Placebo effects and the endocrine system: the role of oxytocin

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Chapter 7

Summary and General Discussion

The aim of the current dissertation was to explore the link between the endocrine system and placebo effects with a focus on the hormone oxytocin. Two perspectives were examined: the possibility to elicit placebo effects in the endocrine system (**Part I**) and influencing placebo and nocebo effects by hormones (**Part II**).

With regard to the first perspective where the focus was on eliciting placebo effects in the endocrine system, we have systematically reviewed studies on classical conditioning of endocrine responses in animals and humans in **Chapter 2**. Classical conditioning is one of the underlying mechanisms of the placebo effect and with this systematic review we aimed to investigate whether it was possible to elicit hormonal changes through this type of placebo manipulation. Overall, we demonstrated that a substantial body of evidence exists on the possibility to trigger endocrine changes with conditioning in animals and that the results of animal studies correspond to the results from human research. However, we have also found that human research is quite limited, as evidence of classically conditioned hormonal responses in humans exists so far only for insulin, cortisol, and growth hormone. No standard protocol for eliciting conditioned endocrine response was found: different studies used different numbers of sessions, different conditioning procedures and different conditioned stimuli. In general, our review demonstrated that there is a large potential for endocrine conditioning, however, no consensus yet exists in this field regarding a standard conditioning design and not all endocrine systems have been investigated, especially in humans. In **Chapters 3** and **4** we described the results of a large randomized controlled trial (RCT) in which we aimed to expand the knowledge from our systematic review and investigated the possibility to condition a hormone that has not been studied before in this context, oxytocin. In **Chapter 3** we described the results of the RCT demonstrating that we succeeded to classically condition endogenous oxytocin release and that this conditioned response became extinct within three evocation days. We showed conditioned effects in endogenous oxytocin levels; however, conditioning did not influence participants' responses to a social task on the evaluation of faces or a pain sensitivity task, which have both been shown to be sensitive to oxytocin effects previously. **Chapter 4** focused on the imaging part of this RCT. The data showed that there was some indication for conditioned oxytocin response in the brain, particularly in the amygdala and

the superior temporal gyrus in response to pictures of fearful faces, stimuli that are commonly used in oxytocin research. However, the conditioned response was relatively small in comparison to actual oxytocin effects and did not generalize to other tasks previously shown to be affected by oxytocin.

Part II of this dissertation was dedicated to the link between placebo and nocebo responses and oxytocin. **Chapter 5** presents results of an RCT in females in which we investigated whether it was possible to enhance placebo effects for pain and itch by using oxytocin. In this study we demonstrated that giving verbal suggestions regarding the pain- and itch-relieving properties of a nasal spray induced a significant placebo analgesia but did not affect itch sensitivity. Importantly, there was no boosting effect of intranasal oxytocin on the placebo effect found. To address the possible limitations of this study, we designed a follow-up study that is described in **Chapter 6,** in which we explored the effects of intranasal oxytocin on placebo and nocebo effects for pain. In contrast to the study described in Chapter 5, this trial included male participants, a higher dosage of oxytocin, and the placebo effects were induced by a combination of verbal suggestions and classical conditioning. The results of this trial are congruent with the results of the study described in Chapter 5: we demonstrated that the combination of verbal suggestions and classical conditioning induced significant placebo and nocebo effects for pain, but that oxytocin did not influence either effect or the extinction of both. With these two studies we conclude that there is no evidence to state that different dosages of oxytocin influence placebo and nocebo effects in male and female samples.

To sum up, the current dissertation described the link between placebo effects and the endocrine system, particularly oxytocin. We demonstrated that there is accumulating evidence in the literature that placebo effects can be elicited in the endocrine system. Moreover, we described results of a randomized controlled trial in which we successfully conditioned oxytocin release. Finally, we showed a lack of evidence that oxytocin can influence placebo and nocebo effects.

General discussion

The aim of this dissertation was to investigate the connection between placebo effects and the endocrine system with a focus on the hormone oxytocin. We looked at this link from two perspectives: eliciting placebo effects in the endocrine system, particularly oxytocin, and influencing placebo effects by oxytocin. First, we did a systematic review of the literature to summarize the existing evidence on classically conditioning the endocrine system. Then, we performed a randomized controlled trial and studied the possibility to classically condition oxytocin effects. We looked at the effects of classical conditioning on endogenous oxytocin release, performance of a social task, pain sensitivity, and brain activation. Furthermore, in two randomized controlled trials we studied whether oxytocin increased placebo and decreases nocebo responses in pain and itch. To summarize, the performed studies provide a novel perspective on the link between placebo effects and oxytocin, and emphasize the importance of studying the connection between the endocrine system and placebo effects. In this chapter we discuss the results of the dissertation, mention several limitations that should be addressed in future research, and discuss clinical implications and scientific relevance of this work.

