Chapter 5

Enhancing placebo effects in somatic symptoms through oxytocin

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Abstract

Objective: Placebo effects relieve various somatic symptoms but it is unclear how they can be enhanced in order to maximize positive treatment outcomes. Oxytocin administration may potentially enhance placebo effects but few studies have been performed with conflicting findings. The study aim was to investigate the influence of positive verbal suggestions and oxytocin on treatment expectations and placebo effects for pain and itch.

Methods: 108 Female participants were allocated to one of four groups: 1) oxytocin with positive verbal suggestions, 2) placebo with positive verbal suggestions, 3) oxytocin without suggestions, and 4) placebo without suggestions. The administration of 24 IU oxytocin or a placebo spray was preceded by positive verbal suggestions regarding the pain- and itch-relieving properties of the spray or no suggestions, depending on group allocation. Pain was assessed with a cold pressor test and itch was assessed with histamine iontophoresis.

Results: Positive verbal suggestions induced expectations of lower pain (F= 4.77, p= .031) and itch (F= 5.38, p= .022). Moreover, positive verbal suggestions elicited placebo analgesia (F= 5.48, p= .021), but did not decrease itch. No effect of oxytocin on the placebo effect or on expectations was found.

Conclusions: Positive suggestions induced placebo analgesia but oxytocin did not enhance the placebo effect. Study limitations are that we only included a female sample and a failure induce placebo effect for itch. Future studies should focus on how oxytocin might influence placebo effects, taken into account the role of gender, dose-dependent effects and various expectation manipulations.

Trial registration: The study was registered as a clinical trial on www.trialregister.nl (number 6376).

Key words: placebo effect, oxytocin, pain, itch

Abbreviations: CPT = cold pressor test; HI = histamine iontophoresis; NRS = numerical rating scale

Introduction

The placebo effect, which represents a positive outcome after receiving an inert treatment, has been repeatedly shown to relief a variety of symptoms or conditions such as pain, depression, anxiety, addiction, and Parkinson's disease amongst others (1-4). An important mechanism that has been suggested to underlie placebo effects is that of positive outcome expectations that are linked to better treatment outcomes (2,5,6,7).

In experimental or clinical settings, positive outcome expectations can be induced by verbal suggestions regarding the positive effects of a treatment. Placebo effects induced by verbal suggestions of positive outcome expectations have been extensively investigated in the context of placebo analgesia (1,8). Numerous studies demonstrated that mere positive suggestions about the analgesic properties of a placebo treatment may result in decreased pain (9-11). Placebo effects of positive verbal suggestions in the context of itch, a symptom closely related to pain (12), have been less studied. Initial studies have shown that verbal suggestions can increase itch, indicative of a nocebo effect (13), whereas verbal suggestions combined with conditioning, another important proposed mechanism underlying placebo effects, can reduce itch, indicative of placebo effects in itch (14). Inconclusive findings have been reported on the effects of verbal suggestions alone in inducing placebo effects for itch. Bartels and colleagues (13) and Van Laarhoven and colleagues (12) found no decrease in itch after giving participants positive verbal suggestions. However, Darragh and colleagues (15) used specific 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.

Despite an increasing body of literature on placebo effects induced by positive verbal suggestions, it is currently not yet clear what exact neurobiological mechanism drives the possible effects of verbal suggestions. Moreover, it is currently unknown how placebo effects can be enhanced in order to obtain the best therapeutic results.

