VS histamine iontophoresis				
Mean itch	3.29 ± 1.53	4.21 ± 1.96	< .001	0.51
Self-rated skin response (SS-10) [a, d]	23.60 ± 11.88	27.56 ± 12.71	< .001	0.39
Wheal area [cm^2]	8.19 ± 3.18	7.92 ± 3.42	.24	0.15
Flare area [cm^2]	41.66 ± 13.33	41.71 ± 13.82	.65	0.05
Change in skin temperature [°C] [c, e]	1.20 ± 1.19	1.20 ± 1.09	.39	0.14

Note (**Table 1**). [a] Misery et al. [35]. [b] n=1 missing due to technical difficulties with the infrared thermometer. [c] calculated as post-iontophoresis temperature – pre-iontophoresis temperature. [d] n=1 missing on the post-VS SS-10. [e] n=2 missing due to technical difficulties with the infrared thermometer.

Clinical and self-rated skin response to histamine

Participants in the combined positive VS groups rated their skin response as less severe compared to the combined negative VS groups, as indicated by small-to-medium-sized significantly lower scores on the SS-10 in the positive VS groups (M=23.60±11.88) compared to the negative VS groups (M=27.56±12.72); $F(1,107)=13.58$, $p<.001$, Cohen's d=0.39. When open-label and closed-label contexts were separated, similar group differences were found, with somewhat larger effects found for the closed-label context (closed-label: $F(1,52)=7.23$, $p=.010$, Cohen's d=0.45; open-label: $F(1,52)=6.09$, $p=.017$, Cohen's d=0.33; see **Supplementary Table E1**). No differences were found for clinical skin response outcomes of wheal and flare area, or skin temperature change between the combined positive and combined negative VS groups (all $p\geq.24$) or between the separate open- and closed-label groups (all $p\geq.23$). An overview of the within-group baseline-to-

post-VS-change for each variable is provided in **Table 2** (combined groups) and **Supplementary Table E2** (separate groups). In short, no significant within-group changes were found for clinical skin response in the combined groups ($p \geq .063$), except for wheal area and skin temperature change in the combined negative VS groups, which decreased significantly from baseline (both $p \leq .001$). When open-label and closed-label contexts were separated, similar decreases were demonstrated in the negative VS groups ($p \leq .009$), except for change in skin temperature in the open-label context ($p = .071$).

Wellbeing: Positive Affect (PA)

No effect of the combined-groups x time interaction on PA was found ($p = .81$), indicating that verbal suggestions did not influence affect during the laboratory session. No main effect of group was found ($p = .51$), but PA changed significantly over time ($p < .001$, see **Supplementary Figure S1**). Post-hoc Bonferroni tests indicated that PA following baseline iontophoresis was significantly higher compared to all other measurements (all $p < .002$), and that other measurement moments did not differ significantly over time (all $p > .99$). Next, analyses were separated for open-label and closed-label contexts. In the open-label context, PA following baseline iontophoresis was higher compared to the two subsequent measurements (all $p < .001$), whereas in the closed-label context, PA following baseline iontophoresis was higher compared to the first and third (post-VS) measurements (all $p \leq .017$; see also **Supplementary Figure S1**). Results for two additional wellbeing scales are discussed in the **Supplementary Material**.

Table 2. Within-group baseline-to-post-verbal suggestions (VS) changes on histamine iontophoresis outcomes for the combined open- and closed-label positive VS groups and the combined negative VS groups.

	Combined open- and closed- label positive VS groups (*n*=55)				**Combined open- and closed-label negative VS groups (*n*=56)**			
	n	Mean change	*t*	*p*	*n*	Mean change	*t*	*p*
Mean itch	55	-0.68	4.97	< .001	56	0.22	-1.24	.22
Self-rated skin response (SS-10) [a]	55	-7.32	6.92	< .001	55	-2.29	2.48	.016
Wheal area [cm^2]	55	-0.73	1.90	.063	56	-1.35	4.64	< .001
Flare area [cm^2]	55	-1.25	1.14	.26	56	-0.46	0.44	.66
Change in skin temperature [°C] [b, c]	54	-0.19	1.18	.25	56	-0.47	3.46	.001

Note (**Table 2**). Mean change was calculated as post-verbal suggestions score – baseline score, with negative values indicating a decrease from baseline, and positive scores indicating an increase from baseline. [a] Misery et al. [35]. [b] n=2 missing due to technical difficulties with the infrared thermometer. [c] calculated as post-iontophoresis temperature – pre-iontophoresis temperature.

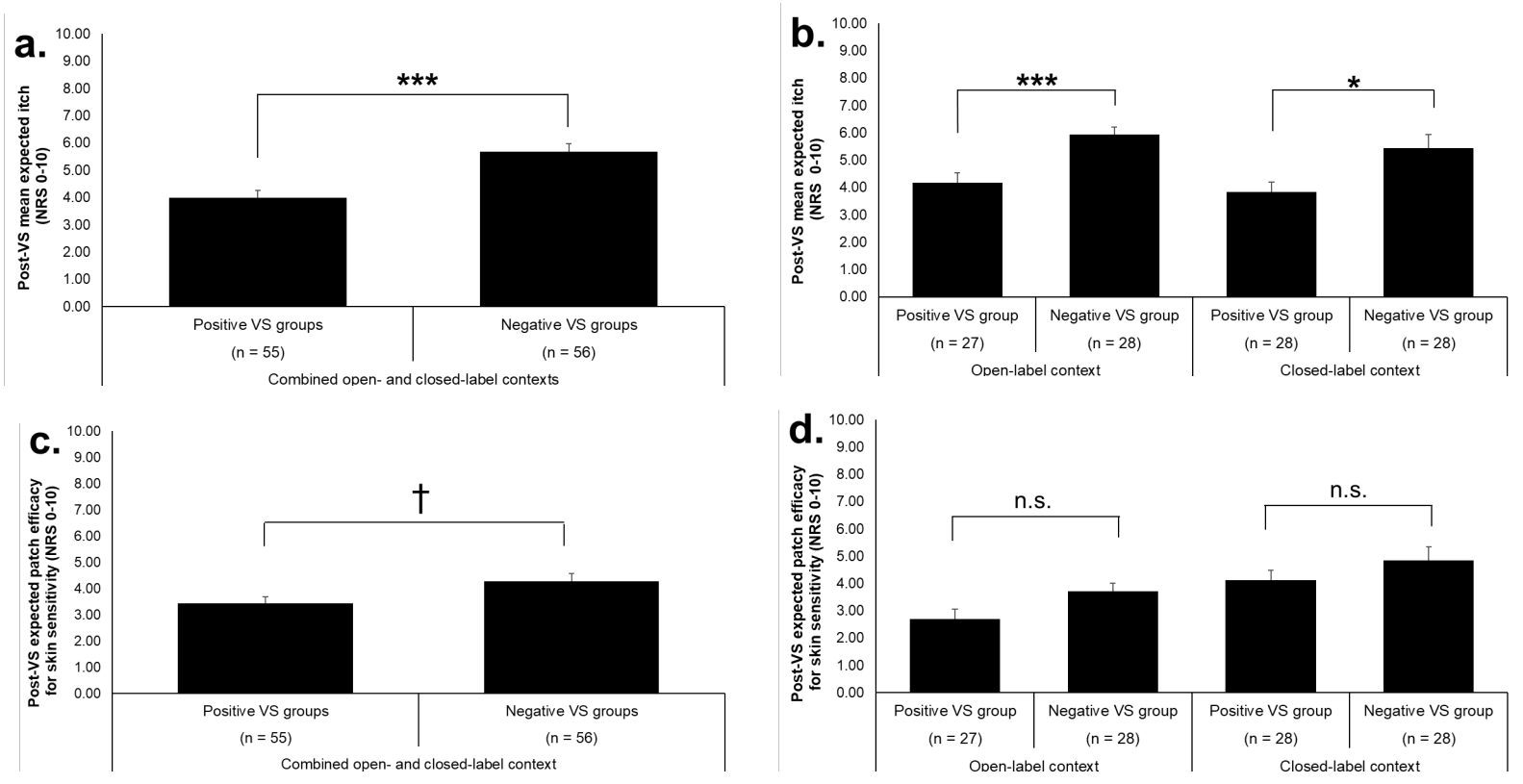
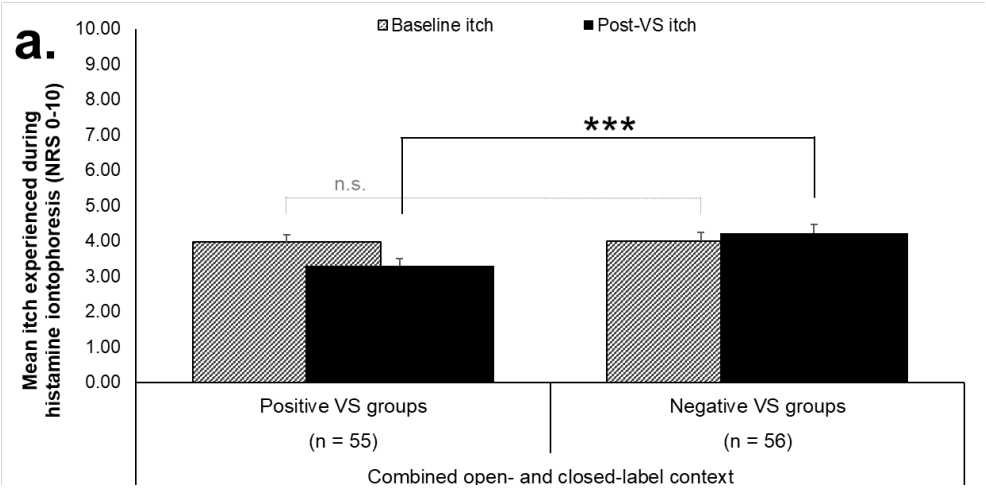


Figure 2. Mean Numeric Rating Scale (NRS) score with the standard error of the mean (SEM) for post-verbal suggestions (VS) itch expectation in [A] the combined open- and closed-label positive and negative VS groups, and [B] the separate open-label and closed-label positive and negative VS groups; and mean NRS with SEM for post-VS expected patch efficacy for skin sensitivity in [C] the combined open- and closed-label positive and negative VS groups, and [D] the separate open-label and closed-label positive and negative VS groups. *** $p<.001$, ** $p<.01$, * $p<.05$, † $p<.10$, n.s. non-significant.



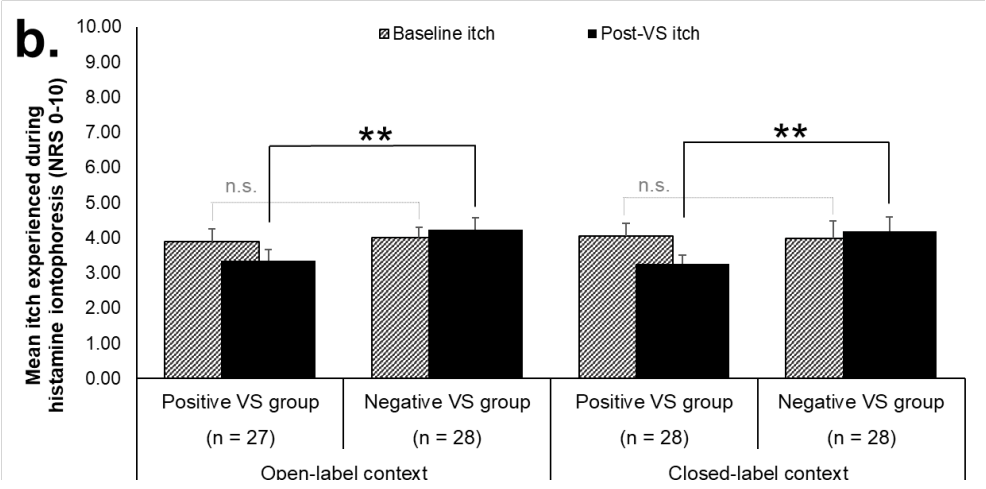


Figure 3. Mean Numeric Rating Scale (NRS) score for itch experienced during histamine iontophoresis for the baseline and post-verbal suggestions (VS) measurements, with the standard error of the mean (SEM) for [A] the combined open- and closed-label positive and negative VS groups, and [B] the separate open-label and closed-label positive and negative VS groups. *** p<.001, ** p<.01, * p<.05, † p<.10, n.s. non-significant.

DISCUSSION

The current study investigated whether positive and negative verbal suggestions regarding a sham transdermal patch for both open-label and closed-label contexts were able to influence self-reported itch during an experimental histamine test. Overall, the study findings illustrate that both open- and closed-label positive suggestions are able to influence expectations for itch and mean itch experienced during an experimental itch induction test compared to negative suggestions. The effects on itch expectations appear larger for the open-label context, whereas for self-rated perceived itch, the effects were larger when suggestions were given for the closed-label context. Secondary analyses indicated that itch decreased significantly following positive suggestions for both open-label and closed-label contexts, but that negative suggestions failed to increase itch. No effects on clinical skin response were found, but participants rated their own skin response as less severe following positive compared to negative suggestions for both open-label and closed-label contexts.

That positive suggestions are able to reduce itch is in line with findings of some, but not all previous studies [8,10-12,14]. The discrepancies in study findings in the literature may be explained by the strength and duration of verbal suggestions. Most of the studies on placebo effects in itch induce positive expectations by using brief suggestions of low or reduced itch [11,12,14]. In line with this, Bartels et al. [10] demonstrated that a combination of learning and suggestions was able to induce placebo effects, but brief suggestions alone could not. On the other hand, Darragh et al. [8] combined verbal suggestions with an information leaflet, which may have contributed to the strength of suggestions. The current study combined positive suggestions about itch with the cover story that a caffeine patch would influence cognitive abilities. That caffeine is able to impact, for example, focus and attention may be commonly accepted knowledge, which may in turn have contributed to the believability of the suggestions for itch.

Negative verbal suggestions did not increase experienced itch, which is not in line with previously conducted research [7,9,41-43]. However, previous studies have induced nocebo-like effects by giving suggestions directly about the itch elicitation methods. Potentially, suggestions regarding a sham treatment method may not elicit equally strong nocebo effects. A previous study, in which suggestions were given about a sham topical treatment, likewise failed to elicit significant increases in itch following negative suggestions [34]. Moreover, most participants were unfamiliar with the itch induction method, which may have resulted in higher itch scores during the baseline test. This may

complicate the estimation of the nocebo response, as the suggestions could have negated a naturally occurring decrease in itch. Future research may consider adding a no-suggestions (natural history) group to control for such effects and to more explicitly evaluate the size of placebo and nocebo effects.

Self-rated skin response was rated as less severe following both open-label and closed-label positive suggestions compared to negative suggestions. Indications that suggestions may be able to influence self-rated skin response have been found in previous research [13] and are further supported here. Clinical – or physical – skin response to histamine on the other hand was generally not influenced by verbal suggestions, which is in line with existing literature [8,13,44]. Wheal area and skin temperature decreased significantly in the negative VS groups. No differences between positive and negative suggestion groups were found, however, making it unlikely that these decreases were related to the manipulation used in the current study. A single previous study showed medium-sized increases in skin temperature following negative suggestions [34], but these findings could not be replicated here. Overall, the findings further support the notion that verbal suggestions may be more likely to impact subjective sensations such as pain or itch, whereas learning (i.e., conditioning) may be needed in addition to instructions in order to induce placebo effects for physical or physiological parameters.

