CHAPTER FOUR

Human Pharmacological Conditioning of the Immune and Endocrine System: Challenges and Opportunities

Judith Tekampe[*,†], Henriët van Middendorp[*,†], Fred C.G.J. Sweep[†], Sean H.P.P. Roerink[†], Ad R.M.M. Hermus[†], Andrea W.M. Evers[*,†,‡,1]

[*]Health, Medical and Neuropsychology Unit, Faculty of Social and Behavioural Sciences, Institute of Psychology, Leiden University, Leiden, The Netherlands
[†]Radboud University Medical Center, Nijmegen, The Netherlands
[‡]Leiden University Medical Center, Leiden, The Netherlands
[1]Corresponding author: e-mail address: a.evers@fsw.leidenuniv.nl

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Abstract

In this chapter, we review recent studies on conditioned pharmacological effects on immune and endocrine responses in humans, and discuss challenges and opportunities for bringing these effects into clinical practice. By altering physiological mechanisms in part independent of pharmacological agents, pharmacological conditioning has high clinical relevance, as illustrated in some patient studies. Methodological challenges for further investigation include broadening the spectrum of opportunities for conditioned pharmacological effects, by investigating conditioning of substances that have not or not often been used before (e.g., corticosteroids) and unraveling mechanisms by which pharmacological responses become conditioned, thereby identifying characteristics that make conditioning designs effective. As an opportunity to optimize external validity, we introduce a design in which the potential of pharmacological conditioning can be pretested in the laboratory. The feasibility of this design is demonstrated by a pilot study.

International Review of Neurobiology, Volume 138
ISSN 0074-7742
https://doi.org/10.1016/bs.irn.2018.01.002

Pharmacological conditioning occurs when administration of an active pharmacological component (unconditioned stimulus, UCS) is repeatedly paired with the occurrence of another stimulus (conditioned stimulus, CS). Once conditioning has taken place, administration of the previously inert CS will elicit a physiological response similar to the response triggered by administration of the UCS. Pharmacological conditioning has important implications for the study of placebo effects (Colloca, 2014), as the effective qualities of the pharmacological stimulus (UCS) are transferred to a previously inert stimulus (CS). Consequently, conditioned pharmacological effects are also called "learned placebo effects" (Pacheco-Lopez, Niemi, Engler, & Schedlowski, 2007; Schedlowski & Pacheco-Lopez, 2010). A key aspect that makes conditioning and other learning mechanisms particularly interesting when compared to other placebo-inducing mechanisms, such as verbal suggestion, is that placebo effects based on conditioning tend to be larger and can be evoked repeatedly (Colloca, 2014).

One of the most widely known examples of pharmacological conditioning is an experiment by Ader and Cohen (1982). In this study, a special breed of mice, which were prone to develop an autoimmune disease similar to lupus erythematosus, were injected with an immunosuppressant drug, cyclophosphamide (UCS), which helps to suppress the symptoms of their autoimmune disease. This injection was repeatedly paired with administration of a sweet-tasting inert saccharine drinking solution (CS). After several pairings of the two stimuli, administering saccharine solution alone triggered a conditioned immunosuppressive response similar to the effect of cyclophosphamide. The conditioned mice also improved on their autoimmune disease (lower mortality rate, slower development of proteinuria) compared to controls. Later, it was discovered that the same principle could be applied in humans (Olness & Ader, 1992). As shown by this example, pharmacological conditioning has high clinical relevance, as it offers the possibility to alter dysfunctional mechanisms in part independent of pharmacological agents, which may eventually lead to lower health-care costs.

For conditioned pharmacological responses to occur, an association between the sensory input of the CS and the peripheral changes caused by the pharmacological agent (UCS) has to be formed in the central nervous system (CNS). In order to orchestrate an unconditioned response (UCR), the CNS must be able to communicate with the peripheral systems in which the response is to occur (Stockhorst, 2005; Vits et al., 2011). As this bidirectional communication between CNS and periphery is a prerequisite for conditioned pharmacological responses, it provides possibilities to study

the interaction between the CNS and peripheral functions of the immune and endocrine system.