Chapter 5
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Enck and Klosterhalfen (16) proposed that oxytocin, a peptide hormone produced in the hypothalamus, might be a facilitator of the placebo effect. Oxytocin is a well-studied hormone that has many positive behavioral effects. Among others, it increases trust (17), empathy and social learning (16-18). In addition, oxytocin has anxiolytic effects (18-20) and it stimulates the secretion of nitric oxide that has been linked to the relaxation response (21). Oxytocin might therefore maximize positive treatment expectations induced by verbal suggestions or lead to better treatment outcomes by inducing a generally more positive affective state. Few studies have investigated the effect of oxytocin on placebo analgesia, with mixed results. Kessner and colleagues (22) induced placebo analgesia in male participants by giving positive suggestions regarding a placebo cream. Participants who received a 40 IU dose of oxytocin spray, demonstrated a higher placebo response to the cream than participants who received a placebo spray. Colloca and colleagues (23) induced placebo analgesia in both female and male participants by giving positive suggestions about pain-relieving effects of a sham electrode. Contrary to Kessner and colleagues (22), they found no effect of 24 IU of oxytocin on placebo analgesia. They however demonstrated that vasopressin, a peptide hormone closely related to oxytocin, enhanced placebo analgesia, but only in women and not in men. In addition to these findings, a few studies have demonstrated that oxytocin itself can influence pain symptoms (24-27), however, this evidence is inconclusive because other studies (22, 23, 28, 29, 30) found no pain-relieving effects of oxytocin. Considering these mixed results, the question remains whether oxytocin can potentially enhance placebo analgesia. Furthermore, no studies to date have examined the effect of oxytocin on placebo effects for other somatic symptoms, such as itch.

In the present study we investigated whether positive verbal suggestions would induce placebo effects for somatic symptoms of pain and itch and whether oxytocin administration would increase these placebo effects in healthy women. Firstly, we hypothesized that participants who receive positive verbal suggestions would expect and experience less pain and itch in response to validated pain (cold pressor test, CPT) and itch (histamine iontophoresis, HI) tests than participants who received no suggestions. Secondly, we expected that oxytocin would boost the placebo effect induced by positive verbal suggestions and participants who receive oxytocin in combination with suggestions would expect and

experience less pain and itch than participants who receive only suggestions or only oxytocin. Similar effects for both pain and itch were expected. We also explored whether oxytocin in combination with verbal suggestions had an effect on the pain unpleasantness after the CPT, skin redness and wheal size, and skin temperature in response to HI. We further studied whether oxytocin reduced pain and itch sensitivity. Questionnaires were administered to control for potential group differences in psychological characteristics such as anxiety, mood, neuroticism, optimism and extraversion.

Methods

Study design

The study design was a randomized controlled trial. It was approved by the Medical Ethical Committee of the Leiden University Medical Center (number NL55922.058.15). The randomization was performed by the Clinical Pharmacy of the Leiden University Medical Center. After the screening, participants were randomly allocated to one of the following groups: 1) oxytocin with positive verbal suggestions, 2) placebo with positive verbal suggestions, 3) oxytocin without verbal suggestions, and 4) placebo without verbal suggestions. Both participants and experimenters were blind regarding the drug condition. One of the two experimenters was aware of the verbal suggestion condition due to the positive verbal suggestions instructions that were given by this experimenter, however, the second experimenter who performed the pain and itch tests was blind regarding allocation of the condition as to avoid influencing the results of the tests.

Participants

Female participants were eligible to participate when they were healthy and between 18 and 35 years old. The exclusion criteria consisted of: current use of medication, skin conditions such as eczema, current diagnosis of a mental disorder, Raynaud's syndrome, acute or chronic pain complaints, and (intended) pregnancy. Prior to the experiment, participants were screened for these exclusion criteria via an online questionnaire in Qualtrics (Provo, UT). Participants were asked to refrain from taking analgesic medication, drinking alcohol and doing intense physical exercise up to 24 hours before the experiment and drinking caffeine drinks up to 2 hours before the experiment. At the start of the experiment, participants

signed an informed consent form and after completion of the experiment they received a monetary compensation.

Procedure

The data was collected between April, 2016 and April, 2017. The experimental timeline is presented in Figure 1.