While open-label suggestions appear particularly effective in inducing expectations for itch in the current study, effects on experienced itch were somewhat lower than for the closed-label context, though still medium-sized. The current study is one of the first to investigate similar verbal suggestions for both an open-label and a closed-label context. A previous study showed mixed evidence for the effects of open-label and closed-label suggestions on itch, but effect sizes did indicate that verbal suggestions had lower efficacy for itch in an open-label context as well [34]. Findings of the current study are in line with this. Most open-label studies report higher effect sizes than those reported in the current study though [39]. These studies have often used a rationale in which placebo effects were explained as learned Pavlovian responses [21-30]. The rationale in the current study differs from the one used previously, as only placebo and nocebo effects induced by positive or negative information (suggestions) and conscious expectancy were explained. These differences in rationale may as a consequence impact expectations in a different manner. Moreover, the open-label rationale in the current study was added onto a concealed positive or negative verbal suggestion (i.e. that the patch contained caffeine that would impact perception of itch, whereas in truth the patch contained no caffeine). This differs from previous work:

open-label rationales have either been provided immediately and without prior concealed suggestions [21-30], or have been added as an extended explanation of mechanisms onto a very succinct suggestion about to-be-expected effects [34]. Potentially, such a 'placebo-reveal' (i.e., explaining that you provided deceptive information first) may have resulted in smaller placebo responses in the open-label context compared to the closed-label (concealed) context. It has been shown that conditioned analgesia persists after it is revealed that subjects are in fact receiving a placebo [45]. A similar mechanism (i.e., first a placebo effect induction, which persists after the open-label rationale) may have played a role in the current study. Future research could aim to investigate how variations in the open-label rationale could impact its efficacy, for example by immediately integrating the open-label rationale in the suggestions or by investigating the efficacy of various open-label explanations of the placebo effect. Alternatively, participants may have responded differently to the negative suggestions, when they are given under concealed (closed-label) or open-label conditions. This may explain differences in effect size found under the open-label and closed-label contexts in the current study. There is evidence that information framing can influence the size of nocebo effects, with positive framing reducing the occurrence of (nocebo) side effects compared to negative framing [33]. Hypothetically, explaining how nocebo effects are formed may likewise impact how nocebo effects are formed, though this cannot be concluded exclusively based on data of the current study. Rather, future research may aim to clarify the impact of open-label information on the formation of nocebo effects. If it can be shown that open-label information can impact the formation of nocebo effects, this may be a potential method to prevent nocebo effects occurring in clinical practice. Moreover, an open-label rationale and suggestions may then be used to enhance placebo effects and inhibit nocebo effects simultaneously, for example by providing an explanation on the role of expectancy and context in treatment of medical conditions.

Some limitations need to be taken into account for the current study. The study was conducted single-blinded, with the experimenter giving the suggestions also being the one that conducted the tests. Potentially, this may have (unconsciously) impacted the participants' rating of itch during iontophoresis. Future research might consider using a double-blinded approach, for example, by having iontophoresis performed by a second experimenter who is blinded to allocated conditions. Second, participants received histamine iontophoresis twice within two hours, which may have caused habituation. However, the itch stimuli were relatively short (2.5 minutes) and presented almost one hour

apart. Moreover, by design, baseline iontophoresis took place on the dominant arm, and post-suggestions iontophoresis on the non-dominant arm. There are indications that handedness may affect sensory threshold and pain sensitivity, with the non-dominant arm being more sensitive [46,47]. It is likely that differences between both arms in sensitivity to itch would have negated habituation effects. Regardless, future research may aim to further control for these factors, including handedness. The lack of a no-treatment group in the current study complicates an estimation of the true placebo or nocebo response, as itch may have changed from baseline to post-VS regardless of suggestions. Including a no-treatment group, or counterbalancing the baseline and post-suggestion tests, may be a valuable contribution in future research to more explicitly evaluate placebo and nocebo effect sizes.

In short, the current study provides evidence that positive verbal suggestions regarding a sham transdermal patch for both open-label and closed-label contexts can influence expectations, itch experienced during, and self-reported skin response following an experimental histamine test. Future research may aim to investigate how variations in open-label rationale may impact the efficacy of positive and negative suggestions for itch. Potentially, open-label rationales may then be used to enhance placebo effects and inhibit nocebo effects in clinical practice, for example by explaining role of expectancy in treatment.

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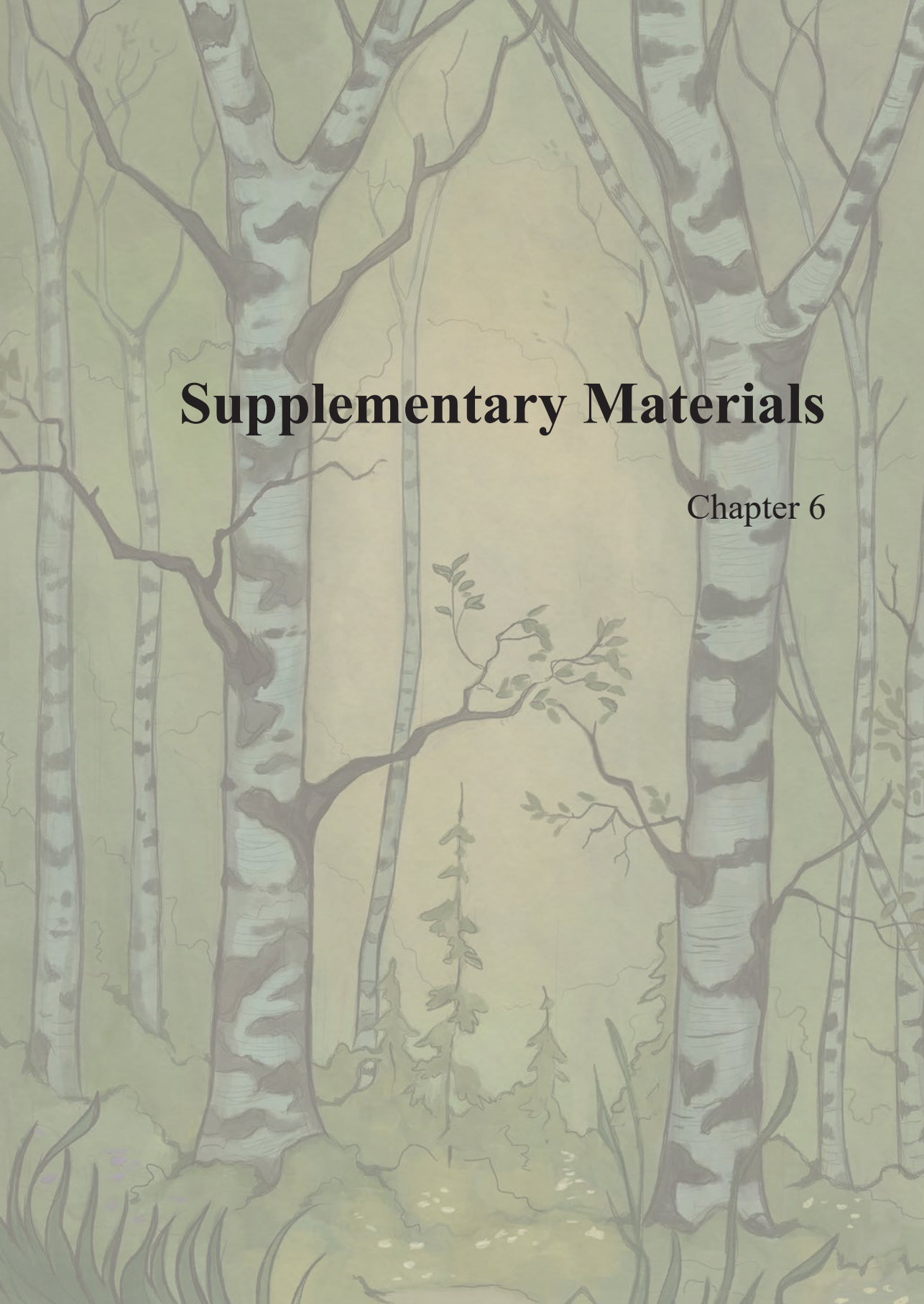


Supplementary Materials

Chapter 6

SUPPLEMENTARY METHODS

Materials and Measures

1. Stroop test and Trail Making Test

As part of the cover story of the patch positively influencing cognition, the Stroop test [1] and Trail Making Test [2,3] were assessed at baseline and following suggestions. Stroop interference scores and percentile scores were calculated controlling for age, sex, and education level. As a large inter-individual variability in the execution of the Trail Making Test was noted (e.g., on noticing and dealing with mistakes during the test – some participants did not correct mistakes whereas others did, thus causing differences on the time spent taking the test and the associated outcome measure), these data were not analysed.

2. Expectations for the cognitive tasks

Prior to the cognitive tasks (both baseline and post-verbal suggestions) participants were asked how well they expected to perform during the tasks by rating the following items on a Numeric Rating Scale (NRS) ranging from 0 ("not good at all") to 10 ("very good"): focus, attention, performance, and speed during the tasks. In addition, expected patch efficacy for focus, attention, and speed was rated following the verbal suggestions using the same NRS.

3. Wellbeing: State Anxiety and General Wellbeing Scales

The State Trait Anxiety Inventory – State Anxiety short scales (STAI-S-s [4]), and seven Numeric Rating Scales (NRS) measuring general wellbeing (relaxed, anxious, serene, agreeable, tense, worried, stressed, see also [5]) on a scale from 0 ('not at all') to 10 ('very much so') were assessed at four moments during the laboratory session: I. pre-baseline iontophoresis (baseline 1), II. post-baseline iontophoresis (baseline 2), III. post-verbal suggestions (post-VS 1), and IV. at the end of the session (post-VS 2). Of the NRS items, negative items were recoded and a total score was calculated by summing all items (with higher scores reflecting higher general wellbeing). Cronbach's alpha for state anxiety ranged from .80 to .83 in the current study. For general wellbeing, Cronbach's alpha ranged .86 to .87.

Statistical Analysis

All analyses were conducted in SPSS 25.0 for Windows (IBM SPSS Inc., Chicago, Illinois, US). As was a priori determined, open-label and closed-label groups were first combined to detect differences between the effects of positive verbal suggestions and negative verbal suggestions with optimal power, and second, repeated for the separate open-label context and the separate closed-label context. Normal distribution of the variables, baseline differences, and assumptions were checked prior to data analysis. General linear model (GLM) analyses of covariance (ANCOVAs) were used to assess group differences in expectations regarding the cognitive tasks. For the expected efficacy of the patch, GLM analysis of variance (ANOVA) was used. Effects of suggestions on Stroop scores were analysed using GLM ANCOVA, and effects of group on wellbeing scales were assessed by mixed between-within-subject RMA. The critical alpha used was α=.05 for the combined group analyses. For the separate open-label and closed-label context analyses, a Bonferroni correction for multiple comparisons was applied ($\alpha/2$=.025).

SUPPLEMENTARY RESULTS

Results

1. Expectations and expected patch efficacy for the cognitive tasks

At baseline, expected attention and performance during the tasks were significantly higher in the combined open- and closed-label negative VS groups (attention: M=7.00±1.39; performance: M=6.80±1.07) compared to the combined positive VS groups (attention: M=6.33±1.56; performance: M=6.26±1.44); $F_{att.}(1,109)=5.55$, p=.020, $F_{perf.}(1,109)=5.09$, p=.026; see **Supplementary Table E3**. Marginal differences were found for expected focus (p=.052) and expected speed (p=.073), respectively. When open-label and closed-label contexts were separated, no baseline differences could be found (all $p\geq$.061; see also **Supplementary Table E4**).

When open- and closed-label groups were combined, no group differences were found for any of the post-suggestions expectation measures (all p>.055), with the exception of expected patch influence on speed; F(1,109)=4.11, p=.045, Cohen's d=0.39 (see **Supplementary Table E3**). Participants in the combined negative VS groups expected the patch to be more effective for speed during the cognitive tasks (M=3.70±2.38), compared to

participants in the combined positive VS groups (M=2.86±1.94). No group differences were found in the separate open-label or closed-label context (all p≥.091; see **Supplementary Table E4**). Within-group baseline-to-post-VS-change indicated no significant changes in expectations regarding the cognitive tasks following suggestions after applying the Bonferroni correction for multiple comparisons (all p≥.042), with the exception of expected speed in the combined positive VS groups. Within these groups, a significant decrease in expected speed was noted (M_{change}=-0.48, t(54)=2.54, p=.014 (see **Supplementary Table E5**). When open-label and closed-label contexts were separated, no significant changes from baseline to post-VS were noted (all p≥.13).

2. Effects on Stroop Test

The combined positive and the combined negative VS groups did not differ on Stroop interference scores or percentile scores at baseline, or following verbal suggestions (all p≥.10). Similar findings were demonstrated when analyses were repeated for the separate open-label and closed-label contexts (all p≥.044). Within-group baseline-to-post-VS-change indicated Stroop interference and percentile scores improved in both the combined positive and the combined negative groups (all p<.001). When groups were separated, similar reductions in Stroop interference and percentile scores were found for the closed-label negative VS and the open-label positive VS groups (all p≤.020). In the open-label negative VS group, no significant change in interference score was found after applying the Bonferroni correction for multiple comparisons (p=.039), however, the percentile score did reduce significantly (p=.011). Stroop interference and percentile scores did not change in the closed-label positive VS group (both p≥.037).

3. Subjective wellbeing: State anxiety and General Wellbeing Scales

No combined-group x time interactions, or main effects of the combined groups, were found for STAI total score or general wellbeing scales (p≥.22). Both state anxiety and general wellbeing changed significantly over time (p≤.012, see **Supplementary Figure S2A** (state anxiety) and **Supplementary Figure S3A** (general wellbeing)). For state anxiety, the baseline measurement was significantly lower than the second measurement (baseline post-iontophoresis; p=.006). No significant differences over time for the other measurements were found (all p≥.080). General wellbeing at the final measurement point

was significantly higher compared to the second and third measurements (both $p \leq .015$). When analyses were conducted for the separate open-label and closed-label contexts, no effects were found for state anxiety (see **Supplementary Figure S2B**). For general wellbeing, the final measurement was significantly higher compared to the baseline post-VS measurement only for the open-label context (see **Supplementary Figure S3B**).

Concluding note on the effects of open-label and closed-label suggestions on expectations regarding cognition, outcomes of the cognitive tasks and wellbeing

Overall, verbal suggestions did not induce differences in expectations regarding the cognitive tasks and patch efficacy for such tasks, with the exception of expected speed during the tasks. The participants in the negative suggestions groups expected the patch to be more effective for speed during the cognitive tasks compared to the positive VS groups. It should be noted though that on general, both groups scored low on expected efficacy for speed (mean of 3.70 and 2.86, respectively, on a 0-10 scale). When groups were separated for open-label and closed-label contexts, no differences in expectations were found. Notably, expectations for the cognitive tasks generally did not reduce after suggestions, indicating that the positive suggestions about the tasks may not have impacted participants' expectations. However, expectations were assessed at baseline before any test was conducted. It may be possible that participants found the Stroop test, Trail Making Test, and filler tasks (i.e. Sudoku's and other puzzles) more challenging than previously anticipated, which may have impacted the scores given post-suggestions. In general, some improvement on Stroop test scores was demonstrated for all groups, which could be due to training effects. Regarding wellbeing, significant changes over time could be demonstrated, but positive and negative verbal suggestions did not significantly impact either state anxiety or general wellbeing. This is in line with the findings for positive affect, which are described in the paper of the current study.