Most experiments investigating conditioned pharmacological responses follow the same basic procedure (Fig. 1, part 2). In the first phase of the experiment, the acquisition phase (also called learning or association phase), an association is formed between the CS, often a gustatory or olfactory stimulus (e.g., taste or smell) and UCS (e.g., an immunomodulatory drug) by paired administration. Often, this paired administration is repeated several times; however, single-trial conditioning, where the CS and UCS are administered together only once, has also proven to be effective in some cases (Barrett, King, & Pang, 2000; Gauci, Husband, Saxarra, & King, 1994; Fig. 1, part 1). In the second phase of the experiment, the evocation phase (also called test phase), the CS is administered alone and it is measured whether or not a conditioned response occurs. When the conditioned response has been evoked by administration of the CS, it will generally decrease in magnitude with every subsequent administration of the CS. This

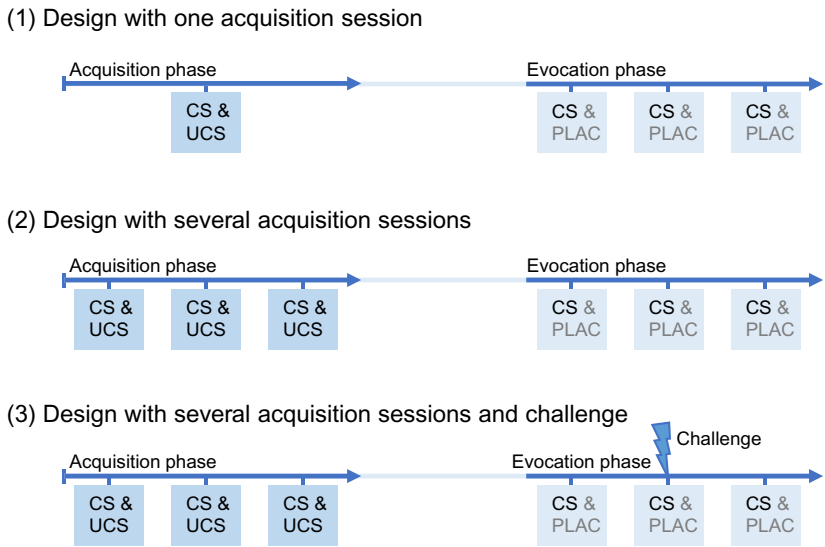

Fig. 1 Examples of different designs used for pharmacological conditioning. The number of expositions to CS and/or UCS and the time interval between expositions and phases may vary between studies. (1) Design with one acquisition session (single-trial conditioning). (2) Design with several acquisition sessions. (3) Design with challenge during the evocation phase to increase external validity. The timing of the challenge may vary between studies. *CS*, conditioned stimulus; *PLAC*, placebo; *UCS*, unconditioned stimulus.

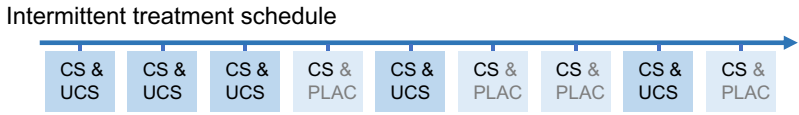

Fig. 2 Intermittent treatment schedule with booster sessions to prevent extinction. This design is used in studies in patient samples. The number of expositions to CS and/or UCS and the time interval between expositions may vary between studies. *CS*, conditioned stimulus; *PLAC*, placebo; *UCS*, unconditioned stimulus.

process is called extinction. One way to prevent extinction (at least to some extent) is to implement an intermittent treatment schedule (e.g., Giang et al., 1996; Longo et al., 1999; Olness & Ader, 1992). In this design (Fig. 2), the CS and UCS are repeatedly administered together to form an association between the two stimuli (similar to an acquisition phase), followed by the CS being administered sometimes alone (similar to the evocation phase in the design described earlier) and sometimes paired with the UCS in a booster session. By paired administration of CS and UCS during the booster session, the association between both stimuli is renewed and the extinction process interrupted.

In this chapter, we briefly review the literature on pharmacological conditioning with the focus on studies in humans to give an overview of what is currently known. We then discuss current challenges and opportunities for clinical application of conditioned pharmacological effects. As part of the solution to some of these challenges, we introduce a design (Fig. 1, part 3) in which the potential of pharmacological conditioning for altering physiological responses involved in pathophysiological processes can be pretested in the laboratory to optimize external validity.

1. PHARMACOLOGICAL CONDITIONING OF IMMUNE AND ENDOCRINE RESPONSES

In the following paragraphs, we will review the recent literature of laboratory studies addressing conditioned pharmacological effects in humans. First we will focus on conditioned immune responses, starting with conditioning in the direction of immunostimulation, followed by immunosuppression and allergic responses. Studies on conditioned endocrine, including glycemic, responses will also be reviewed before finishing this section with a brief discussion of the challenges for future research and clinical application of conditioned pharmacological responses.

