Chapter 6

Effects of oxytocin on placebo and nocebo effects in a pain conditioning paradigm: a randomized controlled trial

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Chapter 6
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Abstract

Oxytocin is a peptide hormone that has been shown to increase trust, decrease anxiety and affect learning as has been observed in conditioning paradigms. Trust, anxiety and learning are important factors that influence placebo effects. In this study we investigated whether oxytocin can increase placebo analgesia, decrease nocebo hyperalgesia, and influence extinction processes of both. Eighty healthy male volunteers were assigned to a 40 IU of oxytocin nasal spray group, or to a placebo control group. Placebo analgesia and nocebo hyperalgesia were induced by a conditioning procedure in combination with verbal suggestions. The results demonstrate that the conditioning procedure successfully elicited significant placebo analgesia and nocebo hyperalgesia responses ($p < .001$). Furthermore, extinction was observed ($p < .001$), although placebo and nocebo responses did not return to baseline and remained significant. Oxytocin did not influence placebo analgesia or nocebo hyperalgesia and had no effect on extinction. This study provides support against the placebo-boosting effects of oxytocin and was the first one to demonstrate that it also did not influence nocebo effects or extinction processes. As managing placebo and nocebo effects has widespread clinical implications, further research should investigate other neurobiological or behavioral pathways to boost placebo and decrease nocebo effects.

Trial registration: The study protocol was preregistered on the website www.trialregister.nl under the number NTR6506.

Perspective: The present study demonstrated that placebo analgesia and nocebo hyperalgesia can be successfully induced by conditioning and verbal suggestions. We could not confirm the hypothesis that oxytocin affects either of these phenomena. Other pharmacological agents and behavioral manipulations for increasing placebo and decreasing nocebo effects should be investigated.

Keywords: oxytocin, placebo effect, nocebo effect, analgesia, hyperalgesia

Introduction

Accumulating findings demonstrate the pain reducing effects of placebo analgesia (1) and pain enhancing effects of nocebo hyperalgesia (2). Expectations and conditioning are two of the most important mechanisms proposed to underlie these phenomena (3, 4). They have been shown to induce robust reductions in pain in experimental and clinical settings (1), while expectations of negative treatment outcomes were associated with higher pain ratings (5). Classical conditioning, a learned association between an initially neutral stimulus (conditioned stimulus, CS) and a physiologically relevant unconditioned stimulus (US), has been also frequently applied to elicit both placebo and nocebo effects for pain (6, 7). Classical conditioning is furthermore characterized by the extinction of the conditioned response with time and when not reinforced (8). This extinction process is particularly found for placebo effects for pain (9, 10). In contrast, a lack of extinction of nocebo effects for pain was repeatedly demonstrated (7, 11). These negative learned associations are particularly relevant for clinical practice as these may be crucial processes underlying various chronic pain syndromes.

Despite the importance of managing placebo and nocebo effects for the treatment of pain, not much research has been done on the neurobiological pathways of these effects as a potential target to enhance placebo effects, and decrease nocebo effects. So far, some evidence exists regarding possibilities to block nocebo effects by proglumide, the cholecystokinin agonist (12), but almost no evidence for the enhancement of placebo effects is available. A recent study aimed to boost placebo analgesia using dopaminergic agonist was not successful (13). Colloca and colleagues, however, found that vasopressin enhances placebo effects in pain but only in women (14). Finding ways to influence placebo and nocebo effects is however crucial for application in clinical practice.

Oxytocin, a peptide hormone produced in the hypothalamus, was proposed as a possible mediator of the placebo effect (15) due to its trust-inducing and stress-relieving properties. A few studies investigated the influence of oxytocin on placebo effects in experimental settings. Kessner and colleagues (16) demonstrated boosting effects of oxytocin on verbally-induced placebo analgesia for experimentally induced pain. In contrast, Colloca and colleagues (14) and Skvortsova and colleagues (17) found no effects of oxytocin on verbally-induced placebo analgesia. These three previous studies used verbal suggestions alone to induce placebo effects. However, it well-known that the combination of verbal suggestions and conditioning induces the largest placebo and nocebo effects (18). No studies so far examined the effects of oxytocin on the placebo effect triggered by classical conditioning along with verbal suggestions and, furthermore, no studies looked at whether oxytocin is able to minimize nocebo effects. As oxytocin facilitates both learning performance during pain conditioning (19) and conditioned fear extinction (20), it is possible that it could affect the conditioning process and also the extinction of placebo and nocebo responses.