Placebo effects in the endocrine system

Our review indicated that placebo effects in the endocrine system have been studied in the context of pharmacological conditioning. In pharmacological conditioning an association between a drug (unconditioned stimulus, US) and an initially neutral conditioned stimulus (CS) is established and further presentation of the conditioned stimulus triggers a physiological response similar to the drug response. Pharmacological conditioning of hormonal responses attracted a lot of interest in the 20th century since Pavlov described the principles of classical conditioning (1): especially a lot of animal research focused on the possibility to trigger hormone release or decrease by classical conditioning. In the 1980s, two reviews were written on this topic, summarizing the accumulating evidence on the endocrine conditioning at that time (2, 3). However, as we have demonstrated in Chapter 2, the interest in this topic significantly dropped in more recent years and only few recent studies looked at conditioning of hormones in humans. In this dissertation we come back to this clinically relevant topic as we believe that being able to modify

hormonal levels with placebo effects might have a lot of potential clinical implications, for example, for patients who require hormonal treatments.

Chapter 2 describes a systematic review of the literature on pharmacological conditioning in the endocrine system and demonstrates that there is a large body of animal research supporting the hypothesis that hormonal levels can be modified by conditioning. Moreover, a smaller number of human studies conducted in this area support the evidence coming from the animal research. Evidence exists for conditionability of corticosterone, insulin, sex hormones, oxytocin, adrenaline, and noradrenaline in animal research (4), but only limited hormones, namely cortisol (5-7), growth hormone (6), and insulin (8), have been investigated in human conditioning research. One of the main questions from the systematic review could not be conclusively answered based on the current evidence base: are all hormonal responses malleable by classical conditioning?

To shed more light on this question, we investigated classical conditioning of a hormone never researched in humans with this paradigm before, oxytocin. The results of this study are described in Chapter 3. We applied a standard conditioning design with acquisition and evocation sessions. During the acquisition session, oxytocin was couple with a conditioned stimulus, a smell of rosewood oil. During the evocation sessions, oxytocin was replaced with placebo and presented with the smell, and several outcome variables were measured. We found a conditioned oxytocin release in saliva after participants received a placebo spray with the smell. This is the first study that investigated the possibility to condition oxytocin placebo release in humans. The only other study that explored this topic was done in rats (9) and also found conditioned oxytocin release in rats in response to placebo given with the CS. Therefore, we were successful to confirm conditionability of oxytocin in humans. Moreover, in Chapter 4, we describe the results of the fMRI scan done on the third evocation day, when the conditioned effect on oxytocin release had already gone extinct. We demonstrated that there is some indication for a conditioned response in the brain, particularly in the amygdala and superior temporal gyrus, although these effects were very small and not generalizable across tasks. This study was the first study about the effects of classical conditioning

with oxytocin on brain activity and even though the effects we found were quite small, these results indicate the potential of studying brain responses to hormonal conditioning.

Optimizing the strength of the conditioned response

It is important to mention that not all studies aimed at hormonal conditioning were successful. For instance, Petrakova and colleagues (10) investigated the possibility to elicit conditioned cortisol release by using intravenous injections of corticotrophin-releasing hormone as an unconditioned stimulus and a distinctive flavored drink as a conditioned stimulus. After two acquisition trials in which they coupled the CS and the US, no change in endogenous cortisol level was found on the evocation trials in response to CS with a placebo. Moreover, Stockhorst and colleagues (11) found conditioned glucose decrease but no conditioned insulin release in a study aimed at conditioning of insulin responses. Classical conditioning, possibly, depends to a large extent on the unconditioned stimulus chosen. The unconditioned response is not necessarily identical to the drug, but rather the changes that happen in the body due to the drug. The afferent signal sent to the brain by these changes constitute the unconditioned stimulus (12). Therefore, to successfully condition changes in certain hormone levels, it is important to understand the physiological action of the US used. In Chapters 2 and 3, we chose intranasal oxytocin as the US. It has been proposed that intranasal oxytocin administration triggers endogenous oxytocin release by feed-forward mechanisms, so that circulating oxytocin stimulates further oxytocin release (13-15). Possibly, because of the direct effects of the intranasal oxytocin on the brain and further endogenous oxytocin release, we were successful in establishing a connection between the US and CS.