1.1 Pharmacological Conditioning of Immune Responses

Research into conditioned pharmacological effects started with the discovery of conditioned immunological responses. First reports of this phenomenon date back to the 1920s or even earlier. In the following years, conditioned responses in the immune system were predominantly investigated in animal studies (see Pacheco-Lopez et al., 2007). In an exemplary study in humans, using a conditioning design with several acquisition and evocation sessions, Buske-Kirschbaum et al. (1992) showed that the increase in the activity of natural killer cells caused by administration of epinephrine could be conditioned. In the acquisition phase of their experiment, they paired a sherbet sweet (CS) with the injection of 0.2 mg of epinephrine (UCS). In the evocation phase, when the CS was administered together with a placebo injection, a conditioned increase in natural killer cell activity (NKCA) was observed. The results of this experiment could only partially be replicated (Kirschbaum et al., 1992). However, more indications for the possibility to condition NKCA by epinephrine and a gustatory CS were found in another study with a discriminant conditioning design. In this study, the administration of one CS, the CS+ (sherbet sweet and white noise), was followed by an injection with the UCS (0.2 mg epinephrine), while the administration of another CS, the CS− (herbal sweet with a specific tone), was followed by no treatment. In the evocation phase, exposure to the CS+ led to an increase in NKCA, while exposure to the CS− had no effect on NKCA (Buske-Kirschbaum, Kirschbaum, Stierle, Jabaij, & Hellhammer, 1994). Taken together, these studies provide relatively strong indications that NKCA can be pharmacologically conditioned in humans.

There are also indications that other proinflammatory immune parameters can be conditioned. One study found higher quinolinic acid and neopterin, and increased CD64 antigen expression after pairing recombinant human interferon gamma (rhIFN-γ, UCS) with the taste of propylene glycol (CS) in an intermittent treatment design (Longo et al., 1999). In another study using a single-trial conditioning design (Fig. 1, part 1), an injection with IFNβ-1a (UCS) was paired with the administration of a distinctively flavored beverage. When the beverage was administered in combination with a placebo injection 8 days later, no conditioned responses were observed (Goebel et al., 2005). Similarly, a study using the same single-trial conditioning paradigm with lipopolysaccharide (UCS) and the same distinctively flavored beverage also found no conditioned immunological effects (Grigoleit et al., 2012). Taken together, these studies suggest that

conditioning proinflammatory immune reactions by pairing immunostimulant drugs with gustatory stimuli is possible, but more evidence is needed to support these findings. Different conditioning designs can be used to investigate these effects, although single-trial conditioning does not seem to be effective for conditioning proinflammatory immune reactions.

Pharmacological conditioning of immune responses in the direction of immunosuppression has also been investigated. In most studies, the oral administration of the immunosuppressant drug cyclosporine A (CsA, UCS) has been paired with a distinctively tasting beverage (CS) in the acquisition phase. When the CS was administered with a placebo during evocation, this consistently led to a conditioned decrease in production of the cytokine interleukin 2 (Albring et al., 2014; Goebel et al., 2002; Ober et al., 2012; Wirth et al., 2011). Some studies also found conditioned reductions in IFN-γ production (Goebel et al., 2002; Wirth et al., 2011) and lymphocyte proliferation (Goebel et al., 2002). All of these are indicators of conditioned immunosuppression. Through variations in the number of evocation sessions between the studies, indications were found that these conditioned immunosuppressive responses may only become apparent after more than one evocation session (Albring et al., 2012). Once conditioned responses have been established, they are relatively stable, as they have shown only to be extinct after 14 evocations (Albring et al., 2014). Also, this type of conditioning can boost the effect of a subtherapeutic dose of CsA to a level that is clinically relevant (Albring et al., 2014), a finding that hints at the possible opportunities for clinical applications based on conditioned pharmacological effects.

Conditioned allergic responses provide more examples of conditioned pharmacological effects. In two studies, allergens (UCS) were applied via a nose spray containing a distinctive smell (CS) (Barrett et al., 2000; Gauci et al., 1994). When the CS was applied alone (nose spray with the smell, but without the allergen) later, conditioned elevations in histamine (Barrett et al., 2000) and tryptase (Gauci et al., 1994) levels in nasal secretion were found. Interestingly, in contrast to studies in conditioned immunostimulation, conditioned responses occurred even after only one acquisition (single-trial conditioning) and were prone to extinction (Barrett et al., 2000). Nonetheless, conditioned responses became larger with more acquisition trials (Barrett et al., 2000). In another study, the dermal application of allergens (UCS) was paired with a visual CS (the color of the vial containing the allergens) during the acquisition phase. In this experiment, no conditioned responses were found (Booth, Petrie, & Brook, 1995). Although

