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Conditioning Immune and Endocrine Parameters in Humans: A Systematic Review

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Abstract
Background: Conditioned pharmacological effects may provide relevant clinical opportunities to improve treatment for patients with a variety of conditions. The aim of this systematic review was to create an overview of studies in this field of research and to investigate whether specific characteristics of the study design make for successful conditioning. ***Methods:*** The protocol of this review was registered in Prospero (PROSPERO 2015: CRD42015024148). A systematic literature search was conducted in the databases PubMed, Embase, and PsychInfo. Studies were included if they were placebo-controlled trials in humans in which the effects of a pharmacological agent on immune or endocrine outcomes (e.g., interleukin-2 and cortisol) were conditioned, using a specific conditioned stimulus. The risk of bias of each study was assessed using the Cochrane risk-of-bias tool. ***Results:*** The final selection included 16 studies. Overall, those studies indicate that conditioning of immunosuppression, conditioning of allergic responses, and conditioning of insulin and glycemic responses is possible. Regarding immunostimulants, antiallergic effects, and cortisol conditioning, the preliminary results are promising, but additional studies are needed. ***Conclusions:*** This systematic review shows classical conditioning of immune and endocrine responses for various pharmaceutical substances. The studies reviewed here indicate that the number of acquisition and evocation sessions, and characteristics of the unconditioned and conditioned stimuli, are important determinants of the effectiveness of pharmacological conditioning on immune and endocrine parameters. In the future, conditioned pharmacological effects may be used clinically as adjunct therapy in various patient populations.

This study was conducted at the Department of Medical Psychology, Radboud University Medical Center, and at the Health, Medical and Neuropsychology Unit, Institute of Psychology, Faculty of Social and Behavioural Sciences, Leiden University.

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Introduction

Learning about associations between external and internal stimuli is of high adaptive value for the survival of any animal [1]. One mechanism by which these associations are learned is Pavlovian or classical conditioning. Classical conditioning is an associative learning process about the temporal and causal relationships of specific stimuli, and it is used to modify behavior, cognitions, and physiological reactions. In the past decades, evidence has also been found for conditioning of physiological responses of the immune and endocrine systems [2, 3]. Conditioning of physiological functions, such as immune and endocrine parameters, can be achieved when the presentation of a conditioned stimulus (CS) is repeatedly paired with the administration of an unconditioned stimulus (UCS). The UCS is mostly a pharmacological agent with specific physiological actions (unconditioned response; UCR). After several paired administrations of CS and UCS during the acquisition phase, in the evocation phase administration of the CS alone is enough to trigger changes in the body (conditioned response; CR) often similar to the changes caused by the pharmacological UCS [3]. When a CR is repeatedly evoked by the administration of a CS without a UCS, extinction occurs by a gradually decreasing CR with every evocation.

That immune and endocrine parameters can be conditioned illustrates a close interaction between the central nervous system and peripheral functions regulating homeostasis and therefore has important implications for the study of placebo and nocebo effects [1, 2]. Placebo and nocebo effects occur when a patient's expectations affect health outcomes [4–6]. These expectations can be either explicit or implicit. Explicit expectations can be induced by, for example, verbal suggestions and affect primarily conscious, subjective processes such as pain. Implicit expectations are thought to be induced primarily by (pharmacological) conditioning and can affect automatic processes such as immune and endocrine parameters [7]. Conditioning of immune and endocrine parameters also has important clinical implications [8–14]. An example of this is provided by a study showing that the magnitude of conditioned antihistamine effects in participants with rhinitis due to house dust mite allergy does not significantly differ from direct antihistamine drug effects [8]. When this effect is found to be retainable, this implies that patients could reduce the amount of medication needed to ameliorate allergic symptoms. Thus, conditioning of such parameters could bear relevance for a wide variety of issues in medical and psychological sciences [15]. However, a large heterogeneity has been reported in the effects found within the field of pharmacological conditioning of immune and endocrine effects [8, 9].

A potentially important explanation for this heterogeneity in studies on conditioned pharmacological effects lies in differences in the design of studies. Studies to date have shown much variability with regard to the number of acquisition and evocation trials as well as with regard to the nature of the CS and UCS used. However, the relevance of these characteristics for finding conditioned pharmacological effects has not yet been systematically investigated. Therefore, the aim of this paper is to create a systematic overview of the existing studies in this field of research and to further elucidate whether there are specific characteristics of the study design that make successful conditioning of pharmacological effects more likely. To achieve this aim, placebo-controlled trials in humans addressing pharmacological conditioning (with a pharmacological agent as the UCS and a specific CS) of immune or endocrine outcomes (e.g., interleukin (IL)-2 and cortisol) will be systematically reviewed with regard to these design characteristics.