Supplementary Table E1. Means, standard deviations, and analysis of (co)variance (AN(C)OVA) outcomes for demographic factors and histamine iontophoresis-related measures for the separate open-label and closed-label positive and negative verbal suggestions (VS) groups.

	Open-label context				Closed-label context			
			AN(C)OVA				AN(C)OVA	
	Positive VS (*n*=27)	**Negative VS (*n*=28)**	***p*-value**	**Cohen's d**	**Positive VS (*n*=28)**	**Negative VS (*n*=28)**	***p*-value**	**Cohen's d**
Demographics								
Sex [male: n (%)]	4 (14.81)	5 (17.86)	.76		4 (14.29)	5 (17.86)	.72	
Age	21.67 ± 2.60	22.29 ± 3.10	.43	0.22	22.11 ± 2.39	21.57 ± 2.95	.46	0.20
Baseline histamine iontophoresis								
Mean itch	3.89 ± 1.35	4.01 ± 1.70	.78	0.08	4.06 ± 1.53	3.99 ± 1.78	.87	0.04
Subjective skin response (SS-10) [a]	30.40 ± 13.38	32.53 ± 13.99	.57	0.16	31.43 ± 13.37	27.05 ± 11.31	.19	0.35
Wheal area [cm^2]	9.15 ± 3.71	8.95 ± 3.52	.84	0.06	8.70 ± 3.08	9.60 ± 4.24	.47	0.24
Flare area [cm^2]	42.25 ± 15.69	42.48 ± 12.54	.95	0.02	44.44 ± 15.92	41.87 ± 13.70	.52	0.17
Change in skin temperature [°C] [b, c]	1.02 ± 1.05	1.44 ± 1.00	.13	0.41	1.76 ± 1.15	1.93 ± 0.95	.56	0.16
Post-VS expectations outcomes for itch								
Expected itch	4.17 ± 1.86	5.93 ± 1.51	< .001	1.04	3.84 ± 1.91	5.44 ± 2.66	.013	0.69
Expected patch effectiveness for skin sensitivity	2.70 ± 2.12	3.71 ± 2.75	.13	0.41	4.13 ± 1.89	4.84 ± 2.24	.20	0.34
Post-VS histamine iontophoresis								
Mean itch	3.34 ± 1.66	4.24 ± 1.76	.008	0.47	3.25 ± 1.42	4.19 ± 2.17	.004	0.54
Subjective skin response (SS-10) [a, d]	23.37 ± 11.73	29.07 ± 12.68	.017	0.33	23.82 ± 12.22	26.00 ± 12.80	.010	0.45
Wheal area [cm^2]	8.15 ± 3.27	7.83 ± 3.02	.75	0.06	8.23 ± 3.15	8.01 ± 3.84	.17	0.25
Flare area [cm^2]	41.25 ± 11.84	42.21 ± 12.28	.90	0.02	42.09 ± 14.88	41.22 ± 15.41	.50	0.09
Change in skin temperature [°C] [c, e]	1.19 ± 1.48	1.07 ± 1.09	.23	0.30	1.21 ± 1.12	1.34 ± 1.10	.92	0.02

Note. [a] Misery and colleagues (6), [b] n=1 missing due to technical difficulties with the infrared thermometer, [c] calculated as post-iontophoresis temperature – pre-iontophoresis temperature. [d] n=1 missing for the Sensitive Scale-10 (SS-10) in the closed-label negative VS group, [e] n=2 missing due to technical difficulties with the infrared thermometer. Cohen's d for ANCOVA was calculated using the covariate-adjusted means (not depicted in the table).

Supplementary Table E2. Within-group baseline-to-post-verbal suggestions (VS) changes on histamine iontophoresis outcomes for the separate open-label and closed-label positive and negative verbal suggestions (VS) groups.

	Open-label context								**Closed-label context**							
	Positive VS group				**Negative VS group**				**Positive VS group**				**Negative VS group**			
	n	Mean change	*t*	*p*	*n*	Mean change	*t*	*p*	*n*	Mean change	*t*	*p*	*n*	Mean change	*t*	*p*
Mean itch	27	-0.55	3.11	.004	28	0.24	-1.02	.32	28	-0.81	3.87	.001	28	0.20	-0.74	.46
Subjective skin response (SS-10) [a]	27	-7.03	5.38	< .001	28	-3.46	2.74	.011	28	-7.60	4.54	< .001	27	-1.09	0.81	.42
Wheal area [cm^2]	27	-1.00	1.73	.095	28	-1.12	2.82	.009	28	-0.47	0.91	.37	28	-1.12	3.69	.001
Flare area [cm^2]	27	-0.16	0.09	.92	28	-0.27	0.19	.86	28	-2.34	1.89	.37	28	-0.65	0.42	.68
Change in skin temperature [°C] [b, c]	27	0.17	-0.67	.51	28	-0.37	1.88	.071	27	-0.55	3.14	.004	27	-0.58	3.03	.005

Note. Mean change was calculated as post-verbal suggestions score – baseline score, with negative values indicating a decrease from baseline, and positive scores indicating an increase from baseline. [a] Misery and colleagues (6), [b] n=2 missing due to technical difficulties with the infrared thermometer. [c] calculated as post-iontophoresis temperature – pre-iontophoresis temperature.

Supplementary Table E3. Means, standard deviations, and analysis of (co)variance (AN(C)OVA) outcomes for expectations and outcomes of the cognitive tests in the combined open- and closed-label positive and negative verbal suggestions (VS) groups.

	Combined open- and closed-label contexts			
			AN(C)OVA	
	Positive VS (*n*=55)	**Negative VS (*n*=56)**	***p*-value**	**Cohen's d**
Baseline expectation outcomes for cognitive tasks				
Expected focus	6.47 ± 1.48	6.97 ± 1.17	.052	0.38
Expected attention	6.33 ± 1.56	7.00 ± 1.39	.020	0.45
Expected performance	6.26 ± 1.44	6.80 ± 1.07	.026	0.43
Expected speed	5.73 ± 1.71	6.25 ± 1.29	.073	0.34
Baseline Stroop test				
Interference score	56.67 ± 7.80	55.38 ± 8.45	.40	0.16
Percentile score	69.91 ± 20.87	65.41 ± 22.26	.28	0.21
Post-VS expectation outcomes for cognitive tasks				
Expected focus	6.33 ± 1.40	6.99 ± 1.41	.10	0.27
Expected attention	6.42 ± 1.60	7.11 ± 1.33	.21	0.20
Expected performance	5.93 ± 1.64	6.57 ± 1.37	.29	0.16
Expected speed	5.24 ± 2.00	6.13 ± 1.43	.055	0.28
Expected patch influence on focus	3.75 ± 2.19	3.81 ± 2.29	.88	0.03
Expected patch influence on attention	3.70 ± 2.28	3.78 ± 2.10	.84	0.04
Expected patch influence on speed	2.86 ± 1.94	3.70 ± 2.38	.045	0.39
Post-VS Stroop test				
Interference score	61.93 ± 9.54	58.86 ± 7.86	.10	0.27
Percentile score	80.87 ± 22.59	75.82 ± 19.00	.43	0.13

Note. Cohen's d for ANCOVA was calculated using the covariate-adjusted means (not depicted in the table).

Supplementary Table E4. Means, standard deviations, and analysis of (co)variance (AN(C)OVA) outcomes for expectations and outcomes of the cognitive tests in the separate open-label and closed-label positive and negative verbal suggestions (VS) groups.

	Open-label context				Closed-label context			
			AN(C)OVA				AN(C)OVA	
	Positive VS (*n*=27)	**Negative VS (*n*=28)**	***p*-value**	**Cohen's d**	**Positive VS (*n*=28)**	**Negative VS (*n*=28)**	***p*-value**	**Cohen's d**
Baseline expectation outcomes for cognitive tasks								
Expected focus	6.26 ± 1.60	6.82 ± 1.33	.17	0.38	6.68 ± 1.34	7.12 ± 0.97	.16	0.38
Expected attention	6.14 ± 1.59	6.75 ± 1.51	.15	0.39	6.53 ± 1.54	7.24 ± 1.25	.061	0.51
Expected performance	6.16 ± 1.45	6.76 ± 1.06	.084	0.47	6.35 ± 1.45	6.83 ± 1.09	.16	0.37
Expected speed	5.71 ± 1.58	6.03 ± 1.41	.44	0.21	5.74 ± 1.86	6.46 ± 1.14	.084	0.47
Baseline Stroop test								
Interference score	57.67 ± 7.32	55.82 ± 7.67	.37	0.25	55.71 ± 8.25	54.93 ± 9.29	.74	0.09
Percentile score	73.48 ± 18.96	67.00 ± 19.97	.22	0.33	66.46 ± 22.36	63.82 ± 24.60	.68	0.11
Post-VS expectation outcomes for cognitive tasks								
Expected focus	6.09 ± 1.53	6.87 ± 1.45	.16	0.35	6.57 ± 1.25	7.11 ± 1.37	.41	0.18
Expected attention	6.34 ± 1.80	7.05 ± 1.30	.34	0.21	6.50 ± 1.40	7.16 ± 1.39	.45	0.17
Expected performance	5.91 ± 1.79	6.68 ± 1.20	.35	0.21	5.94 ± 1.52	6.46 ± 1.55	.61	0.11
Expected speed	5.29 ± 2.22	6.12 ± 1.25	.13	0.32	5.20 ± 1.80	6.14 ± 1.62	.27	0.23
Expected patch influence on focus	3.00 ± 2.22	3.19 ± 2.48	.77	0.08	4.47 ± 1.93	4.44 ± 1.92	.95	0.02
Expected patch influence on attention	2.86 ± 2.11	3.20 ± 2.21	.56	0.16	4.51 ± 2.17	4.36 ± 1.84	.79	0.08
Expected patch influence on speed	2.18 ± 1.79	2.98 ± 2.53	.18	0.37	3.52 ± 1.89	4.42 ± 2.02	.091	0.46
Post-VS Stroop test								
Interference score	64.52 ± 9.24	59.18 ± 8.31	.044	0.48	59.43 ± 9.31	58.54 ± 7.53	.80	0.06
Percentile score	86.07 ± 20.17	76.68 ± 18.86	.21	0.28	75.86 ± 24.00	74.96 ± 19.46	.94	0.02

Note. The critical alpha used following Bonferroni correction for multiple comparisons was $\alpha = (.05/2) = .025$. Cohen's d for ANCOVA was calculated using the covariate-adjusted means (not depicted in the table).

Supplementary Table E5. Within-group baseline-to-post-verbal suggestions (VS) changes for expectations and outcomes of the cognitive tests in the combined open- and closed-label positive and negative verbal suggestions (VS) groups.

	Combined open- and closed- label positive VS groups (*n*=55)				**Combined open- and closed-label negative VS groups (*n*=56)**			
	n	Mean change	*t*	*p*	*n*	Mean change	*t*	*p*
Expectation outcomes for cognitive tasks								
Expected focus	55	-0.14	0.78	.44	56	0.03	-0.15	.89
Expected attention	55	0.09	-0.45	.65	56	0.11	-0.70	.49
Expected performance	55	-0.33	2.09	.042	56	-0.23	1.32	.19
Expected speed	55	-0.48	2.54	.014	56	-0.12	0.68	.50
Stroop test								
Interference score	55	5.26	-4.70	<.001	56	3.48	-3.30	.002
Percentile score	55	10.96	3.95	< .001	56	10.41	-4.01	< .001

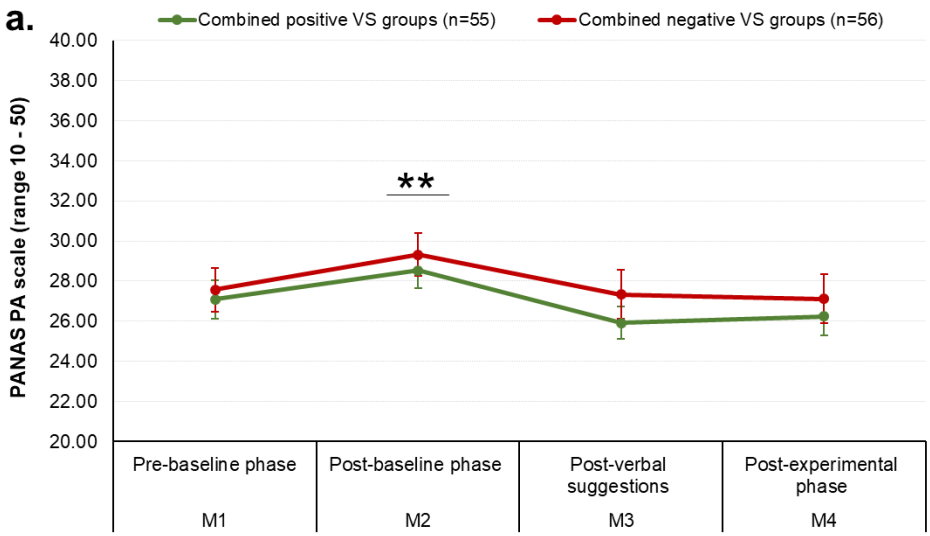
Note. Mean change was calculated as post-verbal suggestions score – baseline score, with negative values indicating a decrease from baseline, and positive scores indicating an increase from baseline.

Supplementary Table E6. Within-group baseline-to-post-verbal suggestions (VS) changes for expectations and outcomes of the cognitive tests in the separate open-label and closed-label positive and negative verbal suggestions (VS) groups.