In this study, we investigated the effects of oxytocin administration on placebo and nocebo effects for pain induced by a combined conditioning and verbal suggestions approach. Moreover, we explored the extinction of placebo and nocebo effects and possible modulating effects of oxytocin on it.

Methods

Study design

A randomized placebo-controlled double-blind design was used for this experiment. Participants were randomly allocated to one of two groups: an oxytocin group, which received 40 IU of oxytocin nasal spray, and a placebo group, which received the same volume of a placebo nasal spray. The study protocol was approved by the Medical Ethical Committee of the Leiden University Medical Center (number NL60185.058.16) and the study was preregistered as a clinical trial on www.trialregister.nl (NTR6506). The randomization was performed by the Clinical Pharmacy of the Leiden University Medical Center using block randomization with the block size of 8.

Participants

Eighty healthy male volunteers between 18 and 36 years old participated in this study. Male participants were recruited since previous studies reported on the oxytocin boosting placebo analgesia in men (16) and not in women (17) and oxytocin fluctuations during the menstrual cycle can be an interfering factor in studying oxytocin effects in women (21). Exclusion criteria were heart and lung diseases, high or low blood pressure, chronic or acute pain complains, heavy use of alcohol or other drugs, current diagnosis of psychiatric disorders, and current use of analgesic medications. Participants were asked to refrain from drinking alcohol and intense physical exercises up to12 hours prior to the experiment and from drinking caffeinated drinks and smoking up to 2 hours before the experiment.

Procedure

Prior to being invited for the experiment, prospective participants were asked to give their informed consent and to complete an online questionnaire on Qualtrics (Provo, UT) to screen for exclusion criteria. All eligible candidates were invited to the laboratory for a 2 hour session.

The experimental timeline is presented in a Figure 1. Upon arrival to the laboratory, participants were given the information about the experiment and were asked to sign a written informed consent form. The experiment was conducted by two experimenters: one gave instructions and the other controlled the equipment. First, participants were asked to fill in several short questionnaires on the computer. Then the pain calibration took place. After determining individual temperatures that elicited low, moderate and high pain levels, participants were administered oxytocin or placebo nasal spray, depending on their group allocation, in a double-blind randomized manner. After the spray administration, participants were provided with verbal suggestions regarding the subsequent conditioning task and a 30-minute waiting time followed to allow oxytocin to reach its peak effects (22). During this break, participants were given

magazines with a neutral content to read. After the break, the conditioning task took place. After the end of the task, participants filled in a closing questionnaire, were debriefed and paid for their participation. The data was collected at the laboratory of the Faculty of Social Sciences of Leiden University between March and November 2017.

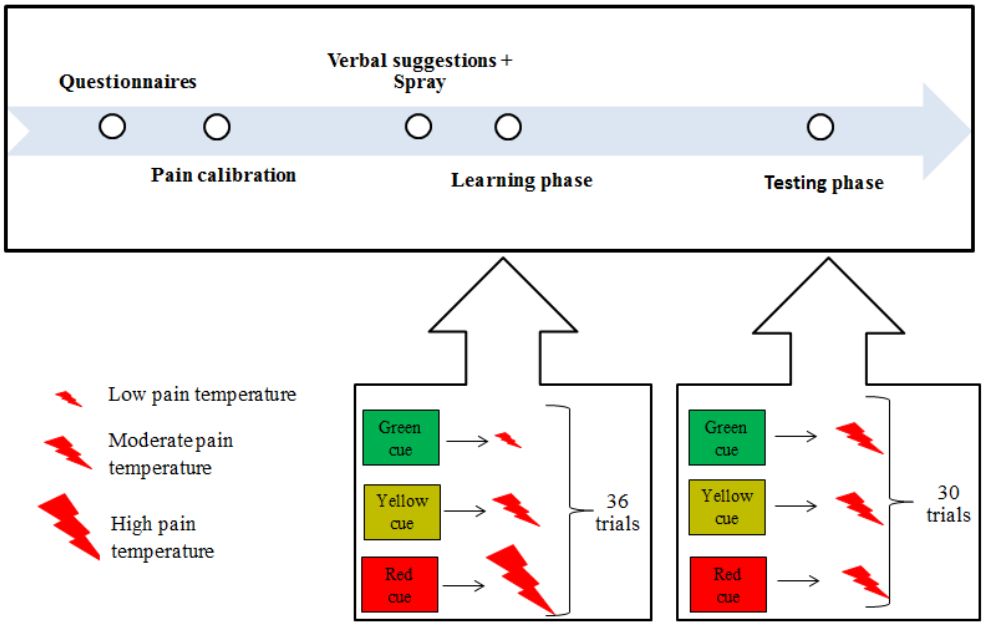

Figure 1. The experimental timeline.