Methods

This review was conducted in accordance with the PRISMA statement [16, 17]. The protocol of this review was registered in Prospero (PROSPERO 2015: CRD42015024148).

Study Criteria and Search Strategy

Studies were eligible for inclusion in this review if they met the following criteria: (1) the study report was written in English, (2) the study was conducted in humans, (3) new data were presented in the study, (4) a pharmacological UCS was used, (5) a well-defined CS was used (distinct taste or smell and not an unspecified laboratory environment), (6) an immune (e.g., IL-2) or endocrine (e.g., cortisol) outcome was assessed, and (7) the study was a placebo-controlled trial.

The inclusion criteria were translated into a search term comprising Medical Subject Headings (MeSH) and text words (tw) and combining conditioning of a pharmacological agent, eligible types of outcome measures, and the UCS used. With this search term, a systematic literature search was conducted in the databases PubMed, PsychInfo, and Embase on June 1, 2015. See online supplementary figure 1 (see www.karger.com/doi/10.1159/000449470 for all online suppl. material) for the search term used for the electronic search in PubMed. The search terms for Embase and PsychInfo were built from this search term. Also, the reference lists and citing articles of all of the articles included in this review were checked in Web of Science for additional articles meeting the inclusion criteria. All of the study titles were screened for thematic relevance by one rater. Two independent raters examined the abstracts of relevant studies according to the inclusion criteria. When an abstract did not contain sufficient information to determine

whether the article fulfilled the inclusion criteria, or if the article was deemed eligible by one or both raters, the full text was examined by both raters. Discrepancies between the two raters were resolved by discussion with a third rater.

Risk-of-Bias Assessment

Two independent raters assessed the risk of bias (RoB) of each included study using the Cochrane risk-of-bias tool [18]. Assessed biases included selection bias (randomization process and allocation concealment), performance bias (blinding of participants and research personnel), detection bias (blinding of the outcome assessment), reporting bias (handling of missing data), and attrition bias (reasons for withdrawal in all conditions). When the two raters did not reach agreement, a third rater was consulted to reach consensus. The RoB was assessed based on the information provided in the article.

Parameters Extracted from the Studies

Regarding effects and design issues, parameters extracted from the papers included the effects of conditioning on immune and endocrine parameters, the number of acquisition and evocation sessions, the characteristics of the CS and the UCS, and the sequence in which the CS and the UCS were presented (i.e., the CS was presented preceding or following the UCS). Also information about responders and nonresponders to the conditioning procedure and the magnitude of the CR was extracted. Other relevant descriptors of the studies included effects of conditioning on self-reported measures, such as self-reported symptoms, and characteristics of the study population, including age, gender, the number of participants, and the type of participants (healthy or patient samples).

Results

Search Results

A total of 12,016 studies were found by searching the PubMed, Embase, and PsychInfo databases. After screening the titles, 153 abstracts remained to be screened by 2 independent raters. Of these, 30 studies were examined in full text. Twelve studies were found to be eligible for inclusion in this review. Screening of the reference lists and citing articles of the included studies in Web of Science yielded another 4 studies eligible for inclusion. The number of excluded studies and the reasons for exclusion are displayed in online supplementary figure 2.

RoB Analysis

The results of the RoB analysis are shown in the RoB graph (online suppl. fig. 3) and the RoB summary (online suppl. fig. 4). Selection and allocation bias were assessed based on the way randomization was achieved and the measures taken to conceal the group allocation. Eleven studies did not provide information on how randomization was achieved and 3 studies did not explicitly state that the allocation of participants was done randomly (unclear RoB, 88%). One study reported that randomization was done by the pharmacy (low RoB, 6%), and one study indicated that randomization was based on the sequence of inclusion of the participants (high RoB, 6%).