The experiment was conducted by a male and a female experimenter. A male experimenter explained the procedure and gave the verbal suggestions, while a female experimenter performed the CPT and HI. Fixed genders of the experimenters were chosen to minimize the possible influence of the gender of the lab personnel on the pain and itch test outcomes. On the test day, the participant was asked to fill in several baseline questionnaires on a computer. Subsequently, the baseline CPT took place. After the test, positive suggestions were given to participants in the two positive suggestions groups and participants received oxytocin or placebo nasal spray depending on group allocation. During a waiting period of 30 minutes allowing oxytocin to reach peak concentration (31), participants filled in some questionnaires and were offered magazines with neutral content to read. After the waiting period, the HI was performed; no baseline HI was performed in order to avoid habituation effects that appear during second HI administration (12). In order to minimize possible crossover effects of the HI on pain sensitivity, an additional waiting period of 10 minutes was completed. After that the post-intervention CPT took place. At the end of the session, participants were asked about their perceived group allocation, debriefed, and paid for their participation in the study.

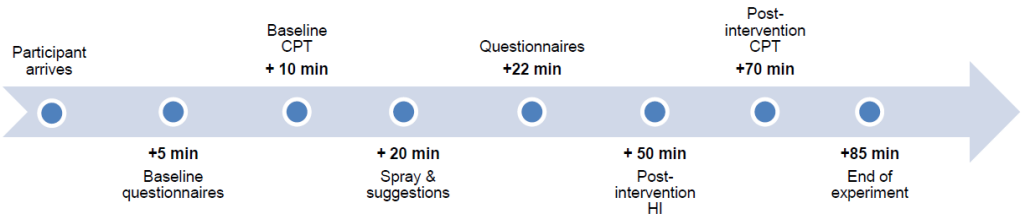

Figure 1. Experimental timeline.

Experimental interventions

Expectation induction. Positive expectations about the nasal spray were induced in the two groups with positive verbal suggestions. Participants received the following verbal suggestions: "Now you will receive a nasal spray. This spray contains oxytocin. It has been demonstrated in previous studies that oxytocin decreases pain and itch sensitivity. We expect that after receiving oxytocin you will also experience less pain during the cold pressor test and less itch during the histamine test". Participants in the groups without suggestions were told the following: "Now you will receive a spray. This spray contains either oxytocin or placebo".

Drug administration. Depending on the group allocation, participants received 24 IU of oxytocin (Syntocinon) or placebo nasal spray. Placebo spray looked and tasted identically to oxytocin and was prepared by the Clinical Pharmacy of the Leiden University Medical Center. The nasal spray was administered by the experimenter with two puffs (one puff per nostril) using a MAD Nasal mucosal atomization device (Teleflex, Inc., Research Triangle Park).

Materials and Questionnaires

Cold Pressor Test

Pain sensitivity was assessed by the CPT. The waterbath consisted of a 2.7 liter styrofoam tank with cold water, which was maintained at a fixed temperature of 4°C. Participants were asked to hold their dominant hand in the water for 1 minute (32). During this minute, every 15 seconds their pain levels were assessed on a numerical rating scale (NRS) with a question "How much pain do you have now?". The participant gave an answer on a 0-10 scale (0 = no pain at all, 10 = worst pain ever experienced). In case participants were unable to hold their hand in the water for the entire minute, they could stop the test at any time. The pain intensity scores in response to the baseline and post-intervention CPT were calculated as the mean scores of the four pain rating measurement points during each CPT. Before the test participants were asked if they experienced any pain and itch and the test was performed only in case of the absence of both. After the test, participants were asked to indicate how unpleasant the test was on a 0-10 NRS scale (0 = not unpleasant at all, 10 = very unpleasant).