Experimental manipulations

Nasal spray. Participants received 40 IU of oxytocin (Syntocinon) or placebo nasal spray depending on group allocation. The spray was administered by the experimenter with four puffs (two puffs per nostril) using a MAD Nasal Mucosal Atomization Device (Teleflex, Inc., Research Triangle Park). The placebo spray looked and tasted identically to oxytocin. The medication was prepared by Clinical Pharmacy of the Leiden University Medical Center that also was responsible for the randomization. The pharmacy assigned participants numbers and assigned participants to oxytocin or control groups. The researchers received the randomization list with the groups assignments only after the end of the study.

Verbal suggestions. After the nasal spray administration, participants received verbal instructions about the experiment. They were told that the aim of the experiment was to investigate how the oxytocin spray

would influence a TENS (transcutaneous electrical nerve stimulation) device. It was explained to them that during the next task, a TENS electrode would be placed on their arm and that this electrode was able to regulate their pain sensitivity levels as it acted on the pain processing pathways. They were told that when they would see a green cue on a computer screen, the TENS would decrease their pain sensitivity, when they would see a yellow cue this would indicate that the TENS would be inactive, and when they would see a red cue this would indicate that the TENS would increase their pain sensitivity. However, the TENS remained inactive during the experiment; the information was used as a cover story for inducing expectations about the influence of TENS on pain sensitivity and thereby possibly strengthening the conditioning procedure.

Pain calibration. Pain stimuli were delivered with a standardized heat pain application device (ATS-II, Medoc Advanced Medical Systems, Ramat Yishai, Israel). The stimuli were applied to the dorsal site of the not dominant arm. During the calibration procedure, three levels of heat stimulation were determined for each participant individually: 1) a temperature that elicited low pain (pain detection threshold, equal to around 1 on a 0-10 numeric rating scale (NRS); 2) a temperature that elicited moderate levels of pain (equal to 4 on the 0-10 NRS); 3) a temperature that elicited high but bearable levels of pain (equal to 7 on the 0-10 NRS). In order to determine these three levels, we applied one sequence of ascending temperatures to participants' arms with a peak temperature lasting for 4 seconds and a between-stimulus interval of 15 seconds. Participants were asked to rate each stimulus on an NRS ranging from 0 to 10 (0 = no pain at all, 10 = worst pain ever experienced). After the pain of 7 was reached, the calibration procedure was stopped.

Pain conditioning task. The pain conditioning task consisted of two phases: a learning phase and a testing phase. In the learning phase, a green cue on a computer screen was coupled to a low pain stimulus, a yellow cue to a moderate pain and a red cue to a high pain stimulus. The learning phase consisted of 36 stimuli (12 of each color/intensity).

In the test phase, only medium pain temperatures were presented coupled with green (placebo condition), yellow (control condition) and red (nocebo condition) lights. The test phase consisted of 30 stimuli (10 of each color). There was no break between the learning and the test phase. The peak temperature lasted 4 seconds with a ramp-up and ramp-down speed of 8 degrees per second and an inter-stimulus interval of 4 seconds. The stimuli were presented in a randomized fixed order: four random sequences of stimuli were created prior to the experiment and each participant was randomly allocated to one of the four sequences. To avoid habituation and sensitization to pain, the thermode was moved to another place on the forearm twice: after the 22nd stimulus of the learning phase and 8th stimulus of the test phase (every 22 stimuli). After each pain stimulus, participants were asked to rate the pain intensity on a 0 (no pain) - 10 (most intense pain imaginable) NRS verbally.

Instruments and materials

Visual stimuli. E-Prime (version 2.0) software installed on a desktop computer was used to present the visual cues (green, yellow, red) during the pain conditioning task. The resolution of the screen was 1280x1024 pixels and participants were sitting approximately 60 cm from the screen. The visual cues were shown two seconds before the start of each heat stimulus. During the cues, the whole screen turned green-, yellow-, or red-colored.

Questionnaires. A short version of the Positive and Negative Affect Schedule (PANAS) (23) was used to measure the mood of participants at baseline. The questionnaire consisted of 10 items: 5 for measuring positive affect and 5 for measuring negative affect. Scores were obtained on a 5-point Likert scale and could range from 5 to 25. Higher scores indicated higher positive and higher negative affect, respectively. The Cronbach's alpha in our sample was .85 for positive affect and.80 for negative affect, indicating a good internal consistency.