The RoB due to a lack of blinding of participants and personnel (performance bias) was considered to be low for 12 studies (75%), given that the outcome measured in these studies was biochemical and therefore less likely to be influenced by bias. In 3 studies investigating allergic patients (19%), administration of the UCS led to either an increase or a decrease in allergic symptoms, making complete blinding unlikely. It is, however, unclear whether this lack of blinding affected the biochemical outcome of the study (unclear RoB). In one study, blinding seemed to be highly unlikely due to the extreme effects of the UCS (lipopolysaccharide) (high RoB, 6%). Because all of the studies included in this review assessed biochemical outcomes, the risk of detection bias was considered to be low. Regarding incomplete outcome data (attrition bias), 9 studies did not provide enough information to assess the RoB (unclear RoB, 56%) and 6 studies reported that the dropout rate was equal for the experimental and control groups (low RoB, 38%). One study stated that only participants who completed all measurements were included in the analysis, without reporting the reasons for dropout or the allocation of subjects who were lost to attrition (high RoB, 6%). Small samples often in combination with unequal group sizes were identified as an additional source of possible bias in 6 studies (unclear RoB, 38%).

Conditioning Paradigms

Detailed information on the design characteristics of the included studies is presented in online supplementary table 1. Eleven of the 16 studies were aimed at conditioning of immune functions (69%) and 5 studies were aimed at conditioning of endocrine functions (31%). Concerning the studies on conditioning of immune functions, 5 studies addressed conditioned immunosuppression (31% of all studies) [19–23], 3 studies addressed conditioned immunostimulation (19%) [24–26], and 3 studies addressed conditioned allergic responses (19%) [9, 27, 28]. Of the studies on conditioning of endocrine outcomes, 4 (25%) addressed conditioned glycemic responses [29–32] and the remaining study addressed conditioned cortisol (6%) [33].

Regarding the general conditioning paradigm, all but one [26] of the studies (94%) [9, 19–25, 27–33] employed a design with an acquisition phase, in which the UCS was repeatedly paired with the CS, and an evocation phase, in

which the CS was presented together with a placebo. The remaining study on immunostimulation employed an intermittent treatment schedule in which the CS was consistently paired with the UCS in the first phase of the study (acquisition phase) and was administered intermittently with or without the UCS in the second phase of the study [26].

Sample Characteristics

The total sample size of the studies varied between 15 [33] and 62 [9], with 5 [28] to 32 [21] participants per group. The participants in 13 studies were healthy volunteers (81%) [19–26, 29–33], whereas 3 studies (19%) included participants who were allergic to either pollen [27] or house dust mites [9, 28].

The age of the participants varied between 18 and 55 years [19, 21–23, 26, 28–30, 33], with the mean age varying between 19 and 31 [9, 19–23, 25, 29–33]. Two studies (13%) did not provide information on the age of the participants [24, 27]. Concerning the gender of the participants, 10 studies (63%) included only male participants [19–23, 25, 29, 30, 32, 33], while 1 study included only female participants (6%) [31] and 3 studies (19%) included male as well as female participants [9, 26, 28] (60% female [9, 26] and 76% female [28]). The 2 remaining studies (13%) did not report the gender of the participants [24, 27].

CR Affecting Immune Parameters

Conditioned Immunosuppression

All of the details of the studies examining conditioned immune responses are described in online supplementary table 2. In terms of CR, all but 1 [19] of 5 studies on conditioned immunosuppression found a significant decrease in stimulated IL-2 in vitro release after evocation in the conditioned group versus the control group [20–23].

Acquisition and Evocation Trials. All of the studies used 4 acquisition trials [19–23]. The number of evocation trials varied between 1 and 14 [19–23], with the study using 1 evocation trial not reporting significant conditioned immunosuppressive responses [19]. Of the 4 studies with more than 1 evocation session, 2 provided no information regarding the time course of extinction [20, 21]. The third study found a significant conditioned reduction in IL-2 after 4 evocation trials and again after 4 more evocation trials, which commenced 11 days later [22]. In the fourth study, using 14 evocation trials, significant conditioned immunosuppression was found after 2 and 4, but not after 14, evocation trials [23].

Conditioned Stimuli. As the CS, a taste stimulus was used in all 5 studies [19–23]. Two studies investigated whether conditioned taste avoidance of the CS occurred [21, 22]. One of these found that the taste of the CS was rated significantly more negatively by the conditioned group compared to the placebo control group [22], whereas the other found no difference in hedonic taste ratings between groups [21].

Unconditioned Stimuli. All of the studies used the immunosuppressant drug cyclosporine A (CsA) as the UCS [19–23]. Administration of CsA led to significant decreases in IL-2 in all of the studies. None of the studies provided information about possible subjectively noticeable effects of CsA, but one study reported that the group receiving CsA scored significantly higher on a side effects questionnaire than the group receiving placebo [23].