Another important factor of classical conditioning is the strength and the duration of the conditioned response. It remains unknown what number of acquisition sessions (the sessions in which the associations between US and CS are established) is necessary to cause a clinically significant and long-lasting conditioned response. From a learning perspective, a larger number of acquisition sessions should lead to a stronger conditioned response (1). However, endocrine conditioning research on this topic remains quite scarce. In the review described in Chapter 2 we found some evidence for a link between the number of acquisition sessions and the strength of conditioned corticosterone responses in animals: conditioned

corticosterone decrease (16) and release (17) were shown to be stronger after a larger number of acquisition trials. No human studies so far looked at the link between the number of acquisition trials and the strength of the endocrine conditioned responses.

In the study described in Chapters 3 and 4 we show that 3 acquisition trials were enough to trigger conditioned oxytocin release. Moreover, we found a fast extinction of this response: on the second evocation day there was only a trend of the conditioned oxytocin release left and on the third evocation day no conditioned response was found anymore in saliva, with only a modest indication of the conditioned response in brain activity. To our knowledge, our study was the first one to examine extinction of the conditioned endocrine responses in humans. It remains unknown if a larger number of acquisition trials would lead to a stronger conditioned response and slow down the extinction process.

Another way to measure a conditioned hormonal response and possibly increase it, would be including a challenge, that would stimulate hormone production, during the evocation session (18). Possibly, during the challenge the effects of classical conditioning would be more visible. An example of such a challenge is proposed in the pilot study of Tekampe (19) where Trier Social Stress test was added to the evocation phase of the cortisol conditioning experiment. In case of oxytocin conditioning, such a challenge would be a task that can stimulate oxytocin production, such as watching an emotional video (20) or receiving a massage (21). However, this remains a hypothesis before more studies investigate the conditioned hormonal responses during such challenges and find the suitable challenges for different hormonal systems.

The role of oxytocin in placebo and nocebo effects

Part II of this dissertation considers the link between placebo effects and the endocrine system from another perspective, namely the extent to which oxytocin can potentially influence placebo and nocebo effects. The hypothesis that oxytocin might be a mediator of the placebo effect was initially proposed by Enck and Klosterhalfen (22). They named several reasons why oxytocin might influence placebo effects: oxytocin has been shown to increase trust (23) and reduce stress (24). Moreover, oxytocin stimulates the secretion of nitric oxide that has been linked to the relaxation response (25), another possible mediator of

the placebo effect. So far, the findings on the possible role of oxytocin were contradictory. Kessner and colleagues (26) have found that exogenous oxytocin boosted placebo analgesia induced by verbal suggestions in men. However, Colloca and colleagues (27) have found no effects of exogenous oxytocin on the placebo effect in pain induced by verbal suggestions in men or in women. Since it is important to find ways to boost placebo effects and decrease nocebo effects, in Chapters 5 and 6 we investigated the possible impact of oxytocin on placebo and nocebo effects. In these two randomized controlled trials we employed two different methods of placebo effect induction: verbal suggestions and classical conditioning, tested female and male samples, investigated placebo effects in two somatic symptoms (pain and itch), and used two different dosages of oxytocin: 24 IU and 40 IU. The results of these two studies are consistent: placebo and nocebo effects for pain were successfully induced by the chosen procedures, but no effect of oxytocin was found.

These two studies have several unique aspects. The trial presented in Chapter 5 was the first study investigating the effects of oxytocin on placebo effects in itch. Most placebo research focuses on pain, and the previous studies of Kessner and colleagues (26) and Colloca and colleagues (27) have also looked at this symptom. Interestingly, our placebo manipulation, giving verbal suggestions about pain- and itch-relieving properties of a nasal spray, induced a significant placebo pain reduction but did not affect itch sensitivity. Research on the possibility to induce placebo effects for itch varies: Darragh and colleagues (28) have used verbal suggestions accompanied with a video about the itch-relieving properties of a placebo cream and demonstrated a decrease in subjective ratings of itch induced by histamine iontophoresis. On the other hand, several studies (29, 30) have failed to induce placebo effects for itch using positive verbal suggestions. Even though pain and itch are closely related symptoms (30), more evidence exists about the possibility to induce placebo effects with verbal suggestions for pain than for itch and our study adds to that.

