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Master thesis

Effects of Anxiety and Stress on the Placebo Response of Oxytocin

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Abstract

Background: The evidence for the possibilities of conditioned endocrine placebo effects is growing. However, the conditioned placebo effect of oxytocin has not yet been researched. Also, research suggests that psychological factors, such as anxiety and stress may influence placebo responses. We examined if it is possible to induce a conditioned placebo response in oxytocin and whether this response is influenced by anxiety and stress.

Methods: In a two-phase conditioning paradigm, 66 women (age: 18-33) were randomly allocated to a conditioned group or to a control group. In the first phase (acquisition phase), participants in the conditioned group received oxytocin nasal spray together with a distinctive smell (conditioned stimulus: CS). Whereas, participants in the control group received a placebo together with the CS. In the second phase (evocation phase), both groups received a placebo in combination with the CS. Salivary oxytocin levels were measured several times throughout both phases. Questionnaires were used to measure levels of anxiety (State-Trait Anxiety Inventory) and stress (Special Events Questionnaire II).

Results: The results of a repeated measures ANCOVA indicated that there was a significant difference in salivary oxytocin levels between conditioned and control group in the evocation phase ($F(1,61) = 7.45$, $p = .008$). Multiple regression analysis (MRA) indicated that anxiety did not moderate the effects of conditioning on oxytocin release ($b = 1.57$, $p = .609$). Nevertheless, MRA indicated that stress moderated the effects of the conditioned placebo effect of oxytocin ($b = .39$, $p = .038$).

Conclusion: Our results indicate that it is possible to induce a placebo effect in oxytocin through the mechanism of classical conditioning. Furthermore, participants with higher stress levels demonstrated a higher placebo effect. These results indicate that stress positively affects the conditioned placebo response in oxytocin.

Keywords: Placebo effect, Oxytocin, Classical conditioning, Anxiety, Stress

1. Introduction

Placebo effects are genuine positive psychophysiological outcomes of a treatment that are attributable to individual treatment expectations and psychosocial context, rather than the action of medications or interventions (Colloca, 2018). According to Gaab et al. (2018), placebos should be understood as a principle rather than a distinct and singular procedure, because placebos come in various forms and shapes. For instance, they can encompass inert substances, such as the classical sugar pill (Gaab, Kossowsky, Ehlert, & Locher, 2019). They can also encompass surgical procedures and acupuncture needles. Jonas et al. (2015) researched placebo effects of surgical procedures in a meta-analysis, in which randomized controlled trials of surgical procedures were included. These were either real surgery procedures or sham surgical procedures for comparison. The observed outcome changes in the sham groups, also referred to as placebo responses, were generally large. In conclusion, the sham surgical procedures were also effective. Kaptchuck et al. (2006), researched the placebo effects of a sham acupuncture needle in a randomized controlled trial in patients with persistent arm pain. The sham acupuncture needle had an effect on self reported pain and severity of symptoms, thus a placebo response occurred. The above described examples are only a few manners of the application of placebos, the possibilities are very broad.

Moreover, there are also various psychological mechanisms trough which placebo responses can be elicited. The most well researched mechanisms are the mechanism of expectations and the learning mechanism of classical conditioning. Benedetti et al. (2003), suggested that placebo responses can be induced by expectations when conscious physiological processes are involved, whereas placebo responses can be induced by classical conditioning when unconscious physiological functions come into play. Expectations, with regards to placebo responses, are conscious events whereby the patient expects a certain benefit (Benedetti & Piedimonte, 2019). The general idea is that thoughts and beliefs about outcome expectations may have positive effects on a certain outcome. In clinical trials, expectations are often induced by verbal suggestion. When this is the case, verbal information is conveyed which may influence the expectations someone has (Benedetti, Mayberg, Wager, Stohler, & Zubieta, 2005; Enck, Benedetti, & Schedlowski, 2008). Placebo effects induced by positive verbal suggestion are extensively studied in pain research. For instance in the study of Skvortsova et al. (2018), in which there were two groups of participants. The first group was an experimental group that received positive verbal suggestions about the active

substance in a nasal spray. The positive suggestion contained the message that previous studies have demonstrated that the active substance in the spray decreased pain sensitivity. The researchers also told participants in this group that it was expected for them to feel less pain during a pain task after the administration of the spray. The other group was a control group and did not receive any verbal suggestions about the active substance in the nasal spray. In this study, positive verbal suggestion successfully elicited placebo analgesia.

Classical conditioning is a process that begins with an unconditioned stimulus (US) and a neutral stimulus (NS). The US evokes a certain natural response, which is called the unconditioned response (UR), the NS doesn't evoke this response. During the process the NS and the US are paired repeatedly. Eventually, the NS alone also evokes the UR. The NS has become a conditioned stimulus (CS) and the response it evokes is called a conditioned response (CR). In experiments the first conditioning phase is called acquisition, this is the pairing phase, where the NS becomes a CS. After the acquisition phase, experiments generally move on to the evocation phase. This is the testing phase, wherein the CS is presented alone. It is measured whether a CR is elicited or not by presenting the CS alone (Colloca & Miller, 2011; Tekampe et al., 2018). Colloca and Miller (2011) described the conditioned placebo effect as a response to an index sign (CS) through which the individual learns to experience a favorable outcome.