the studies on conditioning with allergens have found effects on histamine and tryptase, which are involved in allergic reactions, none has found effects on allergic symptoms (i.e., congestion of the nose or sneezing). However, Goebel et al. (2008) found that allergic symptoms can be targeted when conditioning antiallergic effects. In this study, the administration of anti-allergic medication (desloratadine, UCS) was paired with a distinctively tasting beverage (CS) during the acquisition phase. When the beverage was later administered in combination with a placebo, a conditioned decrease in basophil activation, a reduced reaction to a skin-prick test, and lower symptom scores were found. In an attempt to replicate these promising results in another study, however, the antiallergic effects of conditioning did not exceed those in the placebo group, but both groups improved in symptoms when compared to a natural history group. This finding suggests that conditioning as well as expectation-based effects may be at play (Vits et al., 2013). Concluding, there is at least preliminary evidence that pharmacological conditioning of immune responses in the direction of immunostimulation and immunosuppression, as well as allergic reactions, is possible in the laboratory.

1.2 Pharmacological Conditioning of Endocrine Responses

Not only immune but also endocrine responses can be affected by pharmacological conditioning. Most studies in this direction focus on conditioned responses in blood glucose levels (glycemic responses). Early reports of indications for conditioned glycemic responses in humans were made in 1959, when Russian experimenters found anticipatory hypoglycemic symptoms in schizophrenic patients receiving insulin shock therapy (Stockhorst et al., 1999). After several animal studies in which conditioned glycemic responses were found (Stockhorst, 2005; Stockhorst, Steingruber, & Scherbaum, 2000), the possibility of conditioned responses in humans received the attention of investigators as possible mechanism behind eating disorders (Overduin & Jansen, 1997). More controlled studies in humans yielded mixed results, finding conditioned responses in blood glucose after conditioning with insulin in some participants but not in others (Fehm-Wolfsdorf, Gnadler, Kern, Klosterhalfen, & Kerner, 1993). In the first placebo-controlled experiment in humans, Stockhorst et al. (1999) showed that by repeatedly pairing an injection of insulin (UCS) with the administration of a distinctive smell (CS), the effect of intravenous insulin on blood glucose became conditioned. In a later study, conditioned effects of insulin

on other hormones involved in blood glucose regulation also became apparent (Stockhorst et al., 2004). Interestingly, when intranasal insulin was used as a UCS (Stockhorst, de Fries, Steingrueber, & Scherbaum, 2011), which leads to an increase of the level of insulin in the brain while no changes in blood glucose level occur peripherally, the conditioned response encompasses changes in insulin rather than peripheral blood glucose. This is in contrast to the studies in which intravenous insulin was used as UCS, causing changes in peripheral blood glucose but not in insulin. In these studies, conditioned changes in blood glucose but not in insulin were found (Stockhorst et al., 1999, 2004). This indicates that conditioned pharmacological responses can be very specific, mimicking the bodily reaction the UCS causes (Stockhorst, 2005). Two studies attempted to obtain conditioned changes in blood glucose by using glucose administration as a UCS (Overduin & Jansen, 1997; Stockhorst et al., 2004), which found no conditioned effects. Taken together, these studies provide strong indications that responses involved in blood glucose regulation can be conditioned. Whether these conditioned responses play a role in maladaptive eating behavior and thus eating disorders remains subject to further investigation.

Effects of pharmacological conditioning on other endocrine responses, predominantly corticosterone responses, have been shown in rodents (e.g., Ader, 1976; Buske-Kirschbaum et al., 1996; Janz et al., 1996; Pacheco-Lopez et al., 2004). Conditioning endocrine, specifically hypothalamic–pituitary–adrenal axis (HPA axis) responses, in humans is potentially of great clinical relevance (Sabbioni et al., 1997), as dysfunctions in HPA axis regulation are thought to play a role in various disorders (Fries, Hesse, Hellhammer, & Hellhammer, 2005; Putman & Roelofs, 2011; Yehuda & Seckl, 2011). Only 3 studies so far have investigated conditioned endocrine effects in humans. In the first study, the oral administration of dexamethasone (UCS) was repeatedly paired with the administration of a distinctively tasting beverage (CS) in the acquisition phase. When the CS was administered with a placebo during the evocation phase, indications for a conditioned increase in plasma cortisol were found, but post hoc tests remained inconclusive (Sabbioni et al., 1997). In the second study (Benedetti et al., 2003), sumatriptan was used as a UCS and a conditioned decrease in cortisol and increase in growth hormone were found. Recently, a third study in which corticotropin-releasing hormone was used as UCS was unable to find conditioned responses in cortisol and noradrenaline. Further analysis of the data by a median split revealed indications that participants with high baseline cortisol did show a conditioned increase in cortisol, but participants with

low baseline cortisol did not (Petrakova et al., 2017). In sum, there are some indications for conditioned HPA axis responses in humans, but the precise extent and relevance of these responses remains to be elucidated.