The State Trait Anxiety Inventory – state version (STAI-Ss) (24) was used for measuring baseline state anxiety of the participants. The questionnaire consisted of 6 statements, and participants had to indicate on a scale from 1 (not at all) to 4 (very much) how much this statement applied to them at that specific moment. Scores could range from 6 to 24 with higher scores indicating higher state anxiety. In the present study, the Cronbach's alpha was .81 indicating a good internal consistency.

The revised Life Orientation Test (LOT-R) (25) was used to measure optimism. The LOT-R consists of 3 positive, 3 negative, and 4 filler items and participants had to indicate whether they agree or disagree with each on a scale from 0 (strongly disagree) to 4 (strongly agree). Scores could range from 0 to 24 with higher scores indicating higher optimism. In the present study, the Cronbach's alpha was .75, indicating acceptable internal consistency.

The neuroticism and extraversion scales of the short version of the Eysenck Personality Questionnaire (EPQ-RSS) (26) was used for measuring neuroticism and extraversion. The questionnaire consisted of 24 items: 12 items for measuring neuroticism and 12 for measuring extraversion. The participants were asked to use a dichotomous scale for giving the answers ("yes" or "no"). Scores could range from 0 to 12 with higher scores indicating higher neuroticism and higher extraversion, respectively. The Cronbach's alpha in our sample was .77 for neuroticism, indicating acceptable internal consistency, and .84 for extraversion, indicating a good internal consistency.

Finally, a closing questionnaire was designed to ask participants about their experiences. They were asked to answer the following questions: "Do you think you received oxytocin or placebo?"; "What do you think was the aim of this experiment?"; "Have you heard anything about this experiment from other people? If yes, what?".

Data analyses

The power calculation was performed with software G*Power 3 (27). The input for the power calculation was derived from a study on the effect of oxytocin on placebo analgesia (16) with an effect size of d=0.495. A sample size calculation for a repeated measurements within-between analysis of variance with 2 groups indicated that 48 participants in total (24 in each group) would be needed to obtain a power of .95 at an alpha level of α=0.5. Taking into account the conflicting results of the previous studies and an additional 10% of possible technical failures, we adjusted the total sample size to the conservative number of 80 participants; 40 in each group.

The data analysis was performed using SPSS Statistics version 21 (IBM Corporation) with a two-tailed significance level of alpha < .05. The data was screened for univariate outliers using z-scores. Z-scores above 3.29 or below -3.29 were considered to indicate univariate outliers. One outlier was found for the negative affect score and one outlier for the state anxiety score, with z scores of 6.21 and 5.05 respectively. Non-parametric tests were applied for the analysis of the variables with outliers. Skewness, kurtosis and Shapiro-Wilk tests indicated normality of distribution of all variables. Levene's tests indicated homogeneity of variances in the groups. Sphericity was measured with the Mauchly's test of sphericity and in case of the violation of this assumption, Greenhouse-Geisser corrections were used.

The heat stimuli directly following the repositioning of the thermode, elicited significantly higher pain ratings. For this reason, the two stimuli directly following the repositioning of the thermode, were excluded from the analysis. In total, 35 learning trials and 29 test trials were included in the analysis.

Independent samples t-tests or non-parametric Mann-Whitney tests were used to compare the groups on the baseline and personality characteristics, as appropriate: age, heat pain detection threshold (temperature that was rated as 1 on the VAS pain scale), positive affect, anxiety, optimism, extraversion and neuroticism.

A 2x3 (group (oxytocin vs placebo) x cue color (green vs yellow vs red)) factorial analysis of variance (ANOVA) was used to compare the groups on the mean pain scores in response to green, yellow and red cues in the learning phase of the conditioning task. To examine whether the placebo and nocebo effects were significant and differed between the groups, a 2x3 (group (oxytocin vs placebo) x cue color (green vs yellow vs red)) factorial ANOVA was used to compare the groups on their mean pain ratings in response to the three color cues in the test phase of the conditioning task. Furthermore, to examine the extinction process in more detail, placebo and nocebo effects were calculated for each pair of green-yellow and yellow-red trials of the test phase. Specifically, placebo effects were calculated as the difference between pain ratings in response to the yellow versus the green cues whereas nocebo effects were calculated as the difference between the pain ratings in response to the red versus the yellow cues. In total, 9 placebo and 9 nocebo effects (corresponding to 9 red and yellow or green and yellow comparisons) were calculated. Repeated measures ANOVA was done with time (placebo effect order number) as a within-subject factor,