Some factors can influence the conditioned placebo response, for example psychological factors such as anxiety and stress. Anxiety is a psychological construct that is characterized by feelings of tension, worried thoughts and physical changes, such as an increased blood pressure (Kazdin, 2000). Stress is a psychological state that is characterized by feelings of pressure and physical arousal. Different systems in the human body are activated by stress, such as the sympathetic nervous system (SNS) and the hypothalamic pituitary adrenal axis (HPA-axis). These systems regulate different processes in the body, for example: blood pressure, heart rate and the release of certain stress hormones (e.g. cortisol and adrenaline) (Butler, 1993; Harris, 2020). Vase, Robinson, Verne, and Price (2005) suggested that a reduction in anxiety may be enhancing the placebo effect. In their study to analgesic placebo effects in patients with irritable bowel syndrome, they found that the decrease of anxiety levels was associated with an increase of the analgesic placebo effect. Similar effects were found in stress, a study of Benedetti, Amanzio, Vighetti, and Asteggiano (2006) showed that a reduction of stress was associated with an enhanced analgesic placebo response (Flaten, Aslaksen, Lyby, & Bjorkedal, 2011). However, little research has yet been

done on the influence of these factors on placebo responses. Thus far, the effects of anxiety and stress on placebo responses have only been studied in research on analgesia.

Placebo responses are found in different systems in the human body, including the endocrine system (Skvortsova et al., 2019). The main mechanism through which placebo responses in the endocrine system can be induced is classical conditioning. One study examined if hormonal placebo responses can be induced through the mechanism of (verbally induced) expectations, but it did not find an effect (Benedetti et al., 2003). During hormonal conditioning, in the acquisition phase, the hormone is presented in combination with a NS. This could be a distinctive smell for example. In the acquisition phase, an association between the hormone and the NS needs to be formed. The acquisition phase is over, when the NS became a CS. In the evocation phase, the hormone is replaced with a placebo and presented in combination with the CS. If a hormone is conditioned as described here, an increase of the hormone (CR) should be elicited when presenting the CS alone. Examples of hormones that are proven possible to condition are cortisol, growth hormone and insulin (Benedetti et al., 2003; Johansen, Brox, & Flaten, 2003; Sabbioni et al., 1997; Stockhorst, de Fries, Steingrueber, & Scherbaum, 2011; Stockhorst et al., 2004). In the study of Stockhorst et al. (2011) insulin was conditioned using insulin as a US and a tarry smell (meta-cresol) as a CS. The expected CR to the CS was an increase in insulin, which happened in the evocation phase of this study. Thus, insulin was successfully conditioned here. The current study is the first study that is looking at the possibility of conditioning the hormone oxytocin in humans. The study of Onaka and Yagi (1998) showed that oxytocin can be successfully conditioned in rats. However, this effect extinguished over time when not reinforced.

Oxytocin is a hormone that is produced in the hypothalamus. From here, it transports to the pituitary gland, where oxytocin is stored and secreted (Meyer-Lindenberg, Domes, Kirsch, & Heinrichs, 2011). Oxytocin has effects on maternal behavior: it stimulates uterine contractions during labor and lactation during breast feeding (Veening, de Jong, Waldinger, Korte, & Olivier, 2015). Also, it improves the mother-child bond (Feldman, 2012). More examples of social behaviors in which oxytocin is involved are: facilitating approach behavior and increasing trust (Churchland & Winkielman, 2012; Yan, Yong, Huang, & Ma, 2018). Other positive effects of oxytocin are: increasing how attractive we find others and reducing pain sensitivity (Rash, Aguirre-Camacho, & Campbell, 2014; Theodoridou, Rowe, Penton-Voak, & Rogers, 2009).

There are several studies that have investigated possible implications of oxytocin treatment in clinical practice. For example, Bertsch et al. (2013) found that oxytocin may decrease social threat hypersensitivity in patients with borderline personality disorder. In response to decreasing threat hypersensitivity, anger and aggressive behavior may be decreased in these patients. Also, there is tentative evidence that treatment with oxytocin has improving effects on aspects of social cognition and emotional well-being in individuals with autism spectrum disorder (Anagnostou et al., 2012). Moreover, treatment with oxytocin might improve glucose homeostasis, increase insulin sensitivity and may lead to weight loss in overweight adults (Lawson, 2017). However, it is important to note that the exact implications of oxytocin in clinical practice still need further investigation.

More evident results exist in research of the clinical possibilities on the formation of placebo responses via classical conditioning. For instance, research has demonstrated that placebos can alleviate certain symptoms, such as symptoms of pain, depression and Parkinson's disease (Evers et al., 2018; Forsberg, Martinussen, & Flaten, 2017; Kaptchuk & Miller, 2015). Also, placebos could be useful in pharmacological treatments through the implementation of placebo-controlled dose reduction. This is a procedure in which a certain amount of pharmacological treatment is replaced by a placebo while maintaining the efficacy of the treatment (Doering & Rief, 2012). Previous research has demonstrated that this is possible in children with ADHD. In this research placebos were paired with stimulant medication which elicited a placebo response. This response allowed children with ADHD to effectively be treated on half of their optimal stimulant dose (Sandler, Glesne, & Bodfish, 2010). Placebo-controlled dose reduction also has the potential to alleviate side effects while preserving therapeutic benefits or to reduce health care costs (Doering & Rief, 2012; Tekampe et al., 2018). Lastly, conditioned placebo responses has the potential to be useful as a supporting treatment without increasing the medication dose. This has been demonstrated in a study to immunosuppressive medications in renal transplant patients. In this study, the effects of the medications increased even though the medication dose remained the same (Kirchhof et al., 2018).

Considering the possibilities of placebo treatment via classical conditioning and the tentative possibilities of oxytocin treatment, classical conditioning of oxytocin might be promising. The potential to condition oxytocin release would open additional perspectives for oxytocin treatment in clinical practice. Therefore, it is relevant to elucidate possible factors that may influence the classically conditioned placebo response of oxytocin. As previously mentioned, anxiety and stress may influence placebo responses (Benedetti et al., 2006; Flaten

et al., 2011; Vase et al., 2005). However, possible effects of anxiety and stress on the placebo response of oxytocin have not yet been researched.