Chapter 6 presents the results of the first study that investigated a possible influence of oxytocin not only on placebo but also on nocebo effects for pain. The underlying physiological mechanisms of nocebo effects are in general less investigated and the only study that focused on possible hormonal influences of

nocebo responding showed that nocebo effects might be enhanced through the hormone cholecystokinin (31). We did not have a directional hypothesis about whether oxytocin would enhance or decrease nocebo hyperalgesia. We expected that it might speed up the extinction of the nocebo effect for pain, since it has previously been shown that oxytocin facilitates the extinction of conditioned fear that was also induced by pain stimulation (32). However, our hypotheses were not confirmed: no effect of oxytocin on either the size of placebo and nocebo effects or their extinction was found.

Possibly, minor differences in the study designs between our studies and the study of Kessner and colleagues (26) could explain the differences in the results. For instance, in the study of Kessner and colleagues there was a male experimenter while we had two experimenters, one male and one female working together. It has previously been shown that the laboratory personnel gender can influence pain reports of participants (33) and possibly, it could also influence placebo effects; however, this has not been investigated before. Additionally, the precise methods of placebo effect induction differed between the studies: Kessner and colleagues used verbal suggestions about a placebo ointment, while we gave verbal suggestions about a nasal spray (Chapter 5) and used conditioning with suggestions about a (nonfunctional) transcutaneous electrical nerve stimulation device (Chapter 6). Nevertheless, to be able to use oxytocin in clinical practice, it is important that its effects are clinically significant and reliably generalizable across different samples and placebo effect induction procedures. The results of Part II of the dissertation, however, point at a lack of evidence that oxytocin might play a role in placebo and nocebo effects. The results of the two conducted studies were consistent: neither the gender of the sample nor the dose of oxytocin made a difference. Possibly, oxytocin does not influence placebo and nocebo effects at least in the described procedures, leaving unanswered whether other hormones could be instigated in future research as possible enhances of the placebo effect.

Limitations

The current dissertation presents novel theoretical and empirical evidence about a link between the endocrine system and placebo effects. However, several limitations of this research have to be mentioned.

First of all, due to the lack of research in human studies on pharmacological conditioning of the endocrine system, we were unable to find an optimal conditioning protocol even after performing a systematic review of the available evidence. Therefore, we cannot say whether the design applied in our study on oxytocin conditioning was optimal to elicit the strongest conditioned oxytocin release. We have chosen three acquisition sessions, as this was the average number of acquisition sessions in the human endocrine conditioning studies described in our systematic review. Indeed, by applying this design we succeeded to classically condition oxytocin release. This response was, however, short-lasting and only a modest indication for a conditioned response in brain activity was found. Potentially, a longer acquisition phase would trigger a stronger and more long-lasting conditioned effect, but more research is needed to demonstrate that. Additionally, partial reinforcement, e.g. presenting the unconditioned stimulus at certain points of the evocation phase to reestablish the association between US and CS, could be applied to decrease extinction processes (34). So far this principle has not been studied in the context of hormonal conditioning. To be able to apply conditioning of hormones in clinical practice, it is necessary to be able to reliably induce clinically significant hormonal release and to find ways to avoid fast extinction of the conditioned hormonal responses.

Moreover, the brain mechanisms that trigger endocrine conditioning remain an underexplored topic. Our study from Chapter 4 was a first attempt to explore the brain responses accompanying conditioning of, in this case, oxytocin. The timing of the fMRI experiment is an important limitation of the current work, as the conditioned response in saliva had already gone extinct by the third evocation day when the scanning was done. However, including an fMRI scanning into a conditioning design might be an important confounding factor, as the whole MRI environment can be stressful for participants and might elicit hormonal changes, such as an increase in cortisol levels. Therefore, to minimize these possible confounding effects, we performed the scanning at the end of the whole experiment. Possibly, because of the timing, the effects of conditioning on brain activity were weak and not generalizable across the tasks. Another important limitation is the controversy about the efficacy of exogenous oxytocin administration. There is an ongoing debate in the literature about how intranasal oxytocin reaches the brain and via which