group as a between-subject factor and placebo effect as a dependent variable to evaluate the effects of the group and time on the placebo effect. Bonferroni corrected post-hoc tests were applied to investigate the difference between the time moments. The same analysis was run with nocebo effect as a dependent variable. To compare the strength of placebo and nocebo effects, the mean placebo effect was compared to the mean nocebo effect with a one-sample t-test. Finally, to check if the groups differed in their perceived group allocation, a chi-square test was performed.

To examine the effects of psychological and personality characteristics on the placebo effect, linear regression analyses were performed with positive affect, anxiety, optimism, extraversion and neuroticism as independent variables and the placebo effect as a dependent variable. The same analysis was repeated with nocebo effect as a dependent variable.

To examine whether the perceived group allocation has an effect on placebo and nocebo effects, independent samples t-tests were used to compared people who thought they received placebo and people who thought they received oxytocin spray on their placebo and nocebo effects.

Partial eta squared was calculated for analyses as the indication of the effect sizes.

Results

Baseline characteristics

Data of 76 participants were available for the analysis; data of 4 participants (1 from oxytocin group, 3 from placebo group) were excluded due to technical problems during the conditioning task. The baseline and personality characteristics are presented in Table 1. There were no differences between the groups on any of these variables, except for extraversion: participants randomized to the oxytocin group (M = 10.69, SD = 2.68) reported a higher extraversion in comparison to the control group (M = 9.11, SD = 3.43; t (74) = 2.25, p = .027).

	Oxytocin group (n = 39)	Control group (n = 37)	t	p
Age	23.30 (3.44)	23.11(2.94)	0.26	.80
State anxiety *	8.99 (2.46)	8.70 (2.23)	682	.68
Positive mood	31.90 (8.22)	30.81 (7.07)	0.62	.54
Negative mood*	12.56 (2.58)	13.28 (2.83)	611	.24
Neuroticism	2.59 (2.66)	3.62 (2.78)	-1.65	.10
Extraversion	10.69 (2.68)	9.11 (3.43)	2.25	.027
Optimism	22.13 (3.43)	22.27 (3.85)	-.0.17	.87

Pain detection threshold	43.39 (1.72)	43.87 (1.70)	-1.22	.23

Table 1. Baseline and personality characteristics with standard deviations across the groups. *Mann-Whitney U test is presented instead of t-test, as non-parametric test was applied to these variables

Learning phase

The mean levels of pain during each trial of the learning phase are presented in Figure 2. The temperatures that were calibrated to elicit the pain of 1, 4 and 7 on the 11-point NRS, caused lower pain during the learning phase of the conditioning task (pain of 0.5, 2.85 and 5.88 respectively). The 2x3 (group x cue color) factorial ANOVA with a Greenhouse-Geisser correction demonstrated that there was a significant main effect of the cue color on the pain ratings ($F(1.65, 121.86) = 634.27$, $p < .001$, $\eta p2 = .90$), while the effect of the group ($F(1, 74) = 0.69$, $p = .69$, $\eta p2 = .01$) and the group-cue color interaction ($F(1.65, 121.86) = 0.55$, $p = .54$, $\eta p2 = .01$) were non-significant. Post-hoc tests using Bonferroni corrections indicated that participants rated the stimuli following the red cues ($M = 5.88$, $SD = .18$) as significantly more painful than stimuli following yellow ($M = 2.88$, $SD = 0.14$) and green cues ($M = 0.49$, $SD = 0.06$); also the yellow cues stimuli were rated as significantly more painful than the green cues stimuli (all *p*s < .001; Figure 2). Oxytocin did not influence the pain perception during the learning phase.

Test phase

The 2x3 (group x cue color) factorial ANOVA with a Greenhouse-Geisser correction demonstrated that there was a significant main effect of the cue color on the pain ratings ($F(1.41, 104.61) = 61.71$, $p < .001$, $\eta p2 = .46$), while the main effect of the group ($F(1, 74) = 2.31$, $p = .13$, $\eta p2 = .01$) and the group x cue color interaction ($F(1.41, 104.61) = 0.63$, $p = .48$, $\eta p2 = .01$) were non-significant. Post-hoc tests using Bonferroni corrections indicated that participants rated the stimuli following the red cues ($M = 3.37$, $SD = 0.19$) as significantly more painful than stimuli following yellow ($M = 3.07$, $SD = 0.17$) and green cues ($M = 2.62$, $SD = 0.15$), and green cues stimuli as significantly less painful than yellow and red cues stimuli (all *p*s < .001).