2. CHALLENGES

Numerous challenges have to be addressed before the opportunities of pharmacological conditioning are fully unraveled. One of the most important challenges is broadening the spectrum of opportunities for conditioned pharmacological effects, by investigating conditioning of substances that have not or not often been used before (e.g., corticosteroids). Another challenge is the unraveling of the mechanisms by which pharmacological responses become conditioned and thereby identifying characteristics that make conditioning designs effective.

The UCSs used in the studies so far have mostly been immunomodulatory drugs. This is hardly surprising, considering the historical origin of pharmacological conditioning in conditioned immune responses (Pacheco-Lopez et al., 2007). However, the few studies on conditioned endocrine responses show that the potential of pharmacological conditioning is not limited to modulating immune responses. Expanding the focus of laboratory studies from conditioning of the immune system to other regulatory systems is important in revealing more opportunities for clinical application of conditioned pharmacological responses. Conditioning of glycemic responses, for example, might provide insight into the mechanisms involved in onset and maintenance of eating disorders and obesity and eventually may also offer new treatment possibilities. In the same way, conditioned HPA axis responses may bear relevant implications for understanding and ultimately treating stress-related disorders, as dysregulation of the HPA axis seems to play a role in these disorders.

The studies reviewed earlier show differences in the characteristics of the study design potentially related to successful conditioning of pharmacological responses for different UCSs. Regarding the acquisition of conditioned responses, it is generally accepted that studies with more acquisition trials produce conditioned effects more reliably. However, the finding that single-trial conditioning works for some UCSs (allergens) but not others (immunostimulants) seems to indicate that some responses are more readily conditionable than others. This finding corresponds to differences in conditionability of stimuli in fear conditioning studies, where evolutionary factors are thought to play a role (Ohman, 2009). What factors are behind

differences in conditionability in pharmacological conditioning remains to be elucidated. Regarding the evocation and ultimately extinction of conditioned pharmacological responses, more differences between various UCSs become apparent. While conditioned immunostimulation and allergic responses emerge at the first evocation, conditioned immunosuppressive responses induced by CsA conditioning may only show after two evocations (Albring et al., 2012). In contrast to allergic responses that show signs of extinction at the second evocation, these immunosuppressive responses remain rather stable across several evocations (Ober et al., 2012; Wirth et al., 2011). A possible explanation for these differences in UCSs may lie in the mechanisms by which an association between CS and UCS is formed in the CNS and a conditioned response is generated by CNS–peripheral interaction. These mechanisms seem to differ between UCSs (Schedlowski & Pacheco-Lopez, 2010). Potentially these underlying mechanisms also differ depending on the population targeted, with on the one hand allergic patients who are familiar with the UCS as well as UCR and on the other hand healthy volunteers for whom UCS as well as UCR is unfamiliar prior to the study (Tekampe, van Middendorp, Meeuwis, et al., 2017). More research into the underlying mechanisms by which conditioning takes place in different populations may provide insight into the design characteristics that make for successful conditioning and hence point to opportunities for effectively bringing conditioned pharmacological responses into clinical practice.

In order to understand the mechanisms involved in conditioning of pharmacological effects, it is important to understand the exact nature of the CS and UCS. The CSs used in the various studies are mostly gustatory or olfactory (taste or smell) stimuli. Forming an association between the ingestion of a certain substance (identified by its taste and/or smell, CS) and its consequences for homeostasis (the effect of the drug, UCR) is considered to have an adaptive value for the survival of an organism (Pacheco-Lopez et al., 2007; Schedlowski & Pacheco-Lopez, 2010). By integrating the two stimuli, the organism is able to learn which substances produce favorable bodily changes and are thus suitable for ingestion and which substances produce changes that threaten homeostasis and should thus be avoided. The latter phenomenon, conditioned taste avoidance, is frequently observed in animal studies on pharmacological conditioning and regarded as one of the outcomes of pharmacological conditioning (Pacheco-Lopez et al., 2007; Schedlowski & Pacheco-Lopez, 2010). This basic learning mechanism is the reason that gustatory or olfactory stimuli seem to be the first choice

for pharmacological conditioning. In some studies, the gustatory or olfactory stimuli were combined with auditory (Buske-Kirschbaum et al., 1994) or tactile (Buske-Kirschbaum et al., 1992) stimuli. Another study employed a visual CS, but was unsuccessful in finding conditioned responses (Booth et al., 1995). To what extent the sensory domain of the CS is a determinant for the success of the conditioning procedure remains to be elucidated. Regarding the nature of the UCSs, it is argued that not so much the drug itself but the physiological reaction caused by the drug is the UCS (Woods & Ramsay, 2000). This is important in explaining that some conditioned pharmacological responses, such as immunosuppressive responses, are mimicking the effect of the drug, while others are counterregulatory (Ramsay & Woods, 1997).