Conditioned Immunostimulation

The 3 studies on conditioned immunostimulation [24–26] all used different UCS and assessed different outcome measures (see online suppl. table 2). Two of the studies found significant conditioned immunostimulating effects [24, 26] and one did not [25].

Acquisition and Evocation Trials. Of the 3 studies, 2 used a design with a distinct acquisition and evocation phase [24, 25]. Of these, the study using 4 acquisition trials found significant conditioned immunostimulating responses [24], whereas the study using 1 acquisition trial did not [25]. The remaining study [26] used an intermittent treatment design in which the CS was repeatedly paired with the UCS before being presented alone in one of several evocation trials or again paired with the UCS in a 'booster session'. This study found significant conditioned effects on serum quinolinic acid, neopterin, and CD64 expression on monocytes [26]. None of the 3 studies provided information about extinction of the CR [24–26].

Conditioned Stimuli. All 3 studies used a taste stimulus as the CS [24–26], which in one case was a sherbet sweet that also provided tactile stimulation in the mouth [24]. The CS was administered before the UCS in 2 studies [24, 26] and after the UCS in 1 study [25]. Only 1 study provided information about the hedonic qualities of the CS [25]. After the first consumption, the CS was perceived overall as novel, with a relatively neutral odor and a rather unpleasant taste. After conditioning, the participants in the conditioned group rated the odor of the CS as significantly less pleasant than did the participants in the control group, while no conditioned effects on cytokine levels or other study outcomes were found [25].

Unconditioned Stimuli. All 3 studies used different UCS. In all of the studies, administration of the UCS led to immunostimulatory responses on immune parameters, but only one study reported subjectively noticeable symptoms elicited by the UCS [25]. Additionally, in one study, 6 of the total 37 participants (16%) dropped out for reasons including low energy levels, dizziness, blurred vision, acute abdominal pain, and joint pain [26], which may reflect side effects of UCS administration.

Conditioned Allergic Responses

Three studies investigated conditioned allergic or antiallergic effects in participants who were allergic to either pollen [27] or house dust mites [9, 28]. Both studies aimed at allergic effects by conditioning with allergens found significant conditioned effects in immune parameters linked to allergic reactions, but not in allergic symptoms [27, 28]; however, one study found a significant decrease in the peak nasal inspiratory airflow [27]. The remaining study was aimed at conditioning antiallergic effects and found significant reductions in allergic parameters as well as symptoms in both the conditioned and the placebo control groups compared to a natural-history group, but no significant differences were found between the conditioned and placebo control groups [9].

Acquisition and Evocation Trials. Both studies aimed at conditioning allergic effects used 1 acquisition trial and 1 [28] or 2 [27] evocation trials. Regarding extinction, smaller conditioned effects were found in the second compared to the first evocation trial [27]. The study conditioning antiallergic effects used 5 acquisition and 5 evocation trials. After the first evocation, significantly decreased wheal sizes and allergic symptom scores were found in both the conditioned and the placebo control groups when compared to a natural-history group. After the fifth evocation, a significant reduction in symptoms but not in wheal size was found in both groups compared to the natural-history group [9], possibly indicating effects of extinction.

Conditioned Stimuli. Of the 2 studies conditioning allergic responses, 1 used an odor stimulus [27] and the other used a taste stimulus [28] as the CS. In both studies, the CS was administered before presentation of the UCS. No subjective ratings of the hedonic quality of the CS were reported. In the study aimed at conditioning antiallergic effects, the same CS (drink) as in the studies on conditioning of immunosuppressive effects was used and administered together with the UCS [9]. Here, too, no data on the subjective quality of the CS were reported [9].

Unconditioned Stimuli. Both of the studies conditioning allergic effects used intranasally administered allergens as the UCS, which led to an increase in allergic parameters and allergic symptoms [27, 28]. The study aimed at conditioning antiallergic effects used an antihistaminergic agent as the UCS, leading to significant decreases in allergic parameters and symptoms. In that study, symptom scores were also significantly lower in the placebo control group than in the natural-history group [9].