The current study investigates the classical conditioning of oxytocin. This study has two aims. The first aim is to investigate if it is possible to induce a placebo response in oxytocin through classical conditioning. We hypothesized that after repeated pairings of oxytocin with a neutral stimulus in the acquisition phase, the neutral stimulus becomes a conditioned stimulus and will trigger a conditioned response in the beginning of the evocation phase. The unconditioned response to the administration of exogenous oxytocin (US) triggers further (endogenous) oxytocin release by so-called feed-forward mechanisms (van Ijzendoorn, Bhandari, van der Veen, Grewen, & Bakermans-Kranenburg, 2012). Therefore, in this case, the conditioned response would be elicited when an increase in oxytocin levels is evoked in response to the conditioned stimulus. The second aim is to investigate if anxiety and stress influence placebo responses of oxytocin. Hypothesized is that after having experienced more anxiety or stress, participants will show reduced levels of classically conditioned endogenous oxytocin levels compared to participants who show less anxiety or stress.

2. Methods

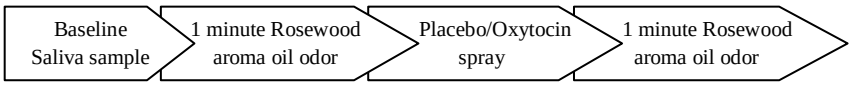
2.1 Study Design

The study was a two-phase randomized controlled trial, it is presented in Figure 1. The study started with a screening session. After the screening session, participants were randomly assigned to one of two groups: a conditioned group or a control group. The first phase was called the acquisition phase. In this phase the odor of rosewood aroma oil (conditioned stimulus, CS) was presented together with the administration of oxytocin in the conditioned group. In the control group, the CS was presented together with the administration of a placebo. The second phase was called the evocation phase. This phase started four days after the last day of the acquisition phase, thus starting on the same day of the week as the acquisition phase. Participants in the conditioned and control groups were given the placebo in combination with the CS during three consecutive days. The study had a double blind design, both the participants and the researchers did not know if participants would receive oxytocin or a placebo in the acquisition session.

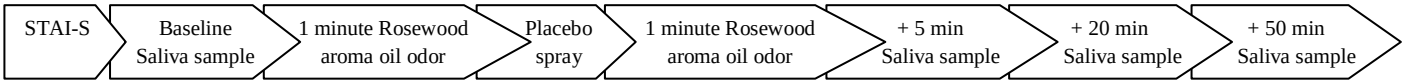
Screening session

- Exclusion criteria check
- Special Events Questionnaire II
- Baseline oxytocin levels

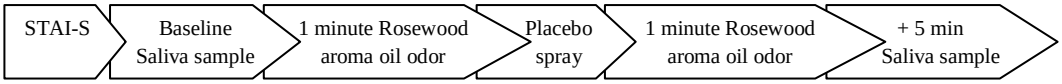
Acquisition sessions (Day 1-3)



Evocation sessions (Day 4-5)



Evocation session (Day 6)



Note. Abbreviation: STAI-S is State-Trait Anxiety Inventory

Figure 1. Study Design

2.2 Participants

Participants in the study were 66 healthy women (*N* conditioned group = 33, *N* control group = 33) between the age of 18 and 33, who use oral contraceptives. The reason for only including women who use oral contraceptives, is that they have stable oxytocin levels throughout the menstrual cycle (Salonia et al., 2005). They were asked to participate through means of flyers spread at Leiden University. Exclusion criteria were serious neurological or psychiatric disorders, substance abuse, (intended) pregnancy or known hypervigilance to one of the ingredients of the oxytocin spray or the odor.

2.3 Procedure

Participants were asked to come to the laboratory for the screening, the acquisition phase and for the evocation phase. The entire experiment took place at a laboratory of the Social Science department of Leiden University.

At the screening, exclusion criteria were checked, then participants were asked to fill in the Special Events Questionnaire II. Also, saliva samples were taken to determine baseline levels of oxytocin.

The acquisition phase lasted three consecutive days. During the acquisition sessions, participants received a nasal spray either with 24 international units of oxytocin or with the placebo, coupled with the odor of rosewood aroma oil. The placebo was a nasal spray, which was identically looking and smelling to the oxytocin nasal spray, only without oxytocin in it. First a baseline saliva sample was taken, to measure oxytocin levels. After this, participants were asked to breathe normally through nasal goggles for one minute before and one minute after the spray administration, to smell the rosewood aroma oil.

The evocation phase also lasted three consecutive days. In this phase, participants were first asked to fill in the State-Trait Anxiety Inventory (STAI-S) during each session. After this, participants in both the conditioned and control group received placebo spray in combination with rosewood aroma oil odor. Saliva samples were taken four times (baseline, 5, 20 and 50 minutes after the placebo administration) to measure oxytocin levels.

2.4 Questionnaires

To measure baseline state anxiety, the STAI-S (α = .82) was used. State anxiety entails a person's current levels of anxiety. This questionnaire consists of six items, which comprise of the following statements: 'I feel calm', 'I am tense', 'I feel upset', 'I am relaxed', 'I feel content', 'I am worried'. Answers are given on a four-point Likert scale. The total score ranges from 6 to 24, with higher scores indicating more anxiety (Marteau & Bekker, 1992).

To measure stress at baseline, a translated (into the Dutch language) and shorter version of The Negative Life Events and Trauma Questionnaire, called Special Events Questionnaire II, was used (Morgan & Janoffbulman, 1994; van Laarhoven et al., 2012). Participants were asked to indicate whether they recently experienced any of the stressful events given in a list. A few examples of stressful events given in this list are: the death of a parent, brother, sister or a partner and being involved in a serious accident. If any of these stressful events were experienced, participants were asked to rate this event on a scale of 0 (the event was experienced as not stressful at all) to 100 (the event was experienced as extremely stressful).