Histamine iontophoresis

Itch was induced by means of HI (12). Histamine dihydrochloride (0.5%) was dissolved in a gel of methylcellulose and propylene glycol in distilled water (manufactured by the Clinical Pharmacy, Leiden University Medical Center) and 2.5 ml was placed in a disposable iontophoresis electrode (IOGEL medium, Chattanooga, Hixson, USA). The electrode was placed on the non-dominant forearm, 2 cm distal to the lateral epicondyle of the humerus. The reference electrode was applied to the skin on the lateral side of the triceps brachial muscle. The histamine solution was delivered with a dose controller (Chattanooga ionto, Chattanooga Group, Hixson, USA) for 2.5 minutes at a current level of 0.4 mA. Itch was measured 6 times during 2.5 minutes (immediately, and after 30, 60, 90, 120 and 150 seconds from the beginning of the test) by asking the participants the following question: "How much itch do you have now?". The participant gave an answer on an NRS scale ranging from 0-10 (0 = no itch at all, 10 = worst itch ever experienced). The itch intensity score was calculated as a mean score of the 6 itch rating measurement points during the HI. Before the test participants were asked if they experienced any pain and itch and the test was performed only in case of the absence of both. Upon completion of the test, the size of skin redness and wheal size were measured with a ruler, and skin temperature was measured using a hand-held infrared digital thermometer (held vertically approximately 1 cm above the area; accuracy ± 2.0 °C, resolution 0.1 °C, BaseTech, Conrad Electronic Benelux B.V.).

Questionnaires

Expectations about pain and itch were measured with questions "How much pain do you expect to experience during the cold pressor test?" and "How much itch do you expect to experience during the histamine test?". Participants gave answers on an NRS from 0-10 (0 = no pain/itch at all is expected, 10 = worst pain/itch ever experienced is expected). Participants answered these questions twice: upon coming to the lab (baseline expectations) and after receiving the spray with or without the combined suggestions (post-intervention expectations).

State anxiety was measured with the short state version of the State Trait Anxiety Inventory, (STAI-Ss) (33). The questionnaire consists of 6 items and the participants were asked to give the answers on a 4-

point Likert scale. The score ranges from 6 to 24, with a higher score indicating higher state anxiety. Cronbach's alpha in our sample (.80) was similar to previously reports (33).

Positive and negative affect were measured with a short version of the Positive and Negative Affect Schedule (PANAS) (34). The short PANAS consists of 5 items for measuring positive affect and 5 items for measuring negative affect on a 5-point Likert scale. The score for both scales ranges from 5 to 25, with higher scores indicating higher positive and higher negative affect, respectively. The Cronbach's alpha for positive affect in our sample was .85 and for negative affect .74 which was similar to previous reports (34).

The short version of the Eysenck Personality Questionnaire (EPQ-RSS; 35) was used for measuring neuroticism and extraversion. Each scale consists of 12 items and the participants were asked to use a dichotomous scale for giving the answers ("yes" or "no"). The total score for neuroticism and extraversion ranges from 0 to 12, with higher scores indicating higher neuroticism and higher extraversion, respectively. The Cronbach's alpha in this sample was lower than previous reports (35): .74 for neuroticism and .42 for extraversion which reflects low reliability.

The revised Life Orientation Test (LOT-R) (36) was used to measure dispositional optimism. The questionnaire consists of 3 positive, 3 negative, and 4 filler items and answers are given on a 5-point Likert scale. The total score ranges from 0 to 24, with higher score indicating higher optimism. In this sample, Cronbach's alpha was .66.

Statistical Analysis

The data analysis was performed using SPSS Statistics version 21 (IBM Corporation) with a two-tailed significance level of alpha < .05. The power calculation was based on two studies: a study on the effect of oxytocin on placebo analgesia (22), which reported an effect size of d=0.495, and a study on the effects of positive verbal suggestions on self-reported pain (1), which found an effect size of d=0.50. G-power (37) analyses yielded a sample size of 23 persons per group to obtain a power of .80 at an alpha level of .05. Taking into account 15% of potential drop outs due to amongst others technical problems, the total sample size was adjusted to a more conservative number of 108 participants.