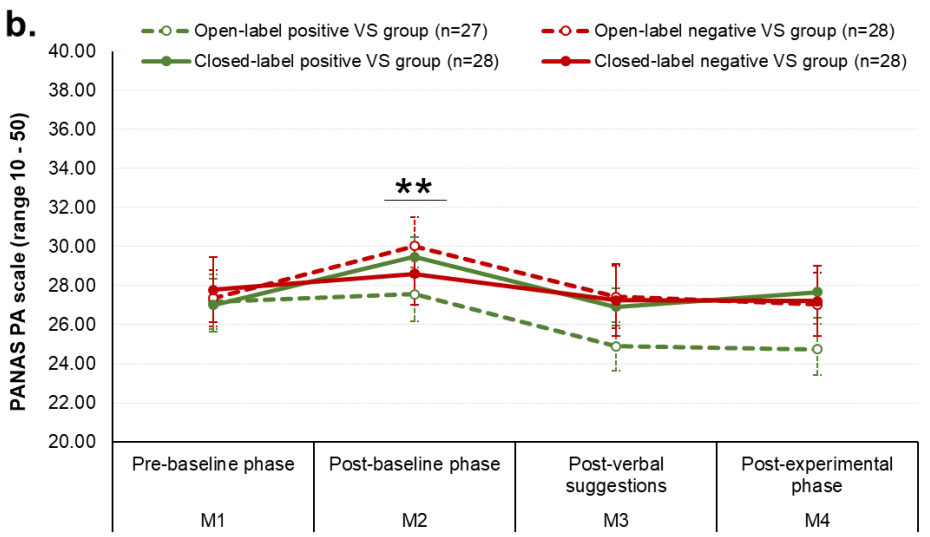
	Open-label context								**Closed-label context**							
	Positive VS group				**Negative VS group**				**Positive VS group**				**Negative VS group**			
	n	Mean change	*t*	*p*	*n*	Mean change	*t*	*p*	*n*	Mean change	*t*	*p*	*n*	Mean change	*t*	*p*
Expectation outcomes for cognitive tasks																
Expected focus	27	-0.17	0.57	.58	28	0.05	-0.20	.85	28	-0.11	0.54	.59	28	-0.003	0.02	.99
Expected attention	27	0.21	-0.73	.47	28	0.30	-1.20	.24	28	-0.03	0.09	.93	28	-0.08	0.42	.68
Expected performance	27	-0.25	1.14	.26	28	-0.09	0.35	.73	28	-0.41	1.76	.089	28	-0.37	1.55	.13
Expected speed	27	-0.43	1.62	.12	28	0.09	-0.31	.76	28	-0.54	1.93	.064	28	-0.33	1.49	.15
Stroop test																
Interference score	27	6.85	-4.82	< .001	28	3.36	-2.17	.039	28	3.71	-2.20	.037	28	3.61	-2.47	.020
Percentile score	27	12.59	-4.02	< .001	28	9.68	-2.75	.011	28	9.39	-2.05	.050	28	11.14	-2.88	.008

Note. Mean change was calculated as post-verbal suggestions score – baseline score, with negative values indicating a decrease from baseline, and positive scores indicating an increase from baseline. The critical alpha used following Bonferroni correction for multiple comparisons was $\alpha = (.05/2)/2 = .0125$.

Supplementary Figure S1. Means + SEMs of the Positive and Negative Affect Schedule (PANAS) subscale 'positive affect' and mixed between-within repeated measures ANOVA (RMA) outcomes for (A) the combined open- and closed label positive VS groups and the combined negative VS groups, and (B) the separate groups.



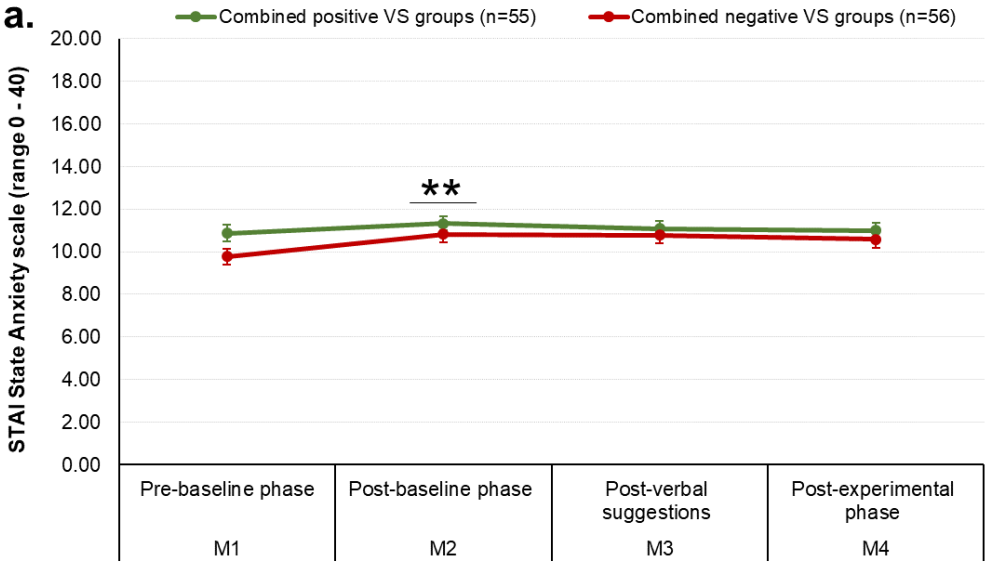
Combined-groups Mixed RMA: group x time n.s.; group n.s.; time $p<.001$.
** Bonferroni post-hoc effect of time: M2 > M1+M3+M4 (all $p<.002$)



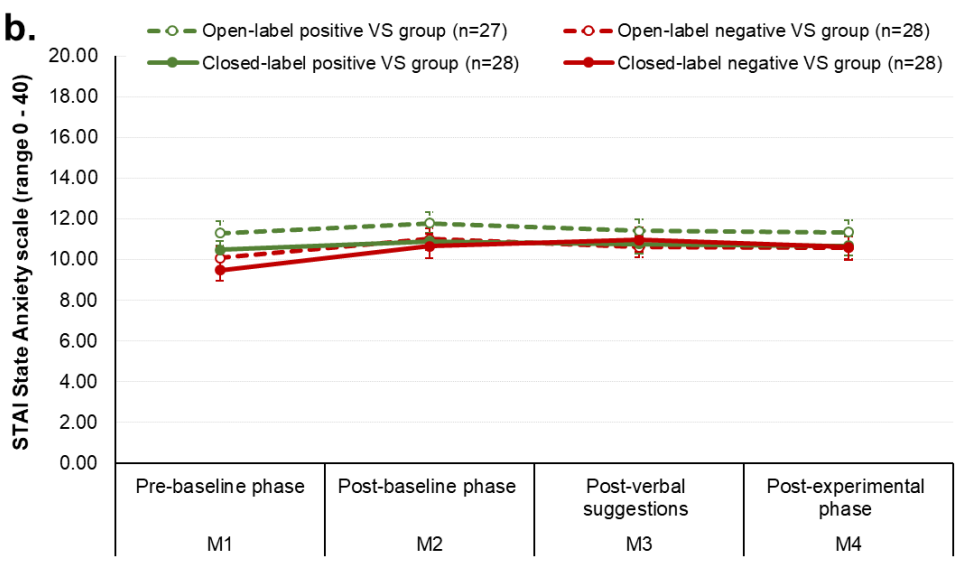
Open-label mixed RMA: group x time n.s.; group n.s.; time $p<.001$.
** Bonferroni post-hoc effect of time: M2 > M3+M4 (all $p<.001$)

Closed-label mixed RMA: group x time n.s.; group n.s.; time $p=.001$.
** Bonferroni post-hoc effect of time: M2 > M1+M3 (all $p<.017$)

Supplementary Figure S2. Means + SEMs of the State and Trait Anxiety Inventory (STAI) subscale 'state anxiety' and mixed between-within repeated measures ANOVA (RMA) outcomes for (A) the combined open- and closed label positive VS groups and the combined negative VS groups, and (B) the separate groups.



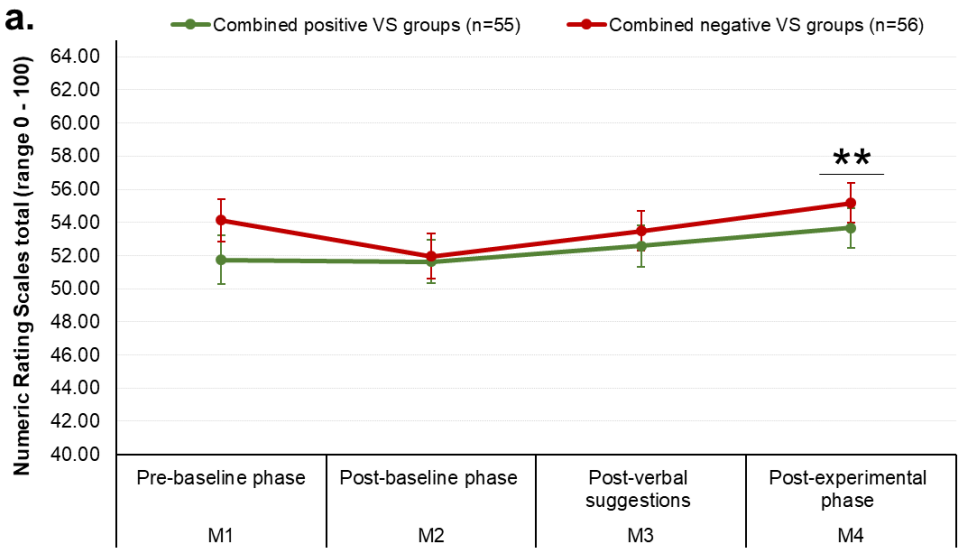
Combined-groups Mixed RMA: group x time n.s.; group n.s.; time *p*=.012
** Bonferroni post-hoc effect of time: M2 > M1 (*p*=.006)



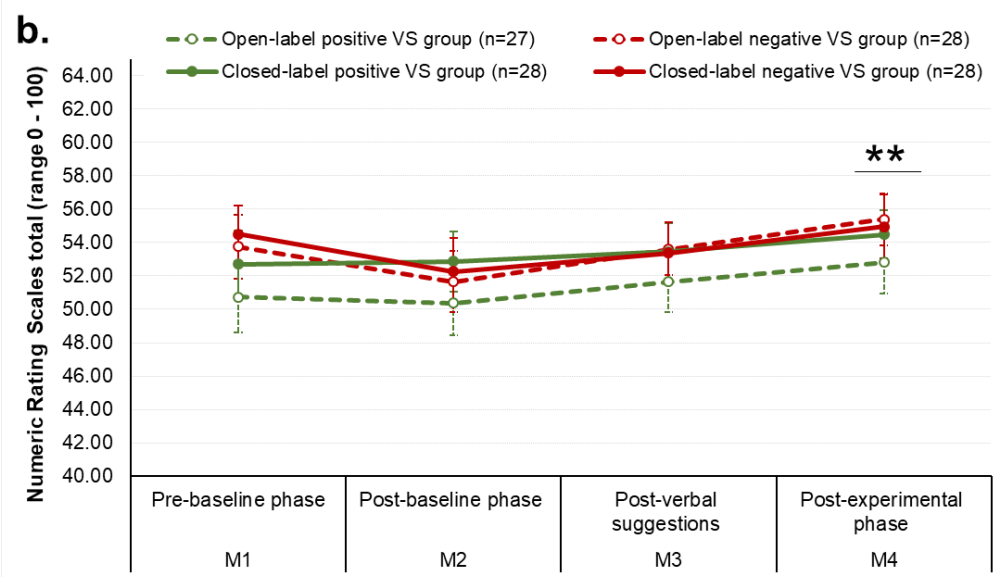
Open-label mixed RMA: n.s.
** Bonferroni post-hoc effect of time: n.s.

Closed-label mixed RMA: n.s.
** Bonferroni post-hoc effect of time: n.s.

Supplementary Figure S3. Means + SEMs of the Numeric Rating Scales (NRS) total score for 'general wellbeing' and mixed between-within repeated measures ANOVA (RMA) outcomes for (A) the combined open- and closed label positive VS groups and the combined negative VS groups, and (B) the separate groups.



Combined-groups Mixed RMA: group x time n.s.; group n.s.; time p=.001
** Bonferroni post-hoc effect of time: M2+M3 < M4 (p<.015)



Open-label mixed RMA: group x time n.s.; group n.s.; time p=.010
** Bonferroni post-hoc effect of time: M2 < M4 (p=.012)

Closed-label mixed RMA: n.s.
** Bonferroni post-hoc effect of time: n.s.

SUPPLEMENTARY REFERENCES

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Chapter 7

Summary and General Discussion

SUMMARY

The aim of this dissertation was to investigate the induction of placebo and nocebo effects for histaminergic itch based on multiple approaches of associative and instructional learning. Pharmacological conditioning and positive and negative verbal suggestions were used to elicit effects in both open-label (i.e., with participants knowing about the placebo or nocebo effect induction) and closed-label (i.e., concealed, or with participants not knowing about the placebo or nocebo effect induction) contexts. Moreover, effects of these approaches on other (psycho)physiological responses to histamine were addressed.

With regard to the dissertation aim, **Chapter 2** examined the existing literature on experimentally elicited placebo and nocebo effects in itch, and itch-related medical conditions and symptoms of the dermis and mucous membranes, as well as in related animal and human models. The systematic literature review covers the methods used to elicit these effects, as well as the general study findings. Broadly, placebo and nocebo effects have been elicited by three techniques, or combinations thereof: verbal suggestions (with or without hypnosis), (classical or operant) conditioning, and social learning (e.g., induction of contagious itch). Overall, these methods were successful in eliciting placebo and nocebo effects for itch and itch-related symptoms within dermatology. However, the review also shows that studies are largely heterogeneous, and that the elicited placebo and nocebo effects are oftentimes conditional: for example, conditioned placebo and nocebo effects are subject to changes in the context in which effects are learned, and verbal suggestions seem to elicit effects only on the short term. A large variety of procedures (i.e., no standard 'conditioning protocol', or standard suggestions) for placebo and nocebo effects induction was found, regardless of which type of technique was used, and effects were investigated in very diverse patient populations, as well as in different animal and human models.

In **Chapter 3,** the results of a randomized controlled study on the classical (pharmacological) conditioning of the antipruritic effects of H1-antihistamines were reported. Pharmacological conditioning is one of the mechanisms by which placebo effects can be induced. Two previous studies have investigated conditioning of antihistamines in allergic patients, but were unable to distinguish between conditioned and other expectancy effects on self-reported allergic symptoms. The study described in this chapter aimed to fill this knowledge gap by investigating conditioned effects for histamine-evoked itch and other histamine-related parameters in healthy volunteers. Although conditioning resulted in

marginal lower itch compared with control, no differences between separate groups were found, nor did conditioning influence other parameters in the study under either open-label or closed-label conditions. Overall, the study provides limited evidence for the antipruritic effects of conditioning with H1-antihistamines.

In **Chapters 4, 5, and 6**, three studies were described in which the effects of verbal suggestions on itch and other (psycho)physiological responses to histamine were examined. In **Chapter 4**, the effects of open-label positive verbal suggestions about low itch were compared with neutral instructions. While no differences between groups were found, expected and experienced itch were significantly related following verbal suggestions exclusively. Moreover, a trend was observed for self-assessed skin condition, with open-label positive suggestions resulting in marginal lower self-assessed skin condition severity compared with neutral instructions. As a whole, these results illustrate a potential role for open-label placebo effects in itch (as evidenced by the association between expected and experienced itch following positive suggestions).

In **Chapter 5**, the effects of open-label and closed-label positive and negative verbal suggestions about the itch-reducing (or –increasing, depending on group allocation) properties of an (inert) tonic on itch were compared. No effects on itch during histamine iontophoresis were found, but itch during a short follow-up period was lower in the positive compared with the negative verbal suggestions groups, both in open-label and closed-label contexts. Further examination of the data indicated that in the positive suggestion groups, itch reduced significantly, whereas in the negative suggestion groups, no changes were found. These results indicate that placebo and nocebo effects may be elicited for itch by verbal suggestions in both open-label and closed-label contexts, though future research on these effects is warranted.

In **Chapter 6,** effects of open-label and closed-label positive and negative verbal suggestions were again compared for itch, with the suggestions being that itch would be influenced as a side effect of a (sham) transdermal caffeine patch. In short, verbal suggestions resulted in significant changes in the amount of itch that was experienced for both open-label and closed-label contexts, thus showing that these effects can be induced when people know about them. As in **Chapter 5**, further examination of baseline-to-post-VS changes shows that itch significantly reduced in the positive VS groups, but did not change following negative suggestions. Taken together, these findings demonstrate

effective placebo and nocebo effect induction for itch under both open-label and closed-label contexts.