From this overview of the research on pharmacological conditioning in humans, it can be concluded that pharmacological conditioning has great potential in affecting immune and endocrine responses. Successfully addressing the challenges discussed earlier will expand our knowledge about the full potential of these responses and, maybe even more importantly, help finding ways to bring conditioned pharmacological responses out of the lab and put them to use in clinical settings.

3. OPPORTUNITIES FOR RESEARCH AND CLINICAL PRACTICE

Conditioned pharmacological effects offer great opportunities for clinical practice. If the effects of drugs can be learned and ultimately produced without the actual presence of the pharmacological agent, this could reduce the amount of medication patients are required to take. As a consequence, pharmacological conditioning might be part of the solution of problems in health-care systems worldwide, such as rising costs or avoidable side effects. When translating findings into opportunities for clinical practice, two main challenges of conditioned pharmacological effects have to be met. The size of the conditioned response has to be clinically relevant (Ader, 2003) and, to be of use for patients requiring medication for longer periods of time, effects of conditioning have to be resistant to extinction (Albring et al., 2014).

Regarding the size of the conditioned responses, pharmacological conditioning in lab research often yields small responses (Tekampe, van Middendorp, Meeuwis, et al., 2017), for example, 10%–15% of the UCR (Stockhorst et al., 1999). It has been argued that this may in part be to

maintain homeostasis, as it seems unlikely that bodily systems will produce responses that will threaten their own health (Ader, 2003). However, this does not necessarily mean that conditioned pharmacological responses cannot be large enough to be clinically relevant. A frequently observed example of the strength of conditioned responses is found in conditioned nausea and vomiting, a phenomenon that frequently occurs in cancer patients receiving chemotherapy. In these patients, stimuli such as the hospital environment (CS) that predict the administration of chemotherapy (UCS) have become associated with the immediate negative side effects of the chemotherapy, including nausea and vomiting. Resultantly, only being in the hospital can evoke these symptoms (Stockhorst, Enck, & Klosterhalfen, 2007). Together with studies on conditioned antiallergic effects, reviewed earlier, these findings show that, despite the relatively small conditioned effects found in laboratory studies (Ader, 2003; Tekampe, van Middendorp, Meeuwis, et al., 2017), conditioned pharmacological responses can elicit clinically relevant symptoms. Further studies in patient samples will be needed to unravel the full potential of pharmacological conditioning in eliciting clinically relevant changes in symptoms.

Extinction could potentially be prevented in two ways. Lab research has shown that conditioned immunosuppressive responses can be prevented from extinction by pairing the CS with a subtherapeutic dose of the UCS during the evocation phase instead of administering the CS alone or with a placebo (Albring et al., 2014). Another way of preventing extinction that has already been tested in patient samples is by using a conditioning design entailing an intermittent treatment schedule (Fig. 2), in which booster sessions are used to avoid extinction (Doering & Rief, 2012). In a case study by Olness and Ader (1992), an intermittent treatment design was used to lower the monthly dose of cyclophosphamide in a patient suffering from lupus erythematosus. To this end, the administration of cyclophosphamide (UCS) was paired with the intake of a specific taste and smell (CS). By using an intermittent treatment schedule, the patient improved clinically while receiving only half of the cumulative dose normally necessary to treat the symptoms. However, the nausea induced by cyclophosphamide became associated with the CS as well, causing the patient to suffer from conditioned nausea and ultimately discontinuing the initiated treatment. Preliminary research also suggests that intermittent treatment designs with cyclophosphamide might help lower the cumulative dose needed to treat symptoms of multiple sclerosis (MS). In a pilot study, the monthly cyclophosphamide doses (UCS) of 10 MS patients were paired with the taste of anise syrup (CS)