The difference scores between the yellow and green trials (placebo effect) and red and yellow trials (nocebo effect) of the test phase are presented in Table 2. The repeated measures ANOVA with a Greenhouse-Geisser correction for the placebo effect showed that the main effect of the group ($F(1, 73) = 0.06$, $p = .82$, $\eta p2 = .001$) and time x group interaction ($F(6.34, 456.80) = 0.80$, $p = .58$, $\eta p2 = .01$) were non-significant; however, there was a significant main effect of time on the placebo effect ($F(6.34, 458.6) = 5.07$, $p < .001$, $\eta p2 = .07$). Post-hoc tests using Bonferroni corrections indicated that the first placebo effect was significantly larger than the following placebo trials.

The repeated measures ANOVA with a Greenhouse-Geisser correction on the nocebo effect showed that the main effect of the group ($F(1, 73) = 0.67$, $p = .41$, $\eta p2 = .01$) and the time x group interaction ($F(6.14,$

448.19) = 0.61, p = .77, ηp2 = .01) were non-significant; however, there was a significant main effect of time for the nocebo effect (F (6.14, 448,9) = 3.67, p = .001, ηp2 = .05). Post-hoc tests using the Bonferroni corrections indicated that only one nocebo effect in the middle of the test phase was significantly smaller than all other nocebo effects. No other significant differences over time were found.

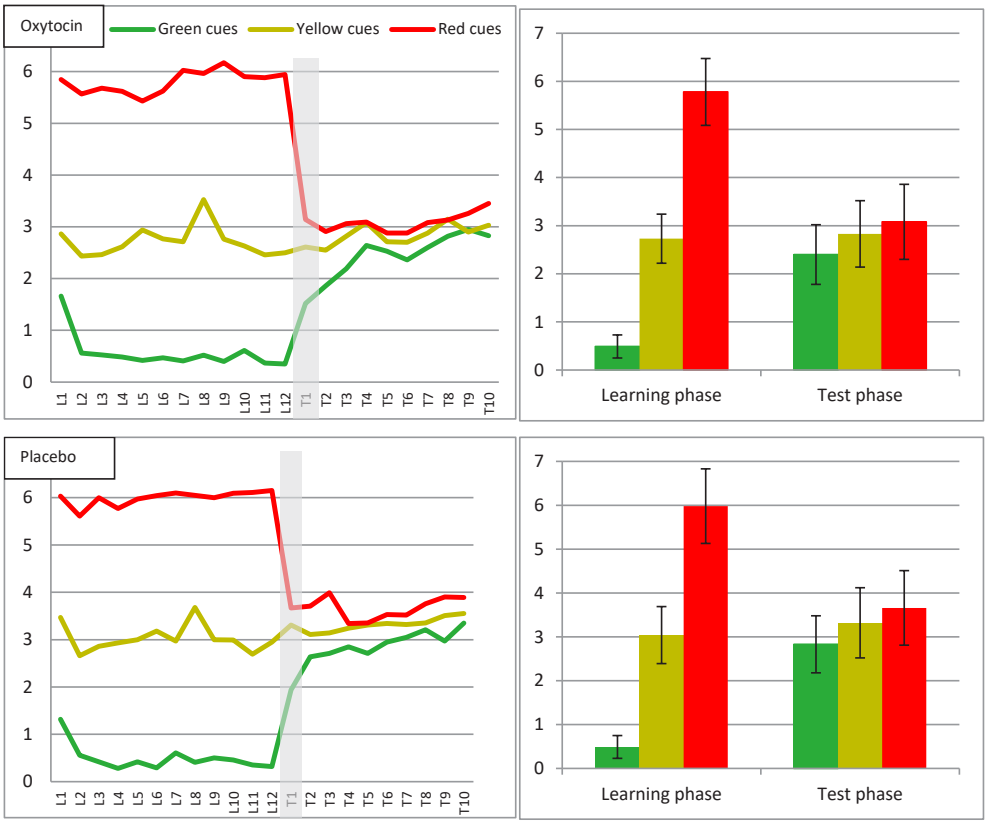

Figure 2. Pain ratings during the learning and test trials in response to green, yellow and red cues. Oxytocin group on the top panel, control group on the down panel, the first test trial is highlighted.