2.5 Statistical analyses

The data analysis was performed using SPSS Statistics Version 25 (IBM Corporation). p-values were reported and considered significant at the < .05 level. Baseline characteristics between the conditioned and control group were compared using independent sample t-test.

Assumptions for performing independent samples t-test were checked. The t-test was robust for a possible violation of normality, because of large enough sample sizes (lowest $N = 32$) (Schmidt, 2018). The assumption of homogeneity was not violated for any of the variables.

To test the first research question: if it is possible to induce a placebo response in oxytocin through classical conditioning, a between subject repeated measures analysis of covariance (ANCOVA) was carried out. Conditioned and control groups were compared on the three oxytocin measurements of the first evocation session (day 4) with the baseline oxytocin measurement of the same evocation session as a covariate. Thus, the three oxytocin measurements were the dependent variables and group was the independent variable. The reason for using the measurements of the first evocation session is that earlier research in rats showed that a placebo response of oxytocin reduces in a period of time when not reinforced (Onaka & Yagi, 1998). Therefore, in the current study, we expect a reduction in a possible placebo response over time as well. We expected the placebo response of oxytocin to be the highest in the first evocation session.

Prior to performing the analysis, the assumptions for repeated measures ANCOVA were tested. The assumption of normality was tested using the Shapiro-Wilk test. The results of this test indicated a violation of normality on oxytocin scores for each group, except for the third measurement in the conditioned group (oxytocin 5 minutes after placebo administration, control group: $W = .619$, $p < .001$, conditioned group: $W = .921$, $p = .022$; oxytocin 20 minutes after placebo administration, control group: $W = .586$, $p < .001$, conditioned group: $W = .875$, $p = .002$; oxytocin 50 minutes after placebo administration, control group: $W = .648$, $p < .001$, conditioned group: $W = .951$, $p = .159$). Log transformations on the oxytocin variables were performed to correct for this violation. Sphericity was measured with Mauchly's test of sphericity. Mauchly's test did not indicate any violation of sphericity ($\chi 2(2) = 2.06$, $p = .358$). Furthermore, assumptions for adding a covariate were tested, they include the assumption of linearity and the assumption of homogenous regression slopes. The assumption linearity was tested by analyzing plots of residuals against predicted values for all four oxytocin measurements (baseline, 5, 20 and 50 minutes after the placebo administration). No signs of non-linearity were found. Lastly, the assumption of homogeneity of regression slopes was tested by computing a separate repeated measures ANCOVA with covariate (baseline oxytocin measurement) times factor (oxytocin measurements 5, 20 and 50 minutes after placebo administration) interaction. The covariate times factor interaction was not significant ($F(2,120) = .96$, $p = .386$), so we assume homogenous regression slopes. Furthermore, the data were screened for outliers on dependent variables, using z-scores. Z-scores above 3 or

below -3, were considered to indicate outliers. When looking at standardized residuals of the dependent variables, the three oxytocin measurements of 5, 20, and 50 minutes after placebo administration, there were five outliers. One outlier was found on the oxytocin measurement 5 minutes after placebo administration, with a z-score of 3.09. Two outliers were found on the oxytocin measurement 20 minutes after placebo administration, with z-scores of -3.33 and 3.28. We did not remove these outliers from our data. They were no extreme outliers and removing them from our data would have affected the generalizability of our study.

To test the second research question: if anxiety and stress influence placebo responses of oxytocin, two moderation analyses were carried out, one for anxiety and one for stress. The variable of anxiety was derived by adding up rated scores of state anxiety (STAI-S) and the variable of stress was derived by adding up rated scores of stressful life events (Special Events Questionnaire II). Moderation analyses were carried out in SPSS using linear multiple regression analyses, to investigate if there are main effects and/or interaction effects. A significant interaction effect would indicate moderation. In SPSS, we recoded conditioned and control group into dummy variables. The conditioned group was assigned to number 1 and the control group was assigned to 0. Also, we centered the continuous variables, these included the first salivary oxytocin measurement after placebo administration and both the anxiety and stress measurements. The last preparatory step we performed in SPSS, before performing the moderation analyses, was creating interaction terms of dummy group times centered anxiety and dummy group times centered stress. In both analyses, the first oxytocin measurement after placebo administration was the dependent variable. In the first moderation analysis, the independent variables were the dummy coded variable of group, centered anxiety and the interaction term of dummy coded group and centered anxiety. In the second analysis, the independent variables were the dummy coded variable of group, centered stress and the interaction term of dummy coded group and centered stress.

Before carrying out the analyses, the assumptions of multiple regression analysis were tested. The assumption of linearity and the assumption of homoscedasticity were tested by analyzing a plot of residuals against predicted values of the dependent variable for both analyses. No systematic deviation from linearity and no signs of heteroscedasticity were found. The *F*-test in both analyses were robust for a possible violation of normality because of a large enough sample size (lowest $N = 32$). Furthermore, the data were screened for outliers on dependent variables, using z-scores. Z-scores above 3 or below -3, were considered to indicate outliers. When looking at standardized residuals of the dependent variable of the first analysis, there was one outlier, with a z-score of 5.46. In the second analysis, there were two

outliers, with z-scores of 3.07 and 4.63. We did not remove them from our data, because that would have affected the generalizability of the study.