(Giang et al., 1996). When in 1 month a subtherapeutic dose of cyclophosphamide was administered together with the CS, 8 of the 10 subjects showed at least a small decrease in white blood cell count, which might reflect a conditioned response. In 4 subjects, this decrease after CS alone was of similar magnitude as the decrease caused by cyclophosphamide administration. Regarding side effects, 7 of the 10 included subjects experienced nausea and/or vomiting as side effect of cyclophosphamide administration. Administration of the CS alone led to the same side effects in 4 of the 10 subjects, providing indications that the negative side effects of the UCS may also become conditioned, but are less prevalent than in usual treatment regimes. Intermittent treatment schedules have not only been used to lower the cumulative dose of cyclophosphamide. In patients using a corticosteroid ointment to control symptoms of psoriasis, a conditioning procedure with an intermittent treatment design was tested. Patients on the intermittent treatment schedule received the full dose of corticosteroid treatment 25%–50% of the time and placebo at other times. Compared to a control group receiving the same cumulative dose, the patients in the intermittent treatment group showed lower symptom severity and less relapse (Ader et al., 2010). Other preliminary reports of positive effects of conditioned pharmacological effects by means of an intermittent treatment schedule in patients come from studies in ADHD (Sandler & Bodfish, 2008; Sandler, Glesne, & Bodfish, 2010) and hypertension (Suchman & Ader, 1992). In an ongoing study, the potential effects of conditioning on the achievement of a higher percentage of drug-free clinical remission with the same cumulative dose of active medication by implementing an intermittent treatment schedule with methotrexate and placebo in patients suffering from rheumatoid arthritis are currently investigated (Manaï, van Middendorp, Veldhuijzen, & Evers, 2017).

Concluding from the limited research in clinical samples so far, conditioned pharmacological effects are potentially large enough to be brought into practice by using intermittent treatment schedules in patients who need to use medication for longer periods of time. As in lab research, conditioned pharmacological effects in patient samples are most frequently investigated in immunomodulating drugs. However, as lab studies show that endocrine responses can be conditioned as well, the clinical potential of conditioned pharmacological responses may not be limited to altering immune responses but may also provide opportunities to alter dysfunctional endocrine responses that might be relevant for eating disorders (glycemic responses) or stress-related disorders (HPA axis responses). It has been argued that

intermittent treatment schedules might also help to reduce the negative side effects of medication (Goebel et al., 2005). As seen in the case of nausea caused by cyclophosphamide, however, negative symptoms can also become associated with, and hence elicited by, the CS (Olness & Ader, 1992). Still these conditioned side effects may be less frequent than the side effects elicited by administration of cyclophosphamide (Giang et al., 1996).

4. BRIDGING THE GAP BETWEEN LAB AND PRACTICE: INCREASING THE EXTERNAL VALIDITY OF LABORATORY STUDIES BY USING REAL-WORLD CHALLENGES

Research in patient samples may eventually make it possible to bring conditioned pharmacological effects out of the lab and into clinical practice. However, the intermittent treatment protocol used in patient studies entails replacing at least some doses of the active drug with an initially inert CS. This involves the risk that—if conditioning is unsuccessful—the patients do not receive the medication necessary to treat their symptoms and deteriorate clinically. A way to pretest in the lab the potential of pharmacological conditioning for altering physiological responses involved in diseases is offered by an adaptation of the experimental design (Fig. 1, part 3). Based on the classical design with acquisition and evocation phase frequently used in previous studies (e.g., Barrett et al., 2000; Buske-Kirschbaum et al., 1992; Goebel et al., 2002; Sabbioni et al., 1997; Stockhorst et al., 1999), this design includes a relevant real-world challenge of the system in which the conditioned response is to occur during the evocation phase to optimize the external validity. This challenge forms a direct test of the possible clinical relevance of conditioned effects. Examples of relevant challenges of the system in which the conditioned response is to occur are a physiological histamine challenge for conditioned antiallergic responses (Meeuwis, van Middendorp, Veldhuijzen, Pacheco-Lopez, & Evers, 2017), exposure to an immunostimulating vaccination after a psychological training aimed to optimize the immune system (Schakel et al., 2017), or exposure to emotional stimuli, infant crying sounds, and pain during an MRI scan after conditioning with oxytocin (Skvortsova et al., 2016).

To illustrate the feasibility of this design in more detail, a pilot study in 10 healthy participants aiming to condition cortisol levels will be described. Cortisol is a key stress-regulatory parameter, suspected to be involved in the onset and maintenance of various stress-related disorders (Fries et al., 2005; Putman & Roelofs, 2011; Yehuda & Seckl, 2011). If cortisol could be