Table 2. Placebo and nocebo effects with standard deviations per test trial across the groups

	Placebo effect		Nocebo effect	
	Oxytocin group (n = 39)	Control group (n = 37)	Oxytocin group (n = 39)	Control group (n = 37)

Test trial 1	1.15 (0.20)	1.27 (0.21)	0.58 (0.21)	0.35 (0.21)
Test trial 2	0.75 (0.19)	0.44 (0.19)	0.35 (0.16)	0.60 (0.16)
Test trial 3	0.50 (0.23)	0.21 (0.23)	0.51 (0.26)	0.88 (0.26)
Test trial 4	0.57 (0.23)	0.53 (0.23)	-0.25 (0.212)	-0.07 (0.22)
Test trial 5	0.13 (0.18)	0.61 (0.18)	0.17 (0.15)	0.08 (0.15)
Test trial 6	0.34 (0.17)	0.46 (0.17)	0.29 (0.14)	0.23 (0.15)
Test trial 7	0.29 (0.18)	0.26 (0.18)	0.01 (0.1)	0.31 (0.16)
Test trial 8	0.25 (0.17)	0.31 (0.18)	0.23 (0.19)	0.39 (0.19)
Test trial 9	0.20 (0.18)	0.31 (0.19)	0.42 (0.20)	0.40 (0.20)

Secondary outcomes

It was demonstrated that the mean placebo effect across all test trials (M = 0.47, SD = 0.50) was larger than the mean nocebo effect (M = 0.30, SD = 0.51; t (75) = 2.40, p = .019). None of the personality characteristics significantly predicted placebo (all p > .08) or nocebo (all p > .19) effects.

Participants in the two groups did not differ in their perceived group allocation (chi square (1, N = 76) = 1.88, p = .171). The majority of participants thought that they received a placebo spray: 71.8% in the oxytocin group and 56.8% in the placebo group. There was no difference between people who thought they received oxytocin (placebo effect: M = .56, SD = 0.71; nocebo effect: M = .48, SD = 0.74) and who thought they received placebo (placebo effect: M= .64, SD = 0.68; nocebo effect: M = .23, SD = 0.60) in their placebo (t (74) = - 0.51, p = .61) and nocebo (t (74) = 1.64, p = .11) effects.

Finally, to explore whether the baseline personality characteristic of extraversion that differed between the groups, influenced the results of the analyses, the same analyses were performed with extraversion as a covariate. As the analyses did not change the results, these results are not reported further.

Discussion

The results of this study demonstrate that conditioning can successfully induce placebo analgesia and nocebo hyperalgesia. Importantly, we showed for the first time that intranasal oxytocin administration did not influence analgesia and hyperalgesia induced by conditioning combined with verbal suggestions or the extinction of these effects.

Our findings regarding on the induction of placebo analgesia and nocebo hyperalgesia by conditioning with verbal suggestions are in line with previous research (1, 2). We used conditioning together with verbal suggestions as it was previously demonstrated that the combination of these two methods is more effective than applying them separately (18). The placebo effect of the first test trial was larger than 1 on the 11-point scale, what is consistent with previous literature (6, 28) and decreased during the test phase showing an extinction pattern. Interestingly, nocebo effects at the first text trial were smaller (0.5 on the 11-point scale), however, they seemed to be affected by extinction less than placebo effects: the greatest extinction of the placebo effect occurred after the first test trial, while the nocebo effect remained stable with only one trial in the middle of the sequence that was significantly lower than others, indicating that the nocebo effect in the present study did not extinct over time. These findings are in line with previous studies that found significant extinction of placebo (9, 10) but not of nocebo effects (7). This phenomenon can be explained by the fact that threatening information is more relevant for an organism's survival from an evolutionary point of view and that is why it should be stored in memory longer than positive information (29). Moreover, Colagiuri & Quinn (30) showed that anticipatory anxiety and elevated autonomic arousal contribute to the persistence of nocebo effects in comparison to placebo effects. Intranasal administration of 40 IU oxytocin did not influence pain-related placebo and nocebo effects and their extinction. These findings are in line with our earlier study in which we also did not find boosting effects of oxytocin on placebo effects for pain and itch induced by verbal suggestions in healthy females and with lower oxytocin doses (17). These findings further confirm previous findings from the study by Colloca and colleagues (14) where no effects of oxytocin on placebo analgesia in either men or women were found. The important addition of the current study is that we employed a conditioning procedure along with verbal suggestions to induce placebo effects and we furthermore explored the effects of oxytocin on nocebo effects. Previous research indicated that oxytocin facilitates learning during fear conditioning when given prior to the conditioning procedure (19) and enhances neural indicators of fear extinction when given prior to the extinction phase (20). We hypothesized that since these effects of fear conditioning were induced by painful stimuli, they could possibly also be applied to the response to a pain conditioning paradigm which essentially represents associative learning. Several explanations can be provided for the lack of confirming these expectations. Placebo analgesia and hyperalgesia are not identical to psychophysiological and neurological responses to fear conditioning used as outcome measures in previous research, such as amygdala activation, skin conductance and reaction times (19, 20). Possibly oxytocin influences neurological conditioned responses but not the conditioned pain perception. By contrast, social aspects seem to play an important role in oxytocin effects. It has been demonstrated that oxytocin enhances learning with social reinforces (emotional faces) in comparison to non-social reinforces (color cues) (31). As the conditioning procedure did not include any social stimuli, possibly