Taken together, the performed studies investigated experimental elicitation of placebo and nocebo effects using various methodological approaches. The studies examined the existing literature on this topic (**Chapter 2**) and whether effects could be elicited by pharmacological conditioning (**Chapter 3**) or by verbal suggestions (**Chapter 4-6**). Finally, they examined the potential of eliciting effects with participants' awareness. In the following section, we discuss the results of this dissertation, mention limitations that may be addressed in future research, and discuss several clinical implications and the scientific relevance of the work.

GENERAL DISCUSSION

The systematic review in **Chapter 2** indicated that placebo and nocebo effects have been investigated in itch and itch-related medical conditions and symptoms of the dermis and mucous membranes using a wide range of induction methods in patient samples, and in relevant animal and human (i.e., healthy participants) models. Three main categories of placebo and nocebo effects induction could be identified: associative learning (i.e., conditioning), instructional learning (i.e., verbal suggestions), and social learning (i.e., social cues). Verbal suggestions were used to investigate placebo and nocebo effects in human trials with study groups of patients, healthy participants, or both. From the systematic literature review, we concluded that there is evidence for the efficacy of verbal suggestions for eliciting both placebo effects and nocebo effects, however, the methods often differ between studies, and effects of suggestions on physiological outcomes are by and large lacking. Secondly, animal and human studies (in healthy participants and patients) showed both placebo (e.g., immunosuppression) and nocebo (e.g., exacerbation of allergic responses, or scratching behavior) effects on physiological and behavioral parameters through classical conditioning. For self-reported outcomes such as allergic symptoms and self-rated itch in human trials, conditioning of negative (nocebo) effects could be demonstrated. However, conditioning of positive (placebo) responses appeared more complicated. One explanation for such a phenomenon may be that learning of negative associations could be more potent and therefore needs less acquisition trials than the learning of positive associations. From an evolutionary perspective, this explanation

would be sensible, considering that rapid learning of and responding to negative stimuli (i.e., threats) might be directly linked to an individual's and species' survival [1-4]. Finally, itch may also be prone to be influenced by social factors, as evidenced by successful induction of contagious itch and the impact that advertisements for different brands of antihistamines were demonstrated to have on reporting of allergic symptoms during antihistamine treatment.

Overall, the existing literature demonstrates ample evidence for placebo and nocebo effects in itch and itch-related conditions and symptoms. However, the body of evidence currently available is also characterized by a large heterogeneity in both methodology and chosen outcome parameters – which makes it challenging to extend findings across dermatological conditions. The current dissertation builds on these previous findings and investigates placebo and nocebo effects for histamine-induced itch in healthy volunteers using conditioning and verbal suggestions. Previous studies used pharmacological conditioning to elicit placebo effects to enhance clinical outcomes in patients diagnosed with psoriasis [5] or allergic rhinitis [6,7]. However, these studies have investigated the efficacy of conditioning for a multitude of symptoms, including itch. This may complicate an exact interpretation of study findings, since symptoms could be susceptible to changes caused by multiple factors that are unrelated to the study aim (e.g., regression to the mean, spontaneous recovery). Moreover, symptoms may be elicited through various pathways (e.g., both histaminergic and non-histaminergic itch pathways). It may then be challenging to ascribe symptom change to a single isolated mechanism such as conditioning with, for example, antihistamines. The effects of pharmacological conditioning of antihistamines have not yet been tested in experimental models that exclusively induce histaminergic itch in healthy volunteers.

Previous work with healthy volunteers also shows that itch may be reduced by providing positive verbal suggestions [8], and that negative verbal suggestions could increase itch [9-11], but itch induction methods differ between studies and it may be challenging to translate study findings to clinical practice. This dissertation extends the previous findings by investigating the efficacy of conditioning and verbal suggestions for itch under open-label conditions (i.e. non-concealed). Potentially, eliciting placebo effects while patients are aware of this may lead to new therapeutic possibilities aimed at maximizing treatment efficacy and minimizing adverse events.

Experimental induction of placebo and nocebo effects for itch

Associative learning: antipruritic conditioning of H1-antihistamines

Previous work shows that allergic responses can be exacerbated by conditioning in patients [12-14], and that immunosuppressive properties of medications may be sensitive to conditioning effects as well. Studies find that the effects of general immunosuppressive agents – for instance, of cyclosporine-A (CsA) – can be mimicked using conditioning mechanisms in humans: when only a conditioned stimulus (CS) is presented, similar effects are found compared with previous exposures, where the CS was presented together with CsA as unconditioned stimulus (UCS) [3,15,16]. For example, conditioning with CsA has been found to result in reduced levels of interleukin-2 and, in some studies, also reductions of IFN-γ [3,17,18]. Considering that exacerbation of allergic responses can also be conditioned, and considering the potential of conditioned (general) immunosuppression, it stood to reason that a reduction of allergic symptoms may also be conditioned. Two studies investigated this hypothesis by classically conditioning the effects of antihistamines in allergic patients, and reported mixed results: although a unique physiological conditioned response (i.e. reduced basophil activation) was found in one study [6], no distinctive effects for self-reported allergic symptoms and physical skin responses were identified [6,7]. It should be noted that one study showed these subjective outcomes reduced over time in both the conditioned and sham-conditioned groups, compared with a natural history group – thus implying that other factors, for example conscious expectancy, could have impacted outcomes [7]. For example, natural fluctuations in allergic symptoms may have been interpreted as medication effects and may thus have potentially interfered with the study protocol.

The study reported in **Chapter 3** builds on the findings of these two studies and investigated whether conditioning with H1-antihistamines could influence itch that was experimentally elicited by histamine and other (psycho)physiological parameters in healthy volunteers. In addition, the study investigated the efficacy of conditioning when participants were aware of the conditioning procedures (open-label). A conditioning protocol was applied with three acquisition moments and three evocation moments. Effects of conditioning on psychological and physiological parameters were examined, as were effects of conditioning during a short term histamine challenge, in which itch was experimentally elicited on the skin of the forearm during a short period of time. Limited evidence for conditioning of H1-antihistamines in reducing histamine-induced itch was

found, while no effects of conditioning were found for any of the other parameters in the study. Potentially, conditioned responses may have been small, as the sample consisted of healthy participants who did not experience allergic symptoms prior to enrolment in the study – this may have led to a situation in which the unconditioned response (effects of levocetirizine) may not have been easily noticeable. Consequently, learned responses would also be small, or associations between the CS and UCS may not have readily formed (as previously discussed in **Chapter 3**). This would be in line with theoretical models that place expectancy at the center of placebo effects as they state that, in order to learn, awareness (of both causes and effects) is needed [19]. There is some evidence that challenges such models, however, as conditioning has been found to result in hyperalgesia and analgesia when the CS was presented on a subliminal (i.e. subconscious) level [19,20]. This would imply that it may hypothetically be possible to unconsciously condition endogenous responses through pharmacological means as well. This notion is supported by the marginal reduction in itch that was found in the conditioned groups of the study in **Chapter 3**. It should be noted though that this reduction in itch was not significant – for clinically relevant effects, awareness may be needed regardless. Alternatively, it may be possible that immunosuppressive conditioning needs more acquisition trials for stronger effects compared to the conditioning of negative events (e.g., allergic responses, other enhanced immune reactions), as immunosuppression may be less sensitive to conditioning [21]. From an evolutionary perspective, rapid learning of negative associations helps in the survival of organisms whereas positive associations may be less relevant and thus less salient for behavioral conditioning [1-4]. Moreover, measures of itch were taken on the third evocation day. It may be possible that (partial) extinction of the conditioned response had already taken place at that moment. For example, this has been shown in a study on conditioned endocrine responses, that used a similar design [22]. Future research could investigate whether antipruritic conditioned effects of antihistamines may be stronger at earlier evocation moments, or investigate what factors could help strengthen placebo effects elicited by antipruritic conditioning of antihistamines (e.g., a longer acquisition phase, itch induction during acquisition to boost learning).

Instructional learning: verbal suggestions about itch and itch-related treatments

Instructional learning, for example by verbal suggestions, may also be a potential mechanism by which placebo (and nocebo) effects could be elicited. As described in

Chapter 2, verbal suggestions can influence levels of itch, but some uncertainty exists about under which circumstances verbal suggestions may induce placebo or nocebo effects. In most experimental studies, the verbal suggestions are modelled after a situation in the clinic. Broadly, three different categories of information modelling can be discerned (see also **Table 1**): I. information about symptoms elicited by a test (**Chapter 4** – as these suggestions are open-label exclusively, this category will be discussed in the following section), II. information about the intentional effects of a treatment method (**Chapter 5**), or III. information about the unintentional effects of a treatment method (e.g., side effects; **Chapter 6**).

In **Chapters 5 and 6**, effects of concealed positive and negative verbal suggestions on itch elicited by a short-term histamine challenge were examined using different categories of information modelling. In **Chapter 5**, participants were told that the effects of a tonic on sensitivity of the skin to itch would be examined. Depending on group allocation, participants were then told that itch would either increase or decrease following the application of a (sham) tonic, making the proposed effects on itch a direct consequence of the intended treatment (Table 1: 'model 2'). In **Chapter 6**, participants were told that the study investigated effects of a transdermal caffeine patch on cognitive functioning, and that *as a side effect*, this would impact sensitivity to somatic symptoms such as itch. As such, proposed positive or negative effects on itch were introduced as an inadvertent consequence of a treatment rather than the intended effects (Table 1: 'model 3'). Overall, both types of suggestions were found to impact itch either during histamine application, or in a short follow-up period after the test. The two ways in which information was provided mirror those often used in consults with patients, where health care providers explain effects of a treatment as well as potential side effects that may be expected.

For itch specifically, there are relatively few studies that have investigated effects of positive verbal suggestions about a treatment on itch. A single study showed that suggestions about a cream were able to elicit placebo effects for itch [8]. The study described in **Chapter 5** is in line with this work and extends these previous findings by showing that verbal information about a different type of topical treatment (i.e., a 'tonic') can also influence itch in a short follow-up period to histamine iontophoresis. It should be noted though that itch during iontophoresis was not significantly influenced by positive and negative verbal suggestions. Potentially, the suggestions may not have been convincing enough to significantly influence itch during the test (e.g., participants were told that the tonic would influence itch, but were not involved about why it would work, or what active

component in the tonic would cause this). Nonetheless, the results of this study as a whole highlight a potential role of verbal suggestions in eliciting placebo and nocebo effects for itch.

In **Chapter 6**, placebo and nocebo effects were elicited by providing information about itch as a side effect of a transdermal caffeine patch. Relatively few studies investigate whether nocebo effects can be elicited by providing side effect information in experimental settings. However, it has been demonstrated that the manner in which side effect information is framed can impact the frequency and severity of several drug-related side effects (see, for example, [34-38]). Although information framing has not been formally investigated for itch yet, the study findings described in **Chapter 6** appear consistent with this line of research. For instance, it is shown that directional (i.e., positive or negative) information about itch as a side effect can directly impact the intensity of itch experienced by participants. Noticeably, significantly reduced itch was found following positive suggestions in **Chapter 6,** but itch did not increase following negative suggestions. This may be explained by the specific study procedures however (i.e., repeated itch provocations may result in lower itch by itself). Overall, the findings in **Chapter 6** show that information about itch as a side effect may impact itch experience.

Taken together, the studies described in **Chapter 5 and 6** demonstrate that providing positive and negative verbal information can influence the experience of itch in experimental settings. This emphasizes that the type and manner in which information is provided could potentially be used to maximize treatment efficacy, by enhancing positive expectations about treatments and eliciting placebo effects. It furthermore shows that it is important to carefully consider the manner in which negative information should be provided in the clinic. Finally, the findings demonstrate that healthcare providers may be able to actively contribute to treatment efficacy by the manner in which they communicate about treatment. However, future research is needed in order to more precisely estimate placebo and nocebo effect sizes, and to investigate whether variations in instructions may impact these effect sizes.

Table 1. Categories of information modelling

Model	Category of information modelling	Situation in the clinic (example)	Experimental placebo or nocebo model (example)	Previous work (example)	Dissertation chapter
1	Information about symptoms that are elicited by a test	Explaining a medical procedure, for example, skin prick tests for allergy, or explaining discomfort caused by blood sampling	Verbal suggestions about a sham device or itch induction method (e.g., "this [device/method] will elicit itch for most people")	*Closed-label* [9-11, 23-27]	Chapter 4 (*open-label exclusively*)
2	Information about the intentional effects of a treatment method	Consults with patients in which information is given about (new) treatments (i.e., what effects to expect from the treatment)	Verbal suggestions about an inert substance, such as a cream or pill, and its effects on experimental elicitation of itch (e.g., "this cream will reduce itch")	*Open-label* [28, 29] *Closed-label* [8, 30-33]	Chapter 5 (*both open- and closed-label*)
3	Information about the unintentional effects of a treatment method [a]	Consults with patients in which information is given about *side effects* that can be expected with a certain treatment, leaflet information	Verbal suggestions about side effects of an inert treatment method (e.g., "this medication can elicit side effects such as headaches")		Chapter 6 (*both open- and closed-label*)

Note: [a] To our knowledge, no previous studies have investigated experimental elicitation of placebo and nocebo effects by verbal suggestions about side effects. However, previous work on information framing and nocebo effects (e.g., [34-38]) suggests that the manner in which information is provided can influence the severity of experienced side effects.

Open-label placebo and nocebo effects

There has been a lot of debate on how to ethically use the knowledge of placebo and nocebo effects in clinical practice [39-42]. Central to this debate is the concept of deception: the notion that the deceptive nature of experimental placebo and nocebo effects induction would complicate direct application of this knowledge in clinical practice, as patients need to be fully informed about treatments for ethical medical treatment [39-42]. Over the years, various solutions to this conundrum have been proposed, including having patients provide consent for informed deception during treatment (e.g., so conditioning mechanisms can be used to enhance placebo effects), or providing patients with minimal information about side effects during consults, and offering them the option to look up information elsewhere (to minimize nocebo effects) [43]. Another promising angle of approach is through eliciting placebo effects without deception [39,44].

Studies have found that open-label placebo effects can be elicited for symptoms of various conditions, including irritable bowel syndrome, allergic rhinitis, chronic low back pain, ADHD, and depression [28,29,45-55]. Central to most of these studies is the combination of giving inert pills and providing a rationale with four key arguments: 1) that placebo effects may be powerful, 2) that these effects may be learned through Pavlovian conditioning, 3) that positive attitudes are not necessary, but may be helpful to induce effects, and 4) that pills need to be taken faithfully (i.e., adherence) [45]. Overall, the studies show promising effects, but with regard to the type of open-label placebo effect induction, little is still known about the underlying mechanisms. A single study has teased apart the effects of the open-label placebo rationale and the inert pills, and found that the inert pills seemed to elicit effects, whereas the added rationale did not significantly contribute to placebo effects [28]. This would imply that effects may be mostly due to previous associations between the medical ritual of ingesting a pill and reduction of symptoms. Another study has shown that only groups that receive a rationale appear to benefit on subjective symptoms, at least when a cream is used as inert substance [52]. These discrepancies may be influenced by previously learned associations between application routes and efficacy for certain types of symptoms [57]. Relatively little is known about how variations in instructions and instruments (i.e., pills, creams, or other medication types) may impact open-label placebo effects, and effects have rarely been investigated for itch or itch-related conditions [28,29]. In the current dissertation, open-label placebo and nocebo effects were induced using various methods, which will be discussed below.