The data was screened for univariate outliers using z-scores. No z-scores above 3.29 or below -3.29 were found indicating the absence of univariate outliers. Skewness and kurtosis and Shapiro-Wilk test were calculated and indicated normality of distribution of all variables. Homogeneity of variances was checked with Levene's tests that indicated homogeneity of variances in the groups. Baseline characteristics (baseline CPT pain score, baseline CPT pain unpleasantness, expected pain, expected itch, state anxiety, mood, neuroticism, extraversion, and optimism) of the participants were compared between the groups with a one-way between-subject analysis of variance (ANOVA). Baseline CPT and post-intervention CPT pain scores were compared with a paired-sample t-test.

The effects of oxytocin and positive verbal suggestions on the pain and itch expectations were analyzed by using two 2x2 factorial ANOVAs with spray (placebo, oxytocin) and positive verbal suggestions (with, without) as between-group factors and pain and itch post-intervention expectations, respectively, as dependent variables in separate analyses.

To test the effects of oxytocin and positive verbal suggestions on the post-intervention CPT pain intensity ratings, a factorial 2x2 ANCOVA was performed, with spray and suggestions as between-group factors and the baseline CPT pain intensity rating as a covariate. A similar analysis was conducted to examine the effects of oxytocin and positive verbal suggestions on the post-intervention CPT unpleasantness rating, including the baseline CPT unpleasantness rating as a covariate.

To assess the effects of oxytocin and positive verbal suggestions on itch induced by the HI, a factorial 2x2 ANOVA was performed with spray and suggestions as between-group factors. Three additional 2x2 ANOVAs were performed to evaluate the effects of the spray and suggestions on the size of skin redness, wheal size, and the skin temperature after the HI.

To check whether the groups differ in their perceived group allocation, chi-square test was performed.

Finally, to assess whether oral contraception use influenced the results of the analyses, the same analyses were performed with pill intake as a covariate. As the analyses did not change the results, these results are not reported further.

As an effect size measure, partial eta squared was calculated for analyses on the primary and secondary outcomes.

Results

Demographics and baseline characteristics

A total of 108 participants (age 22.1 ± 2.4; 57.7% taking oral contraceptives) were equally assigned to the four groups (n = 27 per group). Baseline characteristics of the groups are presented in Table 1. There were no significant differences between the four groups in any of the baseline characteristics. The baseline CPT (*M*= 4.6, *SD* = 2.1) was experienced significantly as less painful than the post-intervention CPT (*M*= 5.0, *SD* = 2.2; *t* (107) = -4.5, *p* < .001).

Table 1. Baseline characteristics

	Oxytocin with positive suggestions (n = 27)	Placebo with positive suggestions (n = 27)	Oxytocin without suggestions (n = 27)	Placebo without suggestions (n = 27)	*F*(1; 104)	*p*
Age	22.2 (2.5)	21.9 (2.4)	22.2 (2.4)	22.2 (2.2)	.10	.96
Baseline expected pain	4.7 (1.8)	4.6 (2.3)	4.8 (2.5)	5.7 (2.1)	1.35	.26
Baseline CPT pain intensity	4.7 (2.3)	4.4 (2.0)	4.6 (2.1)	4.5 (2.2)	.12	.95
Baseline CPT unpleasantness	5.9 (2.4)	6.1 (2.2)	6.2 (2.1)	5.6 (2.6)	0.33	.80
Baseline expected itch	4.9 (2.2)	5.8 (2.5)	5.6 (2.3)	5.8 (2.3)	.10	.40