conditioned, this may have clinical implications for these disorders, but conditioned effects under basal circumstances may not generalize to the cortisol stress response, which is particularly important in this regard (Putman & Roelofs, 2011). In a pilot study in 10 participants (Tekampe et al., 2014; Tekampe, van Middendorp, Sweep, et al., 2017), hydrocortisone (UCS) was paired with a distinctively tasting beverage (CS) 3 times on consecutive days during the acquisition phase. In the evocation phase, again consisting of 3 sessions on 3 consecutive days, the CS was administered paired with a placebo. In the first and second evocation session, conditioned effects (e.g., salivary cortisol levels) were measured under basal circumstances, with participants completing undemanding filler tasks between measurements. In the third evocation session, participants were exposed to a psychosocial challenge by means of the Trier Social Stress Test (TSST). Consisting of a 5-min anticipation time, 6-min presentation, and 4-min mental arithmetic task in front of a critical and nonaffirmative panel of sham experts, the TSST reliably induces an HPA axis response, including elevation in cortisol levels (Denson, Spanovic, & Miller, 2009; Dickerson & Kemeny, 2004). This way, conditioned responses on the cortisol response to stress, which is affected in many stress-related disorders, can be investigated. The pilot study showed the feasibility of the design and preliminary results indicated that conditioning with cortisol may affect cortisol levels under basal circumstances as well as in exposure to psychosocial stress. Particularly after exposure to stress, the conditioned participants additionally appeared to have a lower negative affective response compared to subjects in the placebo group (Tekampe et al., 2014; Tekampe, van Middendorp, Sweep, et al., 2017). This might be a first indication that some effects of pharmacological conditioning specifically become apparent by applying challenges during evocation.

In sum, laboratory studies on pharmacological conditioning in which a challenge of the targeted system is implemented during the evocation phase may provide an opportunity to pretest the clinical potential of conditioned effects and to optimize external validity of designs. An important advantage over studies in patients is that this approach does not entail the risk of clinical deterioration of the disease and that it can be applied in the controlled environment of the laboratory.

5. GENERAL CONCLUSION AND DISCUSSION

Pharmacological conditioning has high clinical relevance for altering physiological mechanisms in the immune and endocrine system in part independent of pharmacological agents. As conditioned pharmacological effects

illustrate the close interactions between the CNS and peripheral functions (Stockhorst, 2005; Vits et al., 2011), they also are of conceptual interest in understanding placebo effects (Schedlowski & Pacheco-Lopez, 2010). Laboratory research has shown that using pharmacological conditioning in humans has great potential in affecting immune and endocrine responses (Sabbioni et al., 1997; Stockhorst et al., 1999, 2011, 2004). Studies on conditioned glycemic responses have illustrated that conditioned pharmacological responses can be very specific (Stockhorst, 2005; Stockhorst et al., 2011) mimicking not so much the direct effect of the drug, such as elevation of a certain hormone level in the blood, but rather the reaction to the drug, such as a counterregulatory response to reduce the hormone level to a physiological quantity. This finding may contribute to the clinical relevance of conditioned pharmacological effects, as they offer the possibility to target specific responses. Most research has focused on conditioned immune responses (Albring et al., 2014, 2012; Barrett et al., 2000; Booth et al., 1995; Buske-Kirschbaum et al., 1994, 1992; Gauci et al., 1994; Goebel et al., 2005, 2008, 2002; Grigoleit et al., 2012; Kirschbaum et al., 1992; Longo et al., 1999; Ober et al., 2012; Wirth et al., 2011) and despite the potential clinical relevance, there is a lack of studies addressing pharmacological conditioning of endocrine functions (Tekampe, van Middendorp, Meeuwis, et al., 2017). Also, conditioned effects found in the lab are generally small and some conditioned responses, such as allergic responses, are prone to relatively quick extinction. Despite these challenges, a small number of studies have brought pharmacological conditioning into clinical practice by using intermittent treatment schedules with preliminary but positive results (Ader et al., 2010; Giang et al., 1996; Olness & Ader, 1992; Sandler & Bodfish, 2008; Sandler et al., 2010; Suchman & Ader, 1992). Studies addressing pharmacological conditioning can be risky in patient samples, as symptoms may flare up if conditioned responses do not occur. A new way to help bridge the gap between laboratory research and clinical practice was found in an adaptation of the experimental design, implementing a challenge of the system in which the conditioned response is to occur to optimize external validity. This design offers the possibility to pretest the clinical potential of pharmacological conditioning for altering physiological responses in the lab and in healthy participants. A pilot study with this design was conducted, aiming to condition cortisol levels by using hydrocortisone as UCS and challenging the HPA axis by implementing a social stress test during the evocation phase. Preliminary results suggest that some effects of pharmacological conditioning might

specifically become apparent by challenging the system that was targeted by conditioning. Future studies including comparable relevant real-life challenges of the conditioned (immune/endocrine) systems offer the opportunity to examine the possible external validity of pharmacological conditioning. If conditioned pharmacological responses can successfully be brought into practice, the ability to transfer the effective qualities of a pharmacological agent to a previously inert stimulus might help reduce the amount of medication patients are required to take and ultimately help lower health-care costs.

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