Associative learning: antipruritic conditioning under open-label conditions

The study described in **Chapter 3** aimed to investigate whether pharmacological conditioning of antihistamines could also be effective when participants knew about the conditioning procedure. While in general, participants in the open-label arm of this trial expected less itch during the histamine challenge compared with the other groups (who were not told about the conditioning procedure, and who were blinded to whether they received active medication), conditioning was able to only marginally influence itch levels. This complicates interpretations of the impact that the open-label rationale may have had on the efficacy of the conditioning procedure. Previous studies show that open-label pills may be used as a dose-extender. For example, Sandler and colleagues [56] showed that subclinical doses (50% decrease) of extended-release mixed amphetamine salts (MAS-XR) could reduce ADHD symptoms in children to a level comparable with a full dose, when MAS-XR was given together with open-label placebo pills as 'dose extenders'. For the open-label placebo pills, an explanation was given to participants of how they may impact treatment by eliciting placebo effects. While no classical conditioning procedure was used in this study, the information it yields may be used for future studies: making use of subclinical doses could potentially strengthen conditioned responses for itch as well. Finally, Schafer and colleagues [58] investigated whether revealing the conditioning procedure to participants would impact conditioned analgesia. They demonstrated that analgesia persisted, even when it was revealed that participants received a placebo, thus indicating that learned placebo effects can be robust. It should be noted though that these instructions were aimed at revealing deception, whereas the instructions used in **Chapter 3** of this dissertation were aimed at convincing participants of the efficacy of conditioning, with the purpose of strengthening expectancy effects and investigating whether placebo effects may be elicited by conditioning under open-label conditions. Revealing previously used deception may impede conditioning (i.e., conditioned effects were halved following the reveal in Schafer and colleagues' experiment [58]), may have the potential to elicit negative thoughts or emotions, and may perhaps erode trust in health care practice in the long term. When conditioning is transparently and adequately explained prior to starting a treatment in which these mechanisms are utilized, such negative consequences could hypothetically be minimized, although this needs to be confirmed by future research.

To our knowledge, the study described in **Chapter 3** was the first to combine classical conditioning with open-label instructions. Variations in the frequency and what type of open-label instructions about conditioning should be provided naturally need to be further investigated, in order to fully gauge the impact of such instructions on the efficacy of conditioning of antipruritic effects. For example, the open-label instructions in **Chapter 3** were repeated with every administration of the CS and UCS or placebo pill. Future research may examine whether this repetition of instructions is necessary. In addition, the current open-label rationale did not specifically touch upon the biological underpinnings of conditioned placebo effects. The level of detail needed to maximize both comprehensibility of the conditioning mechanisms and positive outcome expectations may be examined in future research as well.

Instructional learning: verbal suggestions about itch under open-label conditions

As described above, few studies on open-label placebo effects have made the distinction between effects of the open-label placebo rationale and the inert pills, and the ones that did show that the effects may depend on the type of instrument (e.g., inert pills or creams) used. For example, in one study placebo effects elicited for allergic symptoms were found to be induced by an inert pill, while an added open-label rationale (i.e., explanation of placebo effects) did not elicit effects [28]. Another study reported contradictory findings, however, with an open-label rationale that did elicit placebo effects for pain and an inert cream that did not [52]. Hypothetically, one would imply that associative learning could underlie effects (i.e., placebo effects elicited by performing ritualistic medicinal practices that are strongly associated with symptom relief, for example, taking pills for pain reduction), whereas the other would imply that the explanation of placebo effects underlies the effects (i.e., cognitively modulating expectancies for treatment by explaining the working mechanisms involved and the to-be-expected effects). There are too few studies conducted in this field – with too little variation in instructions and instruments – to draw any firm conclusion on the underlying mechanisms of open-label placebo effects. Moreover, the medical conditions that are studied in this field vary, and little is known about whether open-label instructions can impact itch. The studies described in the current dissertation aimed to investigate whether information provided in an open-label context, modelled after three types of settings in the clinic (see also **Table 1**), could influence the experience of itch in an experimental setting.

In **Chapter 4**, open-label positive suggestions about an itch induction test were compared with neutral instructions. Participants were told that a histamine challenge would elicit little itch in most healthy people, and were given an explanation about how such suggestions may impact experienced itch. This type of information modelling (i.e., providing information about a method that elicits symptoms) has been previously used to test whether (concealed) nocebo or nocebo-like effects can be elicited (see for example, [11,23,25,59]). The study described in **Chapter 4** is, to our knowledge, the first to examine whether this type of modelling could elicit placebo effects in an open-label context. While no direct effects of open-label positive suggestions were found, strong associations between participants' post-suggestions expectations and experienced itch were observed exclusively when open-label suggestions were given. This indicated that participants reported levels of experienced itch close to those that they expected a priori, after open-label suggestions were given. Potentially, giving this type of information, and pointing out the role of expectations in the experience of symptoms, may be helpful when participants or patients already have positive expectations about a treatment. When expectations that patients have prior to treatment are negative however, providing information about the role of these expectations becomes more problematic, as this might only validate that the treatment will likely not work for them. In these cases, interventions aimed at optimizing expectations or at taking away the causes of negative expectations could be more helpful instead. Future research may aim to investigate whether such an approach may be useful to optimize treatment outcomes for itch.

In **Chapters 5 and 6**, positive and negative suggestions were given under open-label conditions as well as closed-label (concealed) conditions. Effects of suggestions on expectations were stronger for the open-label condition, whereas for experienced itch, effects of suggestions under concealed conditions were larger. This apparent contradiction may be explained by the contents of the open-label rationale. In both studies, expectancy is central in the open-label rationale: participants are clearly told that expecting little (or a lot of) itch will influence the intensity of itch that they experience, also when they know about it. This may have primed them to report more profound levels of expected itch when subsequently questioned about their expectations. Regardless of this priming effect however, the studies in **Chapter 5 and 6** show similar patterns in outcomes under both open-label and closed-label contexts. This implies that placebo and nocebo effects occur regardless of whether or not participants were informed about them, and that explicitly informing participants about these effects is not necessarily disadvantageous to clinical

outcomes. However, some caution is needed in drawing this conclusion, as this infers that providing this type of information – that is, explaining the underlying mechanisms of placebo effects – has little actual impact on the formation of expectations about treatment. In **Chapter 5 and 6,** effects of suggestions on expectations were larger in an open-label context however. This suggests that an alternative explanation may be possible: the open-label rationale may have helped in actively shaping placebo and nocebo effects by influencing expectations in a manner that is distinct from concealed placebo and nocebo induction. Speculatively, this would also be in line with previous studies that found that an open-label rationale may (partially) explain placebo effects independently of a previous placebo induction [52,60].

For nocebo effects it is particularly interesting that similar patterns were found for the open-label and closed-label groups. After all, informing patients about nocebo effects has previously been proposed as a potential approach to limit nocebo effects from occurring in clinical practice [61]. This implies that informing about nocebo effects could theoretically have a protective function. However, previous open-label studies [44] show that it does not appear to matter that participants are informed, at least when eliciting placebo effects. This would imply a facilitative (or neutral) role of informing about these effects, which is in contrast with the goal of informing about nocebo effects to prevent them. Findings of the current dissertation likewise support a facilitative (or neutral) role of explaining nocebo effects: the effects of negative suggestions were similar for both open-label and closed-label (concealed) contexts in both **Chapter 5 and 6**. In future research, careful consideration of the manner in which patients or participants can be informed about nocebo effects is necessary, and it should be examined how variations of open-label explanations of nocebo effects may impact the induction of such effects, for example, by comparing different ways of framing this information. It might be especially relevant to examine how variations in explanations of the nature of placebo and nocebo effects in the open-label rationale may impact their effects. Both the current dissertation and previous literature have used an open-label rationale in which an automatic nature for placebo effects is emphasized. While this may be helpful for placebo effects (i.e., they occur regardless of whether you know about them), this may not be the case for nocebo effects. Hypothetically, an explanation of nocebo effects could emphasize active rather than passive components: instead of having these effects be described as automatic responses, focus could be on what can be done about them (e.g., which strategies can be employed to prevent nocebo effects from forming [62-66]). Future research may investigate such strategies.

On a final note, recent findings highlight that open-label placebo effects may depend on a patient's beliefs about placebo effects [47,51]. As described in **Chapter 5**, participants in the open-label groups of this study rated the likelihood that their own experience of itch was influenced by the instructions as rather low. Moreover, effect sizes reported in the current dissertation were generally smaller than in other open-label studies, though this may be due to the other studies being conducted with patient populations rather than healthy volunteers [44]. Future research may aim to investigate for which (patient) subgroups open-label placebos are most likely to be beneficial.

Placebo and nocebo effects in physiological responses to histamine

In line with most previous research [67], no effects of verbal suggestions on physiological responses to histamine were found in the studies described in **Chapter 4, 5 and 6**, with the exception of skin temperature, which changed following suggestions in the study described in **Chapter 5** (i.e., less increase in skin temperature following positive suggestions compared with negative). These findings were not replicated in the study reported in **Chapter 6**, however. It is of note that previous studies found effects of suggestions under hypnosis on skin temperature [68-70]. Moreover, placebo effects have been found for physiological parameters (including skin temperature) that are usually associated with autonomic nervous system (ANS) functioning [71-73]. Indeed, in **Chapter 3**, the only physiological parameter for which group effects were found was heart rate. Any interpretation needs to be made very carefully however, considering that in previous studies suggestions were often made with the intent of changing these parameters (e.g., [68,70]), whereas for the studies in the current dissertation any effect of suggestions (or conditioning) on physiological parameters was treated as a by-product of a placebo response (i.e., the verbal suggestions did not explicitly mention effects on physiological parameters, although effects on other parameters were implied: "you will respond less to the histamine test"). This type of response generalization has been noted before, for example when suggestions of pain were given and skin temperature increased as a results [69], or when suggestions about exaggerated itch following skin prick tests were given and skin reactions were modulated as a result [10].

Generally, placebo and nocebo effects were found more often for subjective symptoms such as itch in the current dissertation, whereas effects on physiological symptoms were more mixed – especially where it concerned placebo and nocebo effects elicited through means

other than conditioning. This is in line with most previous literature and suggests that learning may be necessary in order to facilitate long-term and physiological effects, whereas for subjective symptoms, verbal suggestions may suffice. It has previously been suggested that placebo effects may be elicited by conditioning for unconscious physiological responses, and by expectancy for conscious psychophysiological responses (i.e., pain or itch) [74]. Distinct mechanisms for these effects elicited by conditioning and suggestions have been proposed, but have not been studied extensively so far [19]. For physiological responses to histamine, no comparisons between conditioning and suggestions have been made within a single study so far, which may be remedied in future research.

Limitations

The current dissertation provides novel evidence about placebo and nocebo effect induction for itch and other (psycho)physiological responses to histamine. However, several limitations of the research should be discussed. First, no optimal conditioning protocol could be identified in previous literature (see **Chapter 2**) because the study protocols showed large heterogeneity (related to the specific physiological mechanisms of the used stimuli). This complicated the study design for the study described in **Chapter 3**. It was opted that three acquisition sessions would be sufficient for conditioning to take place. Although stronger learning may occur with an increase of learning trials, gustatory learning is thought to be strongly linked to evolutionary processes and may therefore occur after a single exposure [75]. The decision to measure itch at the end of a three-days evocation phase means that (some) extinction of learned responses could have already occurred. Including histamine tests at earlier time points could potentially have interfered with conditioning effects for other parameters however.

Healthy volunteers were examined in all studies of this dissertation in order to limit the amount of factors that could impact the effects of suggestions and learning on itch. For example, patient groups may have a larger variability in previously learned expectations. These expectations may be especially influenced in patient groups by duration of illness and by previous positive or negative experiences with treatments or the health care system. In addition, by including healthy volunteers only, natural fluctuations in symptom severity or other complications often seen in patient samples were excluded. This may have influenced the study results, as effect sizes could potentially have been smaller due to lower

expected benefit experienced by healthy volunteers. Indeed, participants knew that induced symptoms would be short-term and that they would be able to stop the induction of symptoms at any point in time. This considerably lowers benefits of participating in a study, and patient samples may arguably have a higher wish or desire for improvements in their symptoms, especially when complaints are chronic. Future research may therefore consider investigating placebo and nocebo effects in patient populations for whom itch is a relevant symptom, as placebo and nocebo effects may be more impactful there.

The studies in which suggestions about itch were given were mostly proof of concept studies, in which especially new open-label instructions were tested. Comparisons with previous open-label studies were made in the current dissertation, but some caution is needed, especially given that the content of the rationale differs across studies. For example, it was not possible to use one of the key arguments in previous open-label rationales (i.e., that placebo effects are learned) for the studies described in **Chapter 4-6.** Given that verbal suggestions were used to elicit placebo and nocebo effects**,** together with instruments (e.g., tonic, transdermal caffeine patch) unlikely to have been associated with itch treatment in the past, providing a rationale about learned placebo effects would have been redundant – learning was simply not relevant for the studies described in **Chapter 4-6** and the studies show that open-label effects may also occur without mentioning that placebo effects are learned. However, interpretations need to be made carefully, as demand characteristics may play a role in such studies. The findings of the current studies should therefore be confirmed by future studies, preferably with a double-blind study design. Moreover, future research may consider including a neutral control group, as in the current dissertation positive and negative suggestions were often compared. While this does allow for assessment of the impact of suggestions on itch and other parameters, no estimation of the 'true placebo effect' or the 'true nocebo effect' can be made, as the normal course of repeated tests is unknown.

Finally, limitations concern the power calculations for the secondary hypotheses in the studies described in this dissertation. The effect sizes used as input for these calculations were derived from previous work on placebo and nocebo effects in itch, and resulted in a sample size adequate to test group differences under the separate open-label and closed-label contexts. However, analyses for the secondary outcome measures, such as wellbeing, self-rated and physical skin response to histamine (**Chapter 3-6**), and heart rate, skin conductance, and pulmonary functioning (**Chapter 3**), may have been underpowered. Likewise, limited power may explain why little evidence was found for the moderating role

of personality traits, for example, optimism or neuroticism. Previous work shows indications that personality traits like these may be related to placebo and nocebo responding [10,76,77], but this was not confirmed by the studies described in the current dissertation.