non-social cue based learning mechanisms remained unaffected by oxytocin. On the other hand, some studies (19, 20) found effects of oxytocin on fear conditioning regardless of any social aspect of the CS. Therefore, it remains unclear whether oxytocin influences only the perception of stimuli with a social component.

Another methodological difference of our study from previous research is the dose of oxytocin used. The only experiment that found boosting effects of oxytocin on the placebo effect (16) used 36 IU of oxytocin while two other studies with null findings (14, 17) used a more standard dose of 24 IU. The evidence regarding the correlation between the dose and effectivity of oxytocin is mixed. For example, lower (8 IU) doses of oxytocin have been found to affect amygdala activation in men in response to emotional faces more than higher (24 IU) doses (32), while another study demonstrated that 24 IU had the largest effect on amygdala activation in men in comparison to 12 IU and 48 IU (33). Also, a 24 IU dose seems to have a stronger effect on cortisol levels than a dose of 48 IU (34). On the other hand, 48 IU of oxytocin has been found to improve the performance on a face emotion recognition task, while 24 IU did not (35). Furthermore, a U-shaped relationship between oxytocin dose, social reward, and neural activity has been recently proposed (36), indicating the dose–response relationship is initiated at lower doses in females than males. Considering this mixed evidence, future research needs to determine the exact role of the dose of oxytocin for such phenomena as learning and extinction. Moreover, sex differences may also explain the mixed findings in oxytocin research. For example, oxytocin dampens amygdala activation in response to emotional stimuli in males but enhances activation in females (37). Evidence for opposite effects of oxytocin on social processing in men and women is also present (38, 39). Placebo enhancing effects of oxytocin were previously found in men (16), therefore we recruited a male sample in this experiment. However, we could not confirm these previous results and our results are more in line with our previous study performed in women (17) and with a study by Colloca (14) conducted in both sexes. Sex differences in oxytocin effects remain underexplored and future research should focus on the comparison of male and female samples.

Additionally, we did not find effects of oxytocin on pain sensitivity. A lot of research focused on possible analgesic effects of oxytocin, but the results remain contradictory. A number of studies found pain reducing effects of oxytocin (40-42), however, others could not replicate these results (17, 43-45).

A few limitations of the current study should be mentioned. First of all, repeated administration of the heat stimuli leads to the habituation to pain: the temperatures that during the calibration caused low, medium and high pain levels, during the pain conditioning task elicited lower levels of pain. As participants experienced smaller ranges of pain sensations, it could have decreased the magnitude of placebo and nocebo responses. Despite this fact, this procedure was successful in eliciting placebo analgesia and nocebo hyperalgesia. Additionally, many participants had relatively high pain thresholds and it was

therefore not possible to reach high levels of pain because of the protection mechanisms of the ATS termode that does not allow giving temperatures above certain thresholds. Finally, including only males into the study makes it impossible to generalize the results of the study to females.

To summarize, although placebo analgesia and nocebo hyperalgesia could be successfully induced by conditioning and verbal suggestions, there was no evidence that 40 IU oxytocin given intranasally affected placebo analgesia and nocebo hyperalgesia, nor the extinction of both. Our study adds to the accumulating evidence towards the absence of the ability of oxytocin to influence placebo effects. Researchers interested in possibilities to strengthen placebo effects and reduce nocebo effects might benefit from focusing on other neurobiological and behavioral mechanisms that influence learning mechanisms of placebo and nocebo effects.

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