3. Results

Baseline characteristics are presented in Table 1. Participants were between the age of 19 and 33 and the mean age was 21.4 (*SD* = 2.4). No significant differences between participants in the conditioned and control group were found for age ($t(64) = -.77$, $p = .443$). Also, no significant differences between participants in the conditioned and control group were found for the STAI-S ($t(64) = -.90$, $p = .370$) measuring State Anxiety and for the Special Events Questionnaire II ($t(64) = -.472$, $p = .638$) measuring Stress. Furthermore, no significant differences between the conditioned and control group were found for the covariate, the baseline oxytocin measurement ($t(63) = -1.24$, $p = .221$). Two oxytocin samples were not analyzed due to clotting (one from a participant from the conditioned group and one from a participant from the control group).

Table 1. Baseline Characteristics With Standard Deviations

				Group					
	N	Total	Range	*N*	Conditioned	*N*	Control	*t*	*p*
		M ± SD			*M* ± SD		*M* ± SD		(2-sided)
Age	66	21.4 ± 2.4	18-33	33	21.2 ± 2.8	33	21.7 ± 1.9	-.77	.443
Questionnaires									
State Anxiety (STAI-S)	66	9.9 ± 2.7	6-17	33	9.6 ± 2.9	33	10.2 ± 2.6	-.90	.370
Stress (Special Events Questionnaire II)	66	31.7 ± 41.4	0-150	33	29.2 ± 38.3	33	34.1 ± 44.9	-.47	.638
Covariate: Baseline oxytocin	65	14.2 ± 14.7	1-113	32	11.9 ± 8.4	33	16.4 ± 18.3	-1.24	.221

The results of the repeated measures ANCOVA are presented in Table 2 and Figure 2. They show that, after controlling for baseline levels, there was a significant difference between the conditioned and the control group, with a small to medium effect size ($F(1,61) = 7.45$, $p = .008$, $\eta_p^2 = .109$). The salivary oxytocin levels in the conditioned group ($M = 1.18$,

SD = .05) were higher in comparison to the control group (*M* = .99, *SD* = .05). There was no significant effect of time ($F(2, 122) = .23$, $p = .798$), the oxytocin levels did not significantly differ within participants between the three time measurements. The time x group interaction was also not significant ($F(2,122) = 1.12$, $p = .330$, $\eta_p^2 = .018$).

Table 2. Results Repeated Measures ANCOVA

Source	*df*	*SS*	*MS*	*F*	*p*	η_p^2
Between subjects						
Group	1	1.62	1.62	7.45	.008	.109
Baseline*	1	5.97	5,97	27.49	< .001	.311
Residuals	61	13.24	.22			
Within subjects						
Time	2	.02	.01	0.23	.798	.004
Time x Baseline	2	.07	.03	0.88	.418	.014
Time x Group	2	.09	.04	1.12	.330	.018
Residuals	122	4.63	.04			

Note. $N_{control} = 32$, $N_{conditioned} = 32$; the dependent variables of Time and the covariate were log transformed; Abbreviations: SS is sum of squares, MS is means squares.
*$b_{w5min} = .65$, $b_{w20min} = .64$, $b_{w50min} = .51$

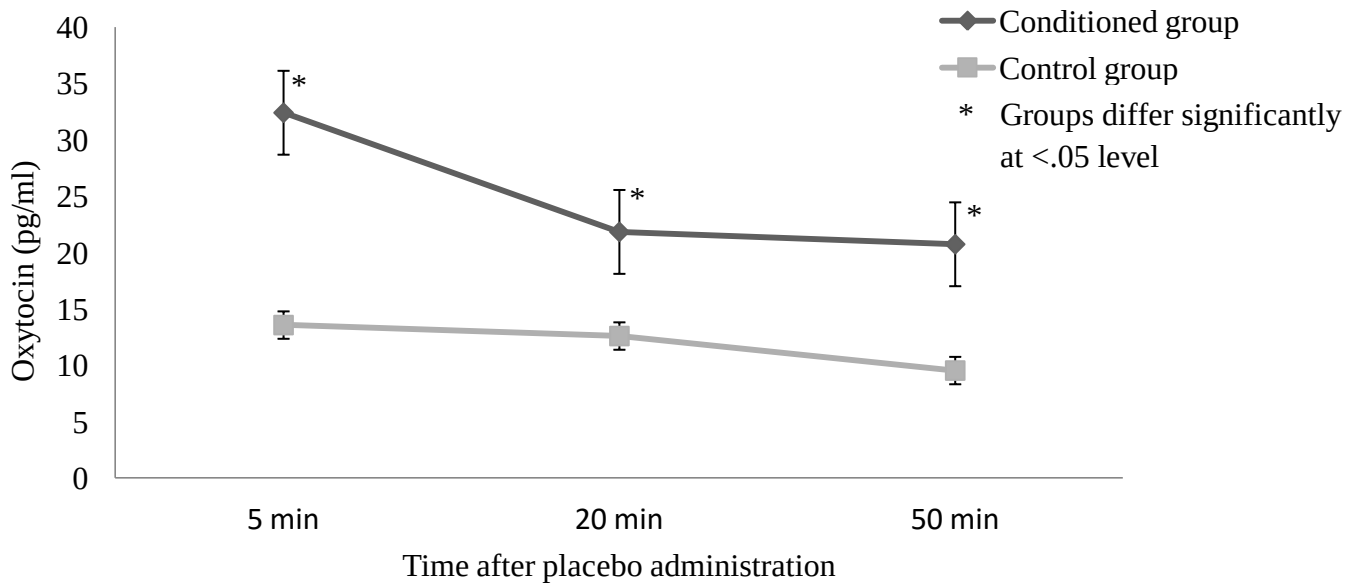


Figure 2. Mean group oxytocin scores by time of measurement, error bars indicate standard error