pathways, central or peripheral, it influences behavior. Leng (35) points out that according to mostly animal studies that measured oxytocin levels in cerebrospinal fluid after intranasal oxytocin administration, only a small fraction of the hormone reached cerebrospinal fluid and it is unclear how these small amounts can influence behavior. Moreover, the timing of the behavioral effects remains a point of discussion. Up to this moment only one study measured levels of oxytocin in cerebrospinal fluid in humans after intranasal oxytocin administration (36). This study found a significant increase in oxytocin levels only 75 minutes after the nasal spray administration, which is later than most behavioral and neural effects that are usually recorded at 45 minutes after administration (e.g., 37, 38, 39) and the conditioned oxytocin release in saliva that was already present at 5 minutes after the CS administration, as we demonstrated in Chapter 3. However, Striepens and colleagues (36) included only 3 or 4 participants per measurement and it remains unclear how generalizable the results collected on such a small sample are. Still, this inconsistency between the peak of cerebrospinal fluid levels and behavioral and neural results is disturbing, and therefore, more research on the pharmacokinetics of intranasal oxytocin is required.

Finally, the studies described in this dissertation failed to find any behavioral effects of oxytocin: exogenous oxytocin administration did not influence perceived facial trustworthiness and attractiveness in the experiment described in Chapter 3, pain sensitivity in Chapters 3 and 5, and no effects of oxytocin on the placebo and nocebo effects were found in Chapters 5 and 6. Regarding the facial trustworthiness and attractiveness task, these were not the primary outcomes of our studies and the experiments were not specifically powered to detect these effects. This could be a possible explanation of the null results. There is also a lot of contradiction in the literature regarding possible analgesic effects of oxytocin: some studies found that oxytocin decreases pain sensitivity (40), while other studies could not confirm these findings (41, 42). Our study contributed to the growing body of evidence about the absence of analgesic effects of oxytocin. Additionally, the effects of oxytocin on brain activity in response to the crying baby sounds and pain stimulation were found only sporadically on the second (group) level analysis and not when a comparison between the groups was made in Chapter 4. The fMRI study from Chapter 4 demonstrated

that oxytocin modified brain responses to the presentation of fearful faces, stimuli previously used in oxytocin research (43-45), and consistently with our hypotheses decreased the activation in the amygdala and the superior temporal gyrus. However, these effects were not found in the other two tasks previously shown to be affected by oxytocin on the between-group comparison. A possible limitation could be the timing of the tasks. Most of the previous research that used these tasks, administered them 45 minutes after oxytocin administration (46, 47). However, our scanning session was longer: the crying sounds task and pain task followed the structural scans and the task with emotional faces. The crying sounds task started around 65 minutes after the spray administration and the pain task- around 80 minutes after. Spengler and colleagues (37) recently showed that the strongest oxytocin effects on brain activation are found in the window between 45 and 70 minutes after oxytocin administration. Therefore, possibly due to the delay in time, the effects of the drug might have already been decreased in our experiment.

Clinical implications

In this dissertation, we addressed several issues that have possible implications, both for the theoretical development of the field and for future implementation of placebo effects in clinical practice.

In Part I of the dissertation, we summarized the knowledge on conditioning of endocrine responses and for the first time demonstrated that oxytocin can be classically conditioned in humans. Hormones underlie almost all physiological processes in the organism: from metabolism to reproduction. Hormonal disruptions are involved in a wide range of disorders: diabetes, thyroid disease, adrenal insufficiency, polycystic ovary syndrome and so on. For the treatment of most of these diseases, patients have to take either synthetic versions of hormones they lack (for example synthetic thyroxine in hypothyroidism) or a medication that regulates the imbalance caused by hormonal malfunctioning (for example, metformin that decreases glucose levels in diabetes type-2). Finding ways to stimulate endogenous production of hormones, therefore, might be very useful for optimizing the treatment of endocrine disorders. For example, patients with diabetes type-2 suffer from malfunctioning of the insulin system and slowly develop inability to secrete insulin. In our systematic review, we demonstrated that there is convincing evidence that insulin effects (including insulin release and glucose reduction) can be classically

conditioned. Boosting the organism's production of insulin by a conditioning manipulation, might have great potential benefits for these patients. Another hormone that has been shown to be malleable by classical conditioning, is cortisol. Classically conditioned cortisol responses might be used to boost psychotherapy of phobic patients as cortisone (a prodrug of cortisol) can have fear-reducing effects in this group of patients (48). Stimulating endogenous oxytocin production with classical conditioning, demonstrated in Chapter 3, has also many possible clinical implications. Oxytocin has been extensively investigated as a treatment for a range of mental disorders, such as autism (49), schizophrenia (50), and post-traumatic stress disorder (51). Potentially, classical conditioning of oxytocin responses could be added to the treatment of these patients to improve their social functioning.