CR Affecting Endocrine Parameters

All of the details regarding the studies examining conditioned endocrine responses are described in online supplementary table 2. Among the 5 studies addressing conditioning of endocrine functions [29–33], 4 studies investigated conditioned changes in glycemic levels [29–32]. Of the 4 studies, 2 [29, 30] found significant conditioned cumulative glycemic decreases from baseline. In one of these studies, a second experimental group was examined with glucose injection instead of insulin as the UCS, and a significant conditioned decrease in the concentration of serum insulin, but no effect on glycemia, was found [30]. Two other studies reported a significant conditioned increase in insulin blood levels but no effect on glycemia [31, 32], C-peptide, or leptin [31]. The remaining study addressed conditioning of plasma cortisol levels as the primary outcome measure [33]. A significant effect indicated an increased cortisol response in the evocation phase for the conditioned group versus the placebo group, but the results of post hoc analyses were nonsignificant [33].

Acquisition and Evocation Trials. Of the 4 studies investigating conditioned changes in glycemia [29–31], the studies using 4 acquisition trials and 1 evocation trial found significant conditioned effects on glycemia [29, 30] and a significant conditioned decrease in the serum insulin concentration in an additional experimental group receiving glucose as the UCS [30]. The remaining 2 studies used 6 acquisition trials and 1 [31] or 6 (in 1 day) evocation trials [32]. Of these, one used intranasal insulin as the CS, and a significant conditioned increase in insulin levels, but no effect on glycemia, was found [32]. In the remaining study, using orally administered glucose as the UCS, no conditioned effects were found [31]. The study aimed at conditioning cortisol used 3 acquisition trials and 1 evocation trial [33]. In that study, an overall increased cortisol response was found, but post hoc analyses were nonsignificant [33]. None of the studies on conditioning endocrine effects provided information concerning extinction.

Conditioned Stimuli. In all 4 studies addressing conditioned glycemic effects, an odor was used as the CS [29–32]. In 1 of these, the odor was combined with a taste stimulus [31]. In 2 studies, the CS was presented both before and during administration of the UCS [29, 30]; in one study administration of the CS preceded that of the UCS [31], and in the remaining study the CS cooccurred with the UCS [32]. None of the studies reported subjective ratings of the hedonic qualities of the CS. In the study aimed at conditioning cortisol levels, a taste administered before the UCS was used as the CS. Data on the hedonic ratings of the CS were not reported, but the authors stated that no indications for conditioned taste aversion were found [33].

Unconditioned Stimuli. In both studies using insulin as the UCS, administration led to a direct decrease in blood glucose levels, which significantly affected counterregulatory hormones and led to an increase in the number of neuroglucopenic symptoms [29, 30]. In the second experimental group in which glucose was administered as the UCS [30], a significant increase in insulin and significant changes in the counterregulatory hormones glucagon and, to a lesser extent, norepinephrine were found [30]. In the third study using insulin as the UCS, the insulin was administered intranasally. This led to an immediate significant increase in peripheral insulin and a significant decrease in glycemia that remained within the euglycemic range [32]. Significant changes in epinephrine, but not in leptin, norepinephrine, or cortisol, were found [32]. In the study using glucose as the UCS, direct effects of UCS administration were not assessed [31]. The study aimed at conditioned effects on cortisol used dexamethasone as the UCS, of which the direct effects were not reported by the authors [33].

Responders and Nonresponders

Of the 16 studies included in this review, 2 (13%) analyzed samples for responders and nonresponders to the conditioning protocol [21, 29]. In the first study on conditioned immunosuppression by CsA, participants who showed a conditioned IL-2 decrease larger than 1 SD were categorized as responders. The ratio of responders to nonresponders was 15:17. Responders could be distinguished from nonresponders by higher levels of state anxiety and plasma norepinephrine at baseline, accounting for 60% of the variance [21]. In the second study on conditioning effects of insulin [29], responders were identified by a median split on the basis of the maximum glycemic decrease shown as a CR during evocation. This study found that responders had previously shown a larger UCR after insulin injection on glycemia and had reported significantly more neuroglycopenic symptoms caused by the insulin UCS during acquisition. Also, they rated the CS (an odor) as significantly more intense than nonresponders [29]. In contrast to the other study [21], responders had significantly lower baseline norepinephrine levels than nonresponders [21, 29].