The results of the moderation analyses are presented in Table 3, Table 4, Figure 3 and Figure 4. The model of anxiety with the interaction term (Table 3; Model 2 and Figure 3) was not significant ($F(3,61) = 1.71$, $p = .174$, $R^2 = .078$, $\Delta R^2 = .004$). This model predicted only 7.8% of the variance in the sample and predicted only 0.4% more than the model without interaction term. The interaction effect between group and anxiety was not significant ($b = 1.57$, $p = .609$). Non-significance of the interaction effect indicates that there is no moderation effect. Anxiety Model 1 only includes the main effects of group and anxiety (Table 3; Model 1), this model is also not significant ($F(2,62) = 2.74$, $p = .093$, $R^2 = .074$). The main effect of group was the only significant effect in this model. ($b = 17.54$, $p = .036$). Given that the control group was coded as 0, the conditioned group as 1 and $b = 17.54$, the predicted mean value of the conditioned group is 17.54 units higher than the mean value of the control group. The main effect of anxiety was not significant ($b = 1.19$, $p = .431$).

Table 3. Moderation Analyses of Anxiety on Placebo Responses of Oxytocin

	b	*SE*	*t*	*p*	R^2	*F*	*p*	ΔR^2
Model 1					.074	2.47	.093	
Intercept	14.25	5.72	2.49	.016				
Group	17.54	8.17	2.15	.036				
Anxiety	1.19	1.51	.79	.431				
Model 2					.078	1.71	.174	.004
Intercept	14.51	5.78	2.51	.015				
Group	17.46	8.22	2.12	.038				
Anxiety	.33	2.26	.15	.884				
Group * Anxiety	1.57	3.05	.51	.609				

Note. $N = 65$; Abbreviation: SE is standard error.

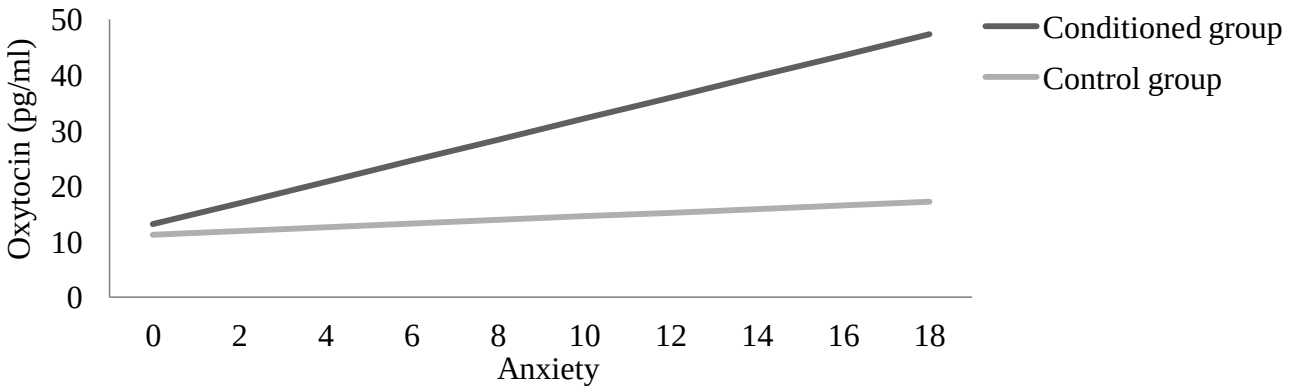


Figure 3. Interaction effect anxiety by oxytocin

The model of stress with interaction term (Table 4; Model 2 and Figure 4) was significant ($F(3,61) = 5.49$, $p = .002$, $R^2 = .212$, $\Delta R^2 = .058$). This model predicted 21.2% of the variance in the sample and predicted 5.8% more variance than the model without interaction term. In this model, the interaction between group and stress was significant ($b = .39$, $p = .038$). Significance of the interaction effect indicates a moderation effect. Table 3 shows an indication of the direction of the interaction effect. Higher levels of stress result in higher levels of oxytocin in the conditioned group in comparison to the control group. The main effect of the variable group (Table 4; Model 2) was also significant ($b = 17.78$, $p = .022$). This was a positive effect, so given that the conditioned group was assigned to number 1 and the control group was assigned to number 0, participants in the conditioned group had higher oxytocin scores than participants in the control group. The main effect of stress was not significant ($b = .08$, $p = .520$).

Table 4. Moderation Analyses of Stress on Placebo Responses of Oxytocin

	b	*SE*	*T*	*p*	R^2	*F*	*p*	ΔR^2
Model 1					.154	5.66	006	
Intercept	14.02	5.46	2.57	.013				
Group	17.83	7.78	2.29	.025				
Stress	.24	.09	2.57	.013				
Model 2					.212	5.49	.002	.058
Intercept	14.42	5.31	2.72	.009				
Group	17.78	7.57	2.35	.022				
Stress	.08	.12	.65	.520				
Group * Stress	.39	.19	2.12	.038				

Note. $N = 65$; Abbreviation: SE is standard error.