Even though conditioned endocrine responses have not been investigated in the context of treatment, there are a few successful examples in which pharmacological conditioning has been shown to be beneficial in clinical practice. Pharmacological conditioning has been applied in placebo-controlled dose reduction studies, i.e., a treatment schedule in which a part of the pharmacological treatment is replaced by a placebo while maintaining the efficacy of the treatment (52). It is hypothesized that patients develop a conditioned reaction in response to their medicine intake, and if a part of the medicine gets replaced with placebo, the treatment efficiency would remain as a result of the conditioned response. Ader and colleagues (53) employed a placebo-controlled dose reduction treatment schedule for the treatment of psoriasis. They demonstrated that the group that received a placebo-controlled dose reduction treatment had a greater skin lesion reduction than the group whose treatment dose was simply reduced, although both groups received the same treatment dose overall, and that this dose-reduction treatment was as efficient as the full-treatment group. Similar results have been demonstrated by Sandler and colleagues (54) in the treatment of attention deficit and hyperactivity disorder. They found that children in a placebo-controlled dose reduction group, where 50% of active medication was replaced with placebo, remained stable during the treatment and did not differ in the severity of symptoms from the group that received the full dose of medication.

Classical conditioning can be used not only for reducing the dosages of medication but also for enhancing the effects of standard treatments. Kirchhof and colleagues (55) have employed a conditioning paradigm to the immunosuppression treatment of renal transplant patients. Immunosuppressive medication was paired with a gustatory stimulus during the acquisition phase and during the evocation phase patients were re-exposed to the gustatory conditioned stimulus. The authors have demonstrated that the group that was exposed to the conditioning procedure in addition to their standard treatment had a reduced T-cell proliferative capacity in comparison to the standard treatment group. These results demonstrate that adding pharmacological conditioning to a standard treatment can improve the efficiency of this treatment. As we have demonstrated in this dissertation, pharmacological conditioning has a potential to elicit conditioned hormonal responses in healthy participants. If this holds true for patients, these methods might be very beneficial for the treatment of hormonal disorders. Placebo-controlled dose reduction and adding conditioning to standard treatment are two methods that can be applied to patients who require hormonal treatments.

Directions for future research

The present dissertation raised several new questions about the link between the endocrine system and placebo effects. First of all, as we have demonstrated in the systematic review, the field of classical conditioning of the endocrine system has a long history and has drawn a lot of attention in animal physiological research. At the same time, human studies on this topic remain limited with a strong focus on the conditionability of cortisol and insulin effects. In Chapter 3, we demonstrated that another hormone, oxytocin, can be also classically conditioned. An important theoretical implication of our finding is that we pointed out that possibly more hormones can be successfully modified by this conditioning paradigm. However, more research into conditioning of other hormones besides cortisol and insulin responses is needed to be able to generalize the animal research to humans.

Another question that was raised from the results of Part I of the dissertation concerns the optimal conditioning design. In the systematic review from Chapter 2, we were unable to describe an optimal conditioning protocol that would guarantee successful and long-lasting endocrine conditioning. The main

reason was the heterogeneity of the designs used in previous research and a lack of studies that would compare effects of several conditioning procedures on the conditioned hormonal responses. There is no agreement on the number of acquisition sessions necessary to induce conditioned endocrine responses and the number of evocation sessions necessary to investigate the extinction of them. It might be, however, impossible to develop one standard protocol, as conditioning depends on various factors: the conditioned stimulus used, the hormonal system investigated, the outcomes measured, etcetera. Nevertheless, it would be very relevant for future research to focus on developing clear conditioning designs per endocrine system.