Magnitude of the CR

Four studies (25%) provided explicit information about the magnitude of the CR [21, 27–29]. In one study using CsA as the UCS, the conditioned immunosuppressive response was about 40% of the immunosuppression caused directly by the UCS [21]. The conditioned effects in responders (defined as participants who showed a conditioned IL-2 decrease larger than 1 SD) were as pronounced as the UCR [21]. In a second study, addressing the conditioned effects of insulin, the conditioned decrease in glycemia was 10–15% of the glycemic decrease caused by insulin administration during acquisition [29]. The 2 remaining studies investigated conditioned allergic responses and found a CR about one third of the size of the UCR [27, 28].

Discussion

Conditioning of immune and endocrine parameters in humans offers new insights into the interaction of the central nervous system with peripheral functions, with promising possibilities for future adjunct therapies in a variety of diseases [8–14]. Based on the studies reviewed here, there is relatively strong evidence that conditioning of immunosuppression using CsA [19–23], conditioning of allergic responses using allergens [27, 28], and conditioning of insulin and glycemic responses using intravenous insulin [29, 30, 32, 34] is possible. Regarding immunostimulants, antiallergic effects, and cortisol conditioning, the results look promising, but additional studies are needed to support evidence for the effectiveness of different UCS [9, 24–26, 33]. An important strength of this review lies in its systematic approach, offering a detailed overview of the existing studies in this field of research. To our knowledge, this is the first systematic review of studies on conditioning of pharmacological effects on immune and endocrine parameters in humans, specifically addressing the current knowledge on the influence of design characteristics on the effects of pharmacological conditioning. Also, this review includes studies in different outcome domains, enabling conclusions that are relevant

for all future studies in conditioned pharmacological effects. However, the large heterogeneity, in combination with the small number of studies included in this review, makes it difficult to draw firm conclusions. Most of the studies included in this review used relatively small samples of healthy volunteers, limiting the reliability and generalizability of the studies on an individual level. Together with the small sample sizes reported by some studies, this might point to a possible publication bias. Concerning risks for other biases, due to incomplete reporting of the study protocol in many of the study reports included in this review, the risks of selection, reporting, and attrition bias were unclear for many studies. For the UCS CsA, allergens, and insulin, however, conditioned effects have been replicated at least once [19–23, 27–30, 32], strengthening the conclusion that the effects of these UCS can be conditioned. Overall, the rigor in study selection, especially with regard to methodological aspects, might have led to the exclusion of some studies in patient samples (e.g., inclusion of a placebo control group might be challenging in patients). However, this same rigor in study selection has the important advantage that the studies included in this review adhered to a relatively high scientific standard, making the conclusions drawn from these studies more reliable.

As previous research has indicated that the characteristics of the study design might have an effect on conditioning [19, 27], studies were reviewed in light of the design that was used, providing some potentially important considerations for future studies. The studies reviewed in this paper suggest that the number of acquisition and evocation trials needed in order to obtain a CR depend on the nature of the UCS and the outcome measure targeted for a CR. Generally, most consistent conditioned effects were found in studies using more than one acquisition trial. However, single trial conditioning (only one acquisition trial) was found to be sufficient to condition allergic responses [27, 28], but not immunostimulation [25, 35]. An explanation for this finding may lie in the populations investigated, with allergic participants and healthy volunteers being included, respectively. It may be easier to condition allergic responses in allergic participants who are familiar with the UCS as well as the UCR than to condition immunostimulation in healthy volunteers for whom both UCS and UCR are novel. Secondly, an organism might less easily show CR that endanger homeostasis [36], like an acute systemic inflammatory response, which was used as a CR in the immunostimulation study with LPS [25]. In the allergen studies, the CR were not accompanied by allergic symptoms [27, 28], suggesting that the CR was not large enough to disrupt homeostasis.

Regarding the number of evocation trials needed to evoke a CR, one evocation trial appears sufficient when conditioning with allergens, insulin injections, and dexamethasone [27–30, 33]. This seems to be in contrast to studies using CsA as the UCS; among these, the only study that did not find a conditioned effect was the one using only one evocation trial [19]. However, an alternative explanation for the lack of a CR in this study might lie in the 12-day lag between acquisition and evocation. Regarding the extinction of CR, more differences between the UCS used were found. While the extinction of conditioned immunosuppression appears to be slow [22, 23], conditioned allergic responses showed signs of extinction during the second evocation [27] and conditioned antiallergic responses were extinct after 5 evocation sessions [9]. The relatively faster extinction of the CR in the studies conditioning allergic responses compared to the immunosuppressive studies may potentially be explained by the use of single-trial conditioning in the allergen studies in contrast to 4 CS-UCS pairings employed by the CsA studies, as there are indications that an increase in the number of acquisition trials results in larger CR [27]. Despite their relevance for the possible clinical application of conditioned effects on immune and endocrine parameters [22, 23], only a few studies have addressed extinction processes. Future studies should consider including more than one acquisition trial to increase the chances of finding a CR and more than one evocation trial to explore the possible effects of extinction.