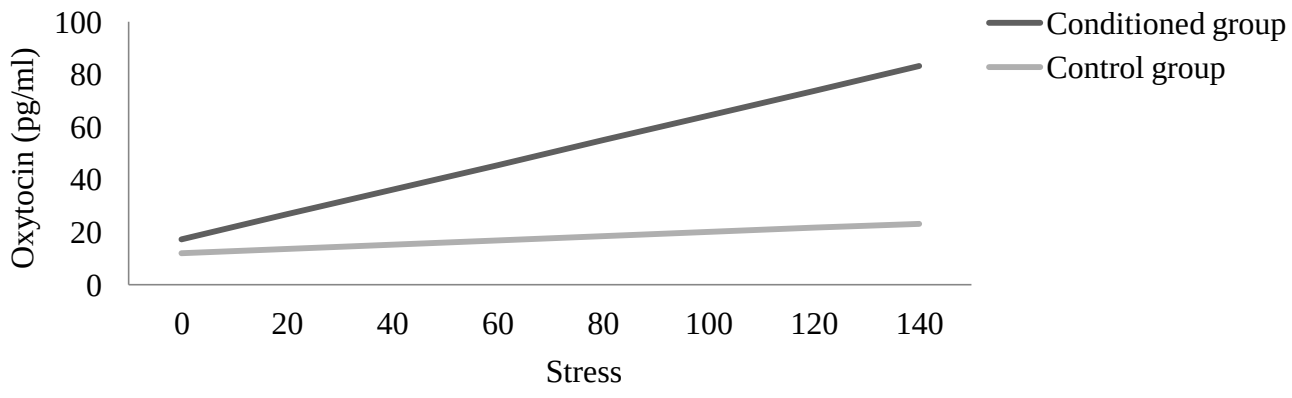


Figure 4. Interaction effect stress by oxytocin

4. Discussion

The present study was two-aimed. The first aim was to investigate whether it is possible to induce a placebo response in oxytocin through the mechanism of classical conditioning. Our results demonstrated that it is possible to condition (endogenous) oxytocin release. After repeated pairings of oxytocin with the smell of rosewood aroma oil odor in the acquisition phase, the aroma oil smell alone triggered a conditioned response in the beginning of the evocation phase. Participants showed a conditioned increase of salivary oxytocin levels. These results were in line with our hypothesis. Our results were also in line with earlier research on the conditioned placebo effect of oxytocin in an animal study. Onaka and Yagi (1998) showed that oxytocin can successfully be conditioned in rats. The current study was the first study to research the classically conditioned placebo effect of oxytocin in humans. Nevertheless, our results were in line with other studies that show supporting results regarding the possibility to classically condition hormones in humans. Other hormones that already were proven possible to condition are cortisol, growth hormone and insulin (Benedetti et al., 2003; Johansen et al., 2003; Sabbioni et al., 1997; Stockhorst et al., 2011; Stockhorst et al., 2004).

The second aim was to investigate whether anxiety and stress influenced placebo responses of oxytocin. The results of the current study showed no effects of anxiety on the conditioned placebo effect of oxytocin. Participants who experienced more anxiety did not show significantly increased or reduced levels of classically conditioned oxytocin levels compared to participants who showed less anxiety. This result was not in line with our hypothesis as well as with earlier research. Earlier research found that decreased anxiety was associated with increased placebo responses in patients with irritable bowel syndrome (Flaten et al., 2011; Vase et al., 2005).

A possible explanation for our results not being in line with our hypothesis or with earlier research could lie in the way of how anxiety is assessed. The study of Vase et al. (2005), on which our hypothesis was based, measured anxiety using the Visual Analog Scale. Participants were asked: ‘How anxious are you about the pain you may experience during this session?’ and rate their anxiety on a scale of 1 (no anxiety) to 10 (the most intense anxiety imaginable). It is notable that in this study, anxiety was closely related to the construct of negative expectation (Vase et al., 2005). In our research anxiety was assessed using the STAI-S, with which we measured state anxiety. The STAI-S measured current levels of anxiety by rating six statements: ‘I feel calm’, ‘I am tense’, ‘I feel upset’, ‘I am relaxed’, ‘I feel content’,

‘I am worried’. Presumably, these questionnaires do not measure the exact same concept of anxiety.

Another difference between the study of Vase et al. (2005) and our study is that anxiety levels were measured at two different points in time during the experiment. This way, the researchers were able to make within-subjects comparisons with regards to anxiety. Whereas, in our study, we only measured anxiety once and made between-subjects comparisons with regards to anxiety. Within-subjects designs have greater control over individual differences than between-subjects designs and therefore have more statistical power. This makes that within-subjects designs are more likely to detect an effect than between-subjects designs. Also, in the study of Vase et al. (2005), anxiety was a less consistent and a weaker predictor than other predictors in the study (the desire and expectation for pain relief). It might be that there is a very small effect of anxiety on placebo effects and that our study did not have enough power to detect it.

Furthermore, in our study, the measures of stress did show an effect on classically conditioned placebo responses, but the effect was not in the direction we expected. We expected that high stress levels would be associated with low placebo responses. However, our results indicated that high stress levels were associated with increased placebo responses. These results were in line with earlier research, in the sense that an association of stress with placebo responses was found. Although, this association was previously found in the opposite direction. Earlier research indicated that decreased stress levels was associated with enhanced placebo responses (Benedetti et al., 2006; Flaten et al., 2011).

A possible explanation of why our results are not in line with our expectations could be that people who experience more stress are more sensitive to placebo manipulation. Previous research investigating placebo effects in visceroception suggested that psychological stress might amplify the placebo effect. In this research 120 participants underwent either the Trier Social Stress Test (stressed condition, $N = 60$) or a simple cognitive task (Control condition, $N = 60$). They were further randomized into groups who either received positive (placebo) or neutral verbal suggestions regarding the treatment expectations (intravenous administration of saline). Before and after receiving the Trier Social Stress Test or the simple cognitive task, participants underwent a visceroception test. They found that the magnitude of change in perceived visceral symptoms (urgency) in response to placebo treatment was affected by acute psychological stress. For, significant differences in urgency between the visceroception tests (before and after) were only found in the stressed condition, not in the

control condition (Roderigo et al., 2017). This is corresponding with our results in such a way that people who experienced more stress, displayed a stronger placebo effect.