Another interesting finding of the RCT described in Chapters 3 and 4 is the possible presence of responders and non-responders in the conditioned oxytocin responses. Overall, a significant conditioned oxytocin release has been found on the first evocation day. However, large standard deviations of oxytocin levels in the conditioned group indicated that not all participants demonstrated a conditioned oxytocin release. This is not the first study on pharmacological conditioning that demonstrated the presence of non-responders. Petrakova and colleagues (10) found overall no significant conditioned cortisol increase in the study aimed at conditioning of cortisol. However, they showed that some of the participants did respond to the conditioning manipulation. Also Ober and colleagues (56) showed a presence of non-responders in a study on classical conditioning of immunosuppression. We were unable to find variables that would explain the differences between responders and non-responders. Neither baseline oxytocin levels nor psychological characteristics of participants such as personality and affect were related to the conditioned response. Several other factors, however, could have played a role. For instance, genetic factors might influence responsiveness to oxytocin conditioning. There is evidence that variations in the oxytocin receptor gene influence the effects of oxytocin administration on brain activity (57). Possibly these genetic variations make some people more sensitive to oxytocin administration and this sensitivity potentially could also influence conditioning with oxytocin. Moreover, baseline hormonal levels might also influence hormonal conditioning. Even though we did not find an association between baseline oxytocin levels and conditioned response, it does not exclude other hormonal factors that could have influenced our results.

For instance, there is a complex interplay between the stress hormone cortisol and oxytocin: these hormones have been shown to have a positive correlation (58) and be released at the same time in response to stress (59), with oxytocin down-regulating cortisol-induced stress responses. Therefore, cortisol might have an influence on the secretion of oxytocin and the variability in baseline cortisol levels could explain responders and non-responders to conditioning with oxytocin. We believe that in order to be able to apply hormonal conditioning to clinical practice, it is crucial to understand why not all people are susceptible to pharmacological conditioning. Therefore, it is important that future research focuses on factors that could help distinguish responders from non-responders.

Another relevant theoretical question was raised in Part II of the dissertation. Despite the lack of evidence that oxytocin influences the placebo and nocebo effect, it is at this moment impossible to rule out any role of oxytocin in placebo responding. In our experiments we used endogenous oxytocin administration to test our hypothesis. Future research could further look into the possibility that endogenous oxytocin levels might underlie placebo effects. As oxytocin is known for its prosocial functions, future research may consider more social aspects in placebo induction, such as warmth of the contact between patient and doctor, trust and empathy (60-62). Possibly, oxytocin might increase placebo effects when a longer and more emphatic interaction between the participant and experimenter/doctor is involved. Additionally, oxytocin might play a role in observational learning of placebo and nocebo effects. It has been demonstrated that oxytocin decreases brain activity in the pain circuitry when seeing pain in others (63); therefore, it might have potential to decrease nocebo effects learned by observation.

It is also important that future studies look for other endocrine moderators of placebo and nocebo effects. For instance, vasopressin seems to be a promising candidate, as Colloca and colleagues (27) have found that it enhances placebo analgesia in women only. Moreover, sex differences in the placebo response have been recently described in placebo responding: men seem to respond stronger to placebo treatment and women to nocebo treatment (64, 65). This can indicate a possible important role of sex hormones in placebo effects.

Finally, there are several possible implications of the knowledge from this dissertation for clinical practice. Since no studies so far applied classical conditioning to patients with endocrine disorders despite the promising results coming from the studies on immune conditioning, it would be relevant for future research to investigate whether classical conditioning can influence hormone levels not only in healthy participants, but also in patients with endocrine disorders, for example in patients with diabetes who suffer from malfunctioning of the insulin system. In case of promising results of such studies, it could be tested whether placebo-controlled dose reduction and conditioning boosting of the treatment can be applied to improve the treatment of endocrine disorders and reduce side effects of the standard treatment schedules.

Conclusions

In the present dissertation we studied the possible connection between placebo effects and oxytocin from two perspectives: conditioning of oxytocin and enhancing placebo effects by oxytocin. We have demonstrated that endocrine parameters can be manipulated by classical conditioning in animals and humans and that it is possible to classically condition endogenous oxytocin release. We furthermore found no support that oxytocin increases placebo and decreases nocebo responses. Understanding how we can influence the endocrine system with placebo effects and, alternatively, how we can affect placebo effects with hormones is important to enable starting to use placebo effects in clinical practice. Applying placebo effects in medical treatments, for example, for endocrine conditions, has a great potential to improve the efficiencies of standard treatment protocols, decrease costs of medications, and minimize side effects.

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