Future research directions

The current dissertation raises several relevant questions that may be further investigated in future research. First, as demonstrated in the systematic review, the field of classical conditioning of immune responses relevant to dermatology has been investigated extensively with animal models. Human trials have focused most on conditioned exacerbation of allergic symptoms, whereas comparatively little is known about how to use classical conditioning mechanisms to enhance treatment efficacy. At the moment, only two studies focused on suppression of allergic symptoms using conditioning mechanisms, but these were unable to distinguish between conditioning and expectancy effects for subjective symptoms. The study described in **Chapter 3** extends these findings by investigating pharmacological conditioning of antihistamine in healthy volunteers. It was demonstrated that conditioning marginally improved itch in response to a histamine challenge. Theoretical implications from this study are that classical conditioning indeed may result in learned suppression of itch or other markers of allergic symptoms, but that, hypothetically, conscious learning (i.e., experiencing reduced symptoms during acquisition) may strengthen these effects. Therefore, effects may be stronger in case of patient studies, as patients could rapidly notice changes in their symptoms, whereas for studies with healthy volunteers, symptoms first need to be deliberately induced. As a first step however, future research may consider strengthening the design used to test pharmacological conditioning with H1-antihistamines. For example, future research may consider including multiple histamine tests, especially given that the timing of conditioned responses for itch, and specifically antipruritic conditioning with antihistamines, has not been investigated systematically. Moreover, including histamine tests in the acquisition phase may help strengthen associative learning for itch in healthy volunteers. It could be possible that in the current, study participants may not have noticed effects of the medication, which would set them up for insufficient learning of associations between CS and UCS. Including multiple tests, or including patients who experience symptoms during acquisition for which they can notice improvements, would help strengthen this type of associative learning. Alternatively,

other outcome parameters could be considered in the future, for example, measuring immune markers related specifically to antihistamine in the blood (e.g., interleukins) [78,79]. These parameters may potentially be more sensitive to relatively smaller conditioned effects compared to subjective or clinical parameters (e.g., itch, pulmonary functioning), but were not measured in the current study.

The conditioning study described in **Chapter 3** showed that conditioning marginally reduced itch for both open-label and closed-label contexts. This raises the question whether deception is necessary for conditioning to occur. Potentially, the conditioning procedure may be explained to participants without losing effects for itch. This needs support of future research, however, as conditioned effects in the current study were marginal and not significant, which hampered assessment of the impact of the open-label rationale. It would be relevant for future studies to further focus on whether and under which circumstances open-label conditioning could reduce itch, as non-deceptive placebo induction may be promising to apply in clinical practice. Regarding open-label placebo effects, another interesting question was raised in **Chapter 4**. It was demonstrated that instructions about low itch and about how participants' expectations impact itch experience led to higher positive associations between expected and experienced itch. However, emphasizing such a relation between expectations and symptomatology may become problematic for nocebo effects, specifically in populations-at-risk, for example, individuals who are highly anxious about receiving medical treatment or have a high fear of side effects. The impact of negative information in these subpopulations may be investigated more thoroughly in future research, for example, by comparing the effects of such instructions across groups with high or low fear of side effects.

Future research may also consider investigating effects of learning and instructions on scratching or other behaviors related to itch. In the systematic literature search in **Chapter 2**, studies are described that show that social cues can impact not only itch (i.e., contagious itch) but also the frequency of spontaneous scratching behavior [80-82]. Scratching has been found to exacerbate itch in skin conditions, and to result in a vicious itch-scratch cycle that can lead to significant impairments for patients (e.g., loss of control, feelings of shame, social isolation) [83]. Therefore, investigating whether placebo and nocebo effects can significantly influence scratching behavior may be a worthwhile approach for future research. So far, a single study investigated whether nocebo effects could generalize from itch to scratching in healthy volunteers, and found no evidence for such response generalization [84]. Future research may investigate whether the elicitation of placebo

effects for itch can also result in reduction of scratching behavior (i.e., response generalization). If it can be demonstrated that placebo effects can generalize from itch to scratching behaviour, this may lead towards new therapeutic possibilities that could target, for example, the itch-scratch cycle.

Finally, the studies described in **Chapter 5 and 6** show that placebo and nocebo effects elicited by verbal suggestions are similar across open-label and closed-label contexts. This raises another theoretical question on the similarity of such open-label and closed-label placebo and nocebo effects. In these chapters, the open-label rationale was provided as an add-on for verbal suggestions about a treatment tool (tonic or caffeine patch). In general, the elicited effects were similar under open-label and closed-label conditions, which has some important implications for research. Careful consideration of the type of information to be provided is necessary. Moreover, the goal that is to be achieved by providing information needs to be considered: if the intention is, for instance, to prevent side effects from occurring, it may not be sufficient to only explain that negative expectations can result in nocebo effects. Hypothetically, such a method could just as likely facilitate nocebo effects (especially when the information about nocebo effects is negatively framed). Rather than explaining that nocebo effects occur through conditioning as a passive, automatic process, it may be more beneficial instead to explain that expectations can be actively used to modulate experience of symptoms [62-66]. Future research could examine whether such an approach can be used to prevent nocebo effects, as well as how this would relate to placebo effects. For instance, it may be worthwhile to investigate whether an open-label rationale that promotes empowerment and active modulation of expectations can enhance placebo effects, or whether it is more prudent for open-label placebo effects to emphasize automaticity of learned responses.

Implications for clinical practice

The results of the current dissertation show that placebo and nocebo effects can be induced for itch and itch-related conditions and symptoms of the mucous membranes using a multitude of methods, including verbal suggestions and conditioning. The information this provides may be used to enhance patients' expectations regarding treatment outcomes in clinical practice, for example, by emphasizing positive information when explaining to-be-expected treatment outcomes to patients and by positive framing of potential adverse effects. For example, when explaining side effects occurrence, it may be useful to discuss

the percentage of people that *do not* experience them rather than the percentage that do [34-38], or to change the manner of informing about side effects of a treatment (e.g., fostering a mindset that side effects may signal that therapies such as immunotherapy work; [85]). Moreover, conditioning mechanisms could be used to maximize placebo effects. For example, by varying the doses of medication, without changing any of the attributes (e.g., the amount, color and shape of pills), conditioned effects could be used to potentially achieve similarly or more effective treatment with lower doses of medication. Several studies already show that this method of conditioned dose reduction can lower medication intake without loss of treatment efficacy for various medical and psychological conditions [5,56]. Findings of the study described in **Chapter 3** indicate that such an approach may potentially be useful to support pharmacological treatment of itch-related conditions as well, however, this needs to be investigated more thoroughly before it can be applied in clinical practice.

In **Chapter 4**, it is demonstrated that open-label positive suggestions about an itch-inducing method can result in positive outcome expectations, and that these in turn are associated with lower itch experience during an experimental itch induction test. This shows that it may be relevant to consider in which ways potentially unpleasant tests and proceedings in health care settings are introduced to patients. Though more research is needed, these findings provide a first indication that it may be helpful to address patients' own expectations and to discuss the impact that these expectations could have on, for example, recovery from medical proceedings, or pain levels during such procedures, especially when patients are highly anxious for invasive procedures. To illustrate, there are studies that show that communication interventions, informational preparation and positive suggestions can influence pain levels [86]. This could potentially be the case for itch as well. Moreover, next to negative emotions (e.g., stress and anxiety), high levels of ruminating (as a chronic negative expectation) have been found to be a predictor for itch in clinical settings as well [87-89]. The findings described in **Chapter 4-6** show that suggestions can impact itch experience, and suggest that providing information about placebo (and nocebo) effects could be a useful way to enhance expectancy effects for itch. In clinical practice this may translate, for example, to psychoeducation regarding the role that expectancy has in harnessing placebo effects for somatic symptoms. Finally, the current studies give some indications that open-label conditioning may potentially be a worthwhile method to facilitate the use of placebo effect mechanisms in clinical practice. If this can be replicated and extended by future research (e.g., with different conditioning paradigms or patient

populations), this may then translate into therapies that utilize conditioned dose reduction in an open-label context. Conditioned dose reduction has already been found effective in psoriasis in a closed-label (i.e., concealed) context [5]. If it is proven effective in an open-label context, medication use could be reduced, and the full potential benefits of placebo effect mechanisms could be reaped in clinical practice as a result.

Conclusion

In the current dissertation, we investigated the experimental elicitation of placebo and nocebo effects for histamine-induced itch and other psychophysiological responses to histamine. Placebo and nocebo effects were examined in a systematic review of the literature, as well as in a series of studies that used multiple induction methods (i.e., classical conditioning, verbal suggestions) for placebo and nocebo effects under both open-label and closed-label (i.e. non-concealed and concealed) conditions. Overall, the dissertation demonstrates that placebo and nocebo effects can be elicited for itch and itch-related parameters by several means. It is shown that histamine-induced itch may be influenced by suggestions under both open-label and closed-label conditions. Moreover, the dissertation shows that potentially, antipruritic effects of antihistamines may be sensitive to conditioning to some extent, though this needs to be investigated further in future research. Placebo (and nocebo) effects can be elicited by conditioning and suggestions with participants' knowledge as well, which is a first step in opening new pathways towards therapeutically applying placebo and nocebo effect knowledge without deception or concealment of methods. Using associative and instructional learning in medical treatments with participants' knowledge may be a promising strategy to maximize placebo effects, minimize nocebo effects, and help in reducing medication use for (chronic) itch and other somatic complaints.

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Nederlandse samenvatting

Placebo-effecten zijn positieve behandeluitkomsten die niet kunnen worden toegeschreven aan actieve behandelonderdelen van bijvoorbeeld farmacotherapie of niet-medicamenteuze therapieën. Zo kunnen placebo-effecten ook ontstaan wanneer een inactieve stof wordt toegediend (d.w.z., een suikerpil oftewel placebopil), en degene die de pil neemt daarbij verwacht dat het een actieve behandeling is, die zal helpen bij het verminderen van klachten. Direct tegenover placebo-effecten staan zogeheten nocebo-effecten, dat wil zeggen, negatieve behandeluitkomsten die niet aan actieve behandelonderdelen toegeschreven kunnen worden. Nocebo-effecten ontstaan door (bewuste of onbewuste) negatieve verwachtingen. Dit kan bijvoorbeeld gebeuren wanneer iemand verwacht dat medicijnen voor vervelende bijwerkingen gaan zorgen (een negatieve behandeluitkomst), omdat ze hierover geïnformeerd zijn of in het verleden vervelende ervaringen mee hebben gehad. Het is ook mogelijk dat negatieve verwachtingen de werking van effectieve behandelingen verminderen. Dit gebeurt bijvoorbeeld wanneer men twijfelt over de effectiviteit van een gegeven behandeling. Er is al veel onderzoek gedaan naar placebo- en nocebo-effecten bij lichamelijke symptomen zoals pijn en er zijn indicaties dat andere lichamelijke symptomen, zoals vermoeidheid, misselijkheid en jeuk, ook onderhevig kunnen zijn aan dit soort effecten. Studies laten bijvoorbeeld zien dat biopsychosociale factoren een grote rol kunnen spelen bij de ervaring van jeuk. Zo is er aangetoond dat enkel praten over jeuk dit symptoom kan opwekken (dit wordt ook wel 'besmettelijke jeuk' genoemd). Door deze grote invloed van biopsychosociale factoren is het mogelijk dat placebo- en nocebo-effecten bij jeuk bijzonder relevant zijn.

Onderzoek toont aan dat placebo- en nocebo-effecten kunnen worden opgewekt door verschillende leermechanismen, waaronder *associatief leren* en *instructieleren*. Bij associatief leren worden verbanden tussen stimuli en daaropvolgende gebeurtenissen aangeleerd. Meer specifiek wordt dit ook wel klassieke conditionering genoemd. Bij medicijngebruik bijvoorbeeld kan het nemen van een pil (stimulus) leiden tot een vermindering van hoofdpijn (de daaropvolgende gebeurtenis). De pil (ongeconditioneerde stimulus) heeft farmacologische eigenschappen die leiden tot vermindering van hoofdpijn; dit noemen we de ongeconditioneerde respons. Het kan zo zijn dat (na verloop van tijd) ook andere eigenschappen van de pil, bijvoorbeeld de vorm of kleur hiervan, in de hersenen geassocieerd worden met pijnvermindering. Dit (de vorm of kleur van de pil) heet de geconditioneerde stimulus. Wanneer alleen de aanblik of het nemen van een pil met dezelfde vorm of kleur leidt tot een vermindering in pijn, spreken we van een aangeleerde (geconditioneerde) reactie – dit is dan een placebo-effect. Een tweede benadering die

binnen dit proefschrift is toegepast is *instructieleren*. Deze vorm van leren omvat het leren doordat informatie of instructies gegeven worden, die de verwachtingen die mensen hebben over toekomstige gebeurtenissen beïnvloeden. In het kader van placebo-effecten kan hier bijvoorbeeld gedacht worden aan verbale suggesties over hoe effectief een procedure of behandeling zal zijn. Wanneer aangegeven wordt dat een procedure tot weinig pijn of jeuk zal leiden, wordt hierdoor een positieve verwachting gevormd. Deze kan er vervolgens toe leiden dat mensen minder pijn of jeuk ervaren dan wanneer verteld wordt dat deze procedure veel pijn of jeuk kan opwekken. De extra pijn- of jeukvermindering die enkel door het geven van deze instructies optreedt wordt ook toegeschreven aan placebo-effecten.

Het primaire doel van dit proefschrift was om te onderzoeken of placebo- en nocebo-effecten opgeroepen kunnen worden voor jeuk (die werd opgewekt door de toediening van histamine op de huid) met behulp van de bovengenoemde leertechnieken. Tevens zijn effecten op andere (psycho)fysiologische reacties op histamine onderzocht. Hierbij zijn placebo- en nocebo-effecten binnen twee verschillende contexten onderzocht. Een van de contexten is hoe onderzoek naar placebo-effecten traditioneel verricht wordt: wanneer mensen niet weten dat er placebo- of nocebo-effecten worden opgewekt en een werkzaam middel verwachten (verborgen inductie van deze effecten; dat wil zeggen, in een gesloten-label context). In een andere context werd onderzocht wanneer men op de hoogte was van de inductie van placebo- of nocebo-effecten (een zogeheten open-label context). In een dergelijke situatie worden placebo- of nocebo-effecten expliciet aan deelnemers uitgelegd en wordt verteld hoe en waarom deze effecten kunnen bijdragen aan een behandeling van jeuk. Studies naar deze open-label inductie van placebo-effecten zijn relatief nieuw: er is nog weinig bekend over open-label placebo-effecten en voor welke symptomen deze kunnen worden geïnduceerd. Wanneer open-label placebo- en nocebo-effecten geïnduceerd kunnen worden voor jeuk, kan onderzoek hiernaar bijdragen aan een vernieuwende en ethische toepassing van de achterliggende mechanismen in de klinische praktijk, bijvoorbeeld door de effectiviteit van bestaande behandelingen te vergroten. Samenvattend werd in dit proefschrift het opwekken van placebo- en nocebo-effecten onderzocht met behulp van verschillende leermechanismen. Hierbij lag de focus in het bijzonder op het lichamelijke symptoom jeuk. De beschreven studies onderzochten de bestaande literatuur over dit thema (**Hoofdstuk 2**), evenals de vraag of placebo- en nocebo-effecten experimenteel opgewekt kunnen worden door (farmacologische) conditionering (associatief leren; **Hoofdstuk 3**) en verbale suggesties (instructieleren; **Hoofdstukken 4-6**). Bovendien werd onderzocht of deze effecten al dan niet opgewekt konden worden wanneer deelnemers

zich bewust waren van de conditionering of betreffende verwachtingsinductie (d.w.z. in een open-label context).