Another possible explanation for discrepancies in different studies could lie in differences in the manner of how stress was included in these studies. In the study of Benedetti et al. (2006) physiological stress is induced using proglumide, a substance that blocks cholecystokinin receptors. Cholecystokinin is a substance that induces subjective and physiological stress, proglumide should inhibit this reaction. In the study of Roderigo et al. (2017) psychological stress is induced by applying the Trier Social Stress Test and in our study existing psychological stress levels are measured by using the Special Events Questionnaire II. Such differences in manners of including stress, for instance differing in psychological or physiological stress, could possibly affect or measure different mechanisms that may affect the placebo effect in a different way.

There are several limitations that need to be addressed. The first limitation is regarding the generalizability to the general population, as only women who take oral contraceptives were included in this study. Enck and Klosterhalfen (2019) argued that gender does play a role in the placebo response. They suggest that the mechanisms through which placebo responses work are predominantly via conditioning in women and via the manipulation of expectancies in men. In our research, the reason for only including women who take contraceptives, was that these women show stable oxytocin levels throughout the menstrual cycle (Salonia et al., 2005). Future research should not limit the research population in such a way, it should include both females and males who do not use medications that regulate the hormonal system. This would contribute to our findings by investigating if comparable results can be found in a more general research population.

Also, we did not measure the expectations of participants regarding the treatment they received. Expectations are an important underlying mechanism of the placebo effect (Benedetti et al., 2005; Benedetti & Piedimonte, 2019; Enck et al., 2008). Although, earlier research has shown that expectations presumably do not have an effect on hormonal secretion, we cannot rule out the possibility that it influenced our results (Benedetti et al., 2003). For instance, expectations might still support the mechanism of classical conditioning (Wager & Atlas, 2015). Future research should take the treatment expectations of participants into account.

Another limitation concerns the used measurements for anxiety and stress. When measuring anxiety we used the STAI-S, meaning that we measured state anxiety. State

anxiety is described as a person's current level of anxiety. For a more complete view of a possible effect of anxiety on the placebo effect of oxytocin, it could be relevant to include trait anxiety as well. Trait anxiety entails a person's general feelings of anxiety (Marteau & Bekker, 1992). When measuring stress we used the Special Events Questionnaire II, this questionnaire measured if participants had experienced certain life events and it measured what the impact of these events was on their lives. So, the level of our measurement of stress was dependent on the given life events. For instance, some participants did not experience any of these events and were marked as low in stress. While other participants did experience several of these life events, their scores of the impact of these events were added up. A questionnaire that measures perceived stress regardless of the environment that someone is in might be more beneficial for our research question. This could for instance be done by using the Perceived Stress Scale by Cohen and colleagues (Cohen, Kamarck, & Mermelstein, 1983; Hellhammer, 2010).

Other limitations are regarding the administration and measurement of oxytocin levels. Thus far, we do not exactly know what happens with the exogenously administered oxytocin in the human body. For instance, it is unknown how much exogenously administered oxytocin reaches the correct part of the brain. It is also unknown whether exogenously administered oxytocin interacts with endogenous levels of oxytocin (McCullough, Churchland, & Mendez, 2013). To investigate mechanisms of the placebo effect of oxytocin it is relevant to know what happens with exogenously administered oxytocin.

Now we know that oxytocin is a hormone that has the potential to be classically conditioned, it can have implications for clinical practice. We know for example that oxytocin treatment may have beneficial effects for patients with borderline personality disorder and patients witch autism spectrum disorder (Anagnostou et al., 2012; Bertsch et al., 2013). We also know that oxytocin treatment might improve glucose homeostasis, increase insulin sensitivity and may lead to weight loss in overweight adults (Lawson, 2017). In clinical practice, the possibility to classically condition oxytocin might be used in placebo-controlled dose reduction. This might have the potential to reduce the dose of the oxytocin in pharmacological treatment, while retaining the efficacy of the treatment (Doering & Rief, 2012). Which could be beneficial in alleviating side effects. Although, according to MacDonald et al. (2011), oxytocin treatment with intranasal oxytocin does not yield serious side-effects. Another benefit of placebo-controlled dose reduction is the reduction of health care costs in pharmacological treatments with oxytocin. Furthermore, the conditioning of

oxytocin might have the potential to be useful as a supporting treatment, without increasing the medication dose (Kirchhof et al., 2018).

Future research should investigate the possibilities of placebo-controlled dose reduction, as well as the possibilities of conditioned supportive treatments in pharmacological treatments with oxytocin. Important to remark is that we should be careful considering these possibilities. The reason for this is that the exact implications of real pharmacological oxytocin treatments in clinical practice are still unclear. Some studies were unable to find (positive) effects of oxytocin treatment (Mameli et al., 2014; Nave, Camerer, & McCullough, 2015). Findings on the clinical relevance of oxytocin are not yet conclusive and need more research to reach consensus. Furthermore, future research could focus on factors that might influence classical conditioned placebo responses. Knowledge about such factors might be important for clinical practice. It could, for example, be useful in determining which factors and to what extend should be present or absent to provide an effective treatment. Our results showed that stress might be an influential factor for the conditioned placebo effect of oxytocin. However, to make a recommendation for clinical practice, more evidence resulting from more thorough research is needed.

In conclusion, this study proves that it is possible to induce a placebo effect in oxytocin through the mechanism of classical conditioning. In addition, our results indicate that some psychological factors may influence the classically conditioned placebo effect of oxytocin. In our study, stress had an effect on the conditioned placebo response of oxytocin. Increased stress levels were associated with enhanced conditioned placebo responses of oxytocin. Anxiety did not have an effect on the conditioned placebo response of oxytocin. This may be promising for clinical practice in the future, as making use of the placebo effect might be an efficient way to reduce medication dosages and to improve pharmacological treatment effects. Herewith, associated factors such as stress, should be taken into account. Yet, more research is needed with more substantial evidence to draw more solid conclusions.

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