Regarding the characteristics of the CS, all studies included in this review used an olfactory CS, presenting either a taste or an odor as a stimulus, finding conditioned immune and endocrine effects for both CS. Although other types of stimuli have also been used (e.g., visual) [37] in conditioning studies, olfactory CS are used most frequently and may be the best choice considering the adaptive value of learning the consequences of ingestion of certain substances for homeostasis, which is reflected in conditioned taste avoidance of the CS [1, 4, 38]. Conditioned taste avoidance might thus only be the behavioral outcome of a CR that affects the immune and endocrine system [4]. For this reason, assessment of the subjective hedonic qualities of the CS as rated by the subject provides additional information on whether or not some kind of conditioning has taken place, even in the absence of conditioned physiological effects [25].

Concerning the sequence in which the CS and the UCS were presented, most studies administered the CS at the

same time as the UCS (56%) [3, 19–23, 29, 30, 32] or before the UCS (38%) [24, 26–28, 31, 33]; only one study administered the UCS before the CS [25]. However, when the route of administration of the UCS is considered, the majority of the studies administered the CS prior to the UCS, as UCS administered via the oral route take longer to be absorbed and thus 'sensed' by the central nervous system than UCS administered intravenously. Because conditioning theory suggests that the CS should predict the occurrence of the UCS, conditioning is theoretically expected to be most effective in studies in which the CS is presented immediately prior to or together with the UCS [39, 40]. The finding that the only study in this review in which the intravenously administered UCS was presented before the CS found no conditioned effects might indicate that in future studies the CS should be administered immediately prior to or together with the UCS if possible.

As this review shows that the characteristics of the design affect the outcome of studies, future articles should describe the used study design thoroughly so that additional factors that determine the effectiveness of conditioning paradigms can be identified. Also, as little is known about the phenomenon of responders and nonresponders, it would be useful if researchers would provide information on this issue. Moreover, it would be advisable to assess possible predictors of responsiveness to conditioning at baseline. Lastly, the vast majority of the studies reviewed here were conducted in healthy male volunteers. Future studies should address less selective samples to improve generalizability and provide information on possible differences in the conditionability in different groups.

As the CS used in the studies reviewed here is an inert substance, CR on endocrine and immune outcomes are frequently referred to as conditioned or learned placebo effects [1, 4, 7]. They illustrate a close interaction between the central nervous system and peripheral functions regulating homeostasis [1, 2]. Conditioned pharmacological effects show that placebo effects not only affect subjective symptoms but can also alter specific peripheral mechanisms that are outside of conscious control [7]. Thereby, they broaden the future clinical possibilities of using placebo effects in clinical practice. Concrete possibilities for clinical applications of placebo effects induced by pharmacological conditioning have been shown by several studies using conditioning protocols as adjunct therapy in patients. With this approach, beneficial outcomes have been reported in patients suffering from psoriasis [12], ADHD [10, 11], systemic lupus erythematosus [14], and multiple sclerosis [13]. The use of conditioning protocols as adjunct therapy has the potential to reduce unwanted side effects [35] and might also improve the cost-effectiveness of expensive treatments, as fewer and lower doses of a certain medication may be needed to achieve the same clinical results [12, 13, 23]. Even though there are indications that conditioning as an addition to pharmacological interventions is effective, complete and long-term replacement of pharmacological treatments with conditioning might not be possible as the effect sizes found for CR are small and CR become extinct over time. Many of the studies in patient samples have methodological shortcomings, such as the lack of placebo control groups, which is also the reason why they could not be included in this review. Future studies in patients should strive to overcome these issues by applying a randomized placebo-controlled design, using distinct CS, and measuring biochemical as well as subjective outcomes.

To summarize our findings, classical conditioning of immune and endocrine responses is possible for various pharmaceutical substances and has great potential for future clinical applications. If future studies would carefully consider characteristics of the study design (i.e., number of acquisition and evocation trials and type of CS) and report about effect sizes, extinction effects, and conditioning responders and nonresponders, this would provide more insight into the full potential of pharmacological conditioning.

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