In **Hoofdstuk 2** is de bestaande literatuur over experimenteel geïnduceerde placebo- en nocebo-effecten bij jeuk, jeuk-gerelateerde aandoeningen en symptomen van de huid en slijmvliezen onderzocht. In deze systematische literatuurstudie werden naast studies bij patiëntpopulaties ook studies naar relevante diermodellen en experimentele studies met gezonde participanten geïncludeerd. De literatuurstudie beschrijft methoden die doorgaans gebruikt worden om placebo- en nocebo-effecten op te wekken. Daarnaast wordt een samenvatting van de onderzoeksresultaten gegeven. In grote lijnen kan worden gesteld dat in de literatuur gebruik is gemaakt van drie leertechnieken (of combinaties hiervan) om placebo- en nocebo-effecten op te wekken: associatief leren (klassieke of operante conditionering), instructieleren (met behulp van verbale suggesties, al dan niet aangeboden met hypnose) en sociaal observatieleren (bijvoorbeeld inductie van 'besmettelijke jeuk'). Over het algemeen konden deze technieken placebo- en nocebo-effecten opwekken voor jeuk en jeuk-gerelateerde symptomen van de huid (zoals zwellingen of laesies). Wel waren de gevonden studies vaak heterogeen in de gekozen inductietechnieken (bijv. conditionering en suggesties), en bleken de geïnduceerde placebo- en nocebo-effecten vaak voorwaardelijk te zijn: zo waren geconditioneerde effecten bijvoorbeeld vaak afhankelijk van de context waarin effecten werden geleerd, en leidden veranderingen in omgevingsfactoren ertoe dat effecten verminderden of verdwenen. Bovendien waren verbale suggesties vaak enkel effectief op korte termijn. Samenvattend laat de systematische literatuurstudie zien dat een grote verscheidenheid aan experimentele procedures is gebruikt binnen de studies; dat wil zeggen, er is geen standaard 'conditioneringsprotocol' of standaard set van verbale suggesties voor het oproepen van placebo- en nocebo effecten te onderscheiden. Bovendien zijn effecten onderzocht in zeer diverse patiëntenpopulaties, en zijn verscheidene diermodellen en experimentele modellen met gezonde participanten gebruikt.

In **Hoofdstuk 3** zijn de resultaten van een gerandomiseerd gecontroleerd onderzoek naar klassieke (farmacologische) conditionering van de jeuk-remmende eigenschappen van antiallergische medicijnen (antihistaminica) gerapporteerd. Twee eerdere studies hebben farmacologische conditionering met antihistaminica bij patiënten met een huisstofmijtallergie onderzocht. Deze studies konden geen onderscheid maken tussen geconditioneerde effecten en anderszins aan verwachting gerelateerde effecten (bijvoorbeeld de verwachtingen die de allergische deelnemers zelf al hadden over de

effectiviteit van antihistaminica, nog voordat zij deelnamen aan het onderzoek) op zelf-gerapporteerde allergische symptomen. De studie uit Hoofdstuk 3 was bedoeld om de kennis opgedaan uit eerdere studies aan te vullen, door geconditioneerde effecten voor jeuk (experimenteel opgewekt door histamine toe te dienen op de huid) bij gezonde vrijwilligers te onderzoeken. Conditionering werd bovendien onderzocht binnen twee verschillende contexten: een open-label context, waarin deelnemers uitleg over conditionering ontvingen en verteld werd dat de effecten van conditionering met antihistaminica in deze studie getest werden; en een gesloten-label context, waarin deelnemers niet wisten dat zij geconditioneerd werden. Deze twee groepen werden vergeleken met twee controlegroepen. Ondanks dat conditionering (in de gecombineerde open- en gesloten-label context groepen) leidde tot een marginale vermindering van jeuk vergeleken met de controlegroepen, werden geen significante verschillen tussen de afzonderlijke groepen gevonden. Tevens werden andere parameters binnen de studie niet tot weinig beïnvloed door conditionering. Over het algemeen biedt de studie beperkt bewijs voor de jeuk-remmende effecten van conditionering met antihistaminica bij gezonde vrijwilligers.

In de hoofdstukken 4, 5 en 6 worden drie gerandomiseerde gecontroleerde onderzoeken beschreven, waarin de effecten van verbale suggesties op jeuk en andere (psycho)fysiologische reacties op histamine werden onderzocht. In **Hoofdstuk 4** werden de effecten van open-label positieve verbale suggesties (over het ervaren van weinig jeuk na een histamine-applicatie op de huid) vergeleken met neutrale instructies. Hoewel er geen significante verschillen in jeuk tussen de groepen zijn gevonden, waren de verwachtingen over jeuk van deelnemers wel significant beïnvloed door de instructies. Zo verwachtten deelnemers na de suggesties minder jeuk te ervaren vergeleken met deelnemers die neutrale instructies kregen. Deze verwachtingen van deelnemers waren significant gerelateerd aan de hoeveelheid jeuk die ervaren werd: hoe minder jeuk deelnemers verwachtten, hoe minder jeuk zij uiteindelijk rapporteerden. Dit werd uitsluitend binnen de positieve suggestie groep gevonden; na neutrale instructies was dit verband niet aanwezig. Bovendien kon een trend worden waargenomen voor zelf-beoordeelde conditie van de huid na toediening van histamine: na open-label positieve verbale suggesties werd de huidconditie door deelnemers marginaal beter beoordeeld vergeleken met de neutrale instructie groep. Samenvattend illustreren deze resultaten een potentiële rol voor open-label placebo effecten bij jeuk.

In **Hoofdstuk 5** werden de effecten van open-label en gesloten-labed positieve en negatieve verbale suggesties over de jeuk-verminderende of -verhogende eigenschappen van een

(niet-werkzame) tonic op jeuk vergeleken. Deelnemers in de open-label groepen ontvingen naast suggesties een extra uitleg over hoe informatie en verwachtingen de ervaring van jeuk kunnen beïnvloeden. Hoewel er geen effect van suggesties op de ervaren jeuk tijdens het toedienen van histamine op de huid (de primaire uitkomstmaat van het onderzoek) werd gevonden, was jeuk tijdens een korte follow-up periode lager in de positieve vergeleken met de negatieve verbale suggestie groepen (zowel voor de open- als gesloten-label contexten). Exploratieve nadere analyse van de data liet zien dat jeuk in de positieve suggestiegroepen aanzienlijk verminderde van de baseline tot post-suggestie test, terwijl in de negatieve suggestiegroepen geen significante veranderingen over tijd werden geobserveerd. De resultaten van deze studie laten zien dat positieve suggesties jeuk kunnen verminderen in vergelijking met negatieve suggesties, zowel wanneer men op de hoogte is van het ontvangen van suggesties (open-label) als wanneer dit niet zo is (gesloten-label).

Hoofdstuk 6 beschrijft de bevindingen van een studie waarin de effecten van open-label en gesloten-label positieve en negatieve suggesties op jeuk opnieuw vergeleken werden, ditmaal met de suggestie dat jeuk wordt beïnvloed als bijwerking van het toebrengen van een transdermale pleister. Deelnemers werd verteld dat deze pleister cafeïne bevatte (terwijl het in feite een niet-medicamenteuze, dat wil zeggen een niet-werkzame, pleister betrof). Verteld werd dat, als bijwerking van deze pleister, ook de gevoeligheid voor lichamelijke prikkels beïnvloed zou worden (in de positieve verbale suggestiegroepen werd verteld dat dit tot minder jeuk zou leiden, en in de negatieve verbale suggestiegroepen werd verteld dat dit tot meer jeuk zou leiden). Net zoals in de studie uit Hoofdstuk 5 ontvingen deelnemers in de open-label groepen extra informatie over de invloed van informatie en verwachtingen op de ervaring van jeuk. Kort samengevat leidden positieve verbale suggesties vergeleken met negatieve verbale suggesties tot een significante afname van de hoeveelheid jeuk die tijdens histamine-applicatie werd ervaren, zowel in een open-label als in een gesloten-label context. Net zoals in het onderzoek uit Hoofdstuk 5 blijkt jeuk aanzienlijk te verminderen in de positieve verbale suggestiegroepen, terwijl in de negatieve suggestiegroepen jeuk onveranderd blijkt. Samengevat tonen deze bevindingen aan dat bepaalde verbale suggesties jeuk kunnen beïnvloeden, ongeacht of men van de verwachtingsinductie door suggesties afweet (open-label) of niet (gesloten-label).

In **Hoofdstuk 7** worden de bevindingen uit het proefschrift besproken aan de hand van de wetenschappelijke literatuur. Samenvattend wordt in de literatuur bewijs voor placebo- en nocebo-effecten bij jeuk (en jeuk-gerelateerde parameters) gevonden. Daarnaast is aangetoond dat jeuk over het algemeen door conditionering en verbale suggesties beïnvloed

wordt, hoewel meer onderzoek nodig is om te bepalen hoe deze leerprocessen werken en dergelijke effecten versterkt zouden kunnen worden. De in het proefschrift besproken studies dragen bij aan kennis ten aanzien van het kunnen opwekken van placebo- en nocebo-effecten, ook wanneer men hiervan weet. De ethische overwegingen die een rol spelen binnen de geneeskunde, bijvoorbeeld de noodzakelijkheid van het zo volledig mogelijk informeren van patiënten over behandelingen en behandelopties, maakt dit type onderzoek bijzonder relevant in de zoektocht naar strategieën om kennis over placebo-effecten toe te passen in de klinische praktijk. Onderzoek naar (met name open-label) placebo- en nocebo-effecten bij jeuk en andere symptomen kan op deze manier bijdragen aan het op een ethische en verantwoorde manier verbeteren van bestaande behandelingen, bijvoorbeeld door deze te optimaliseren met behulp van leertechnieken en informatieverstrekking.

Curriculum Vitae

Stefanie Meeuwis was born on May 1st, 1991 in Oosterhout (NB), the Netherlands. In 2009 she completed her secondary education at the St. Oelbertgymnasium in Oosterhout and started her Bachelor in Psychology at Tilburg University, for which she earned her degree in 2012. Next, Stefanie started the Master program in Clinical Psychology at Tilburg University, for which she graduated with honors (cum laude) in 2013. During this period, Stefanie gained clinical experience at the Trubendorffer addiction clinic in Tilburg, where she also obtained her certificate for psychodiagnostics ('basisaantekening psychodiagnostiek'). She wrote her thesis on depression, anxiety and distress and their association with high-sensitive c-reactive protein and fibrinogen in patients with non-obstructive coronary artery disease, under the supervision of dr. Paula Mommersteeg. After receiving her Master's degree, Stefanie started her PhD-project under supervision of professor Andrea Evers, dr. Henriët van Middendorp and dr. Judy Veldhuijzen. The PhD-project was part of a large project funded by the European Research Council grant of Andrea Evers: "Empowering expectations for health and disease: training the immune and endocrine system". During several international meetings, Stefanie received different poster and presentation awards: for example, she was awarded 'best early career oral presentation' at the European Society for Dermatology and Psychiatry congress, presented a citation poster in the session for 'best conference abstracts' at the American Psychosomatic Society annual conference, and received travel grants for the 9th and 10th World Congress on Itch by the International Forum for the Study of Itch. Next to the research activities, Stefanie supervised multiple bachelor and master students for their thesis projects or internships. Stefanie was also a member of the organising board for the 2nd official Society for Interdisciplinary Placebo Studies Conference which took place in July 2019 in Leiden, the Netherlands.

Currently, Stefanie works as a postdoctoral researcher at Leiden University on several research projects in the area of placebo and nocebo effects and related topics.

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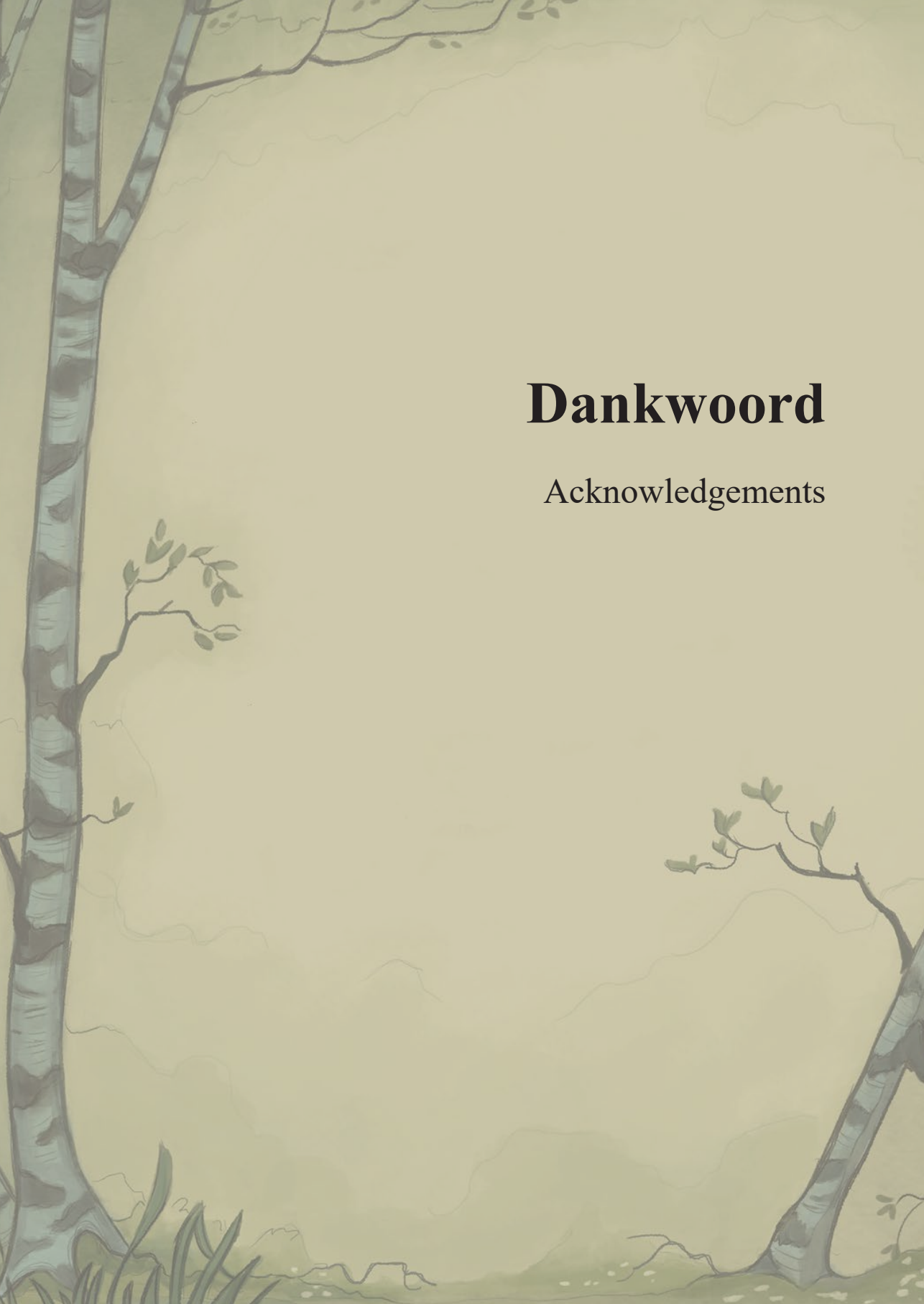

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