State anxiety (STAI-S)	9.8 (2.4)	9.0 (2.1)	9.0 (1.7)	9.8 (2.3)	1.21	.31
Positive mood (PANAS)	27.1 (6.1)	27.2 (6.2)	29.7 (5.0)	27.7 (7.0)	1.07	.36
Negative mood (PANAS)	12.6 (2.3)	11.3 (1.9)	12.7 (3.8)	13.0 (2.4)	2.23	.089
Neuroticism (EPQ-RSS)	4.5 (2.9)	3.7 (2.6)	3.48 (2.5)	4.2 (2.7)	0.80	.50
Extraversion (EPQ-RSS)	8.0 (1.6)	8.0 (1.9)	8.3 (1.6)	8.0 (1.9)	0.21	.89
Optimism (LOT-R)	22.6 (3.0)	22.2 (3.6)	22.2 (3.0)	21.9 (3.2)	0.18	.91

Means and standard deviations are given for all variables. Abbreviations*:* STAI-S- State Train Anxiety Inventory, State-Version; PANAS- Positive and Negative Affect Schedule; EPQ-RSS- Eysenck Personality Questionnaire short version; LOT-R- The revised Life Orientation Test; CPT- cold pressor test.

The effect of verbal suggestions and oxytocin on expected pain

Expected pain scores are presented in Table 2 and Figure 2a. A significant main effect of the positive verbal suggestions on expected pain was found ($F(1, 104) = 4.77$, $p = .031$, $\eta_p^2 = .04$): participants in the positive verbal suggestions groups expected significantly less pain than those in the groups that did not receive suggestions. However, neither a significant main effect of the spray ($F(1, 104) = 0.03$, $p = .86$, $\eta_p^2 < .001$), nor a significant interaction between the spray and the positive verbal suggestions ($F(1, 104) =$

0.19, $p = .66$, $\eta_p^2 = .002$) on expected pain was found, indicating that oxytocin did not influence the expected pain of the participants.

The effect of verbal suggestions and oxytocin on expected itch

Expected itch scores are presented in Table 2 and Figure 2b. There was a significant main effect of the positive verbal suggestions on the expected itch ($F(1, 104) = 5.38$, $p = .022$, $\eta_p^2 = .05$), indicating that participants in the positive verbal suggestions groups expected less itch in comparison to the groups that did not receive suggestions. However, neither the spray ($F(1, 104) = .04$, $p = .84$, $\eta_p^2 < .001$), nor the interaction between the spray and the positive verbal suggestions ($F(1, 104) = .041$, $p = .84$, $\eta_p^2 < .001$) had a significant effect on the expected itch.

Table 2. Somatic symptom outcome parameters

	Oxytocin with positive suggestions (n=27)	Placebo with positive suggestions (n=27)	Oxytocin without suggestions (n=27)	Placebo without suggestions (n=27)
Pain				
Post-intervention expected pain	5.3 (2.5)	5.4 (2.0)	6.0 (1.9)	6.2 (2.3)
Post intervention CPT pain intensity	5.1 (2.2)	4.5 (1.7)	5.4 (1.9)	5.2 (2.4)
Post intervention CPT pain unpleasantness	6.1 (2.4)	5.9 (2.4)	6.8 (2.1)	6.5 (2.3)
Pre- and post intervention difference score	-0.4 (1.0)	-0.1 (1.2)	-0.7 (1.3)	-0.7 (0.9)
Itch				

Post-intervention expected itch	4.2 (2.3)	4.4 (2.3)	5.4 (2.6)	5.4 (2.2)
Post intervention HI itch intensity	3.2 (1.7)	3.1 (1.5)	3.7 (1.7)	3.2 (1.6)
Redness size (mm)	93.9 (15.1)	94.5 (18.4)	94.8 (16.8)	99.1 (17.2)
Wheal size (mm)	58.6 (4.8)	58.8 (5.4)	60.5 (6.7)	58.9 (4.6)
Skin temperature (degrees Celcius)	31.3 (1.5)	31.1 (1.7)	30.9 (1.4)	31.0 (1.3)

Means and standard deviations are given for all variables. Abbreviations: CPT- cold pressor test; HI-histamine iontophoresis; mm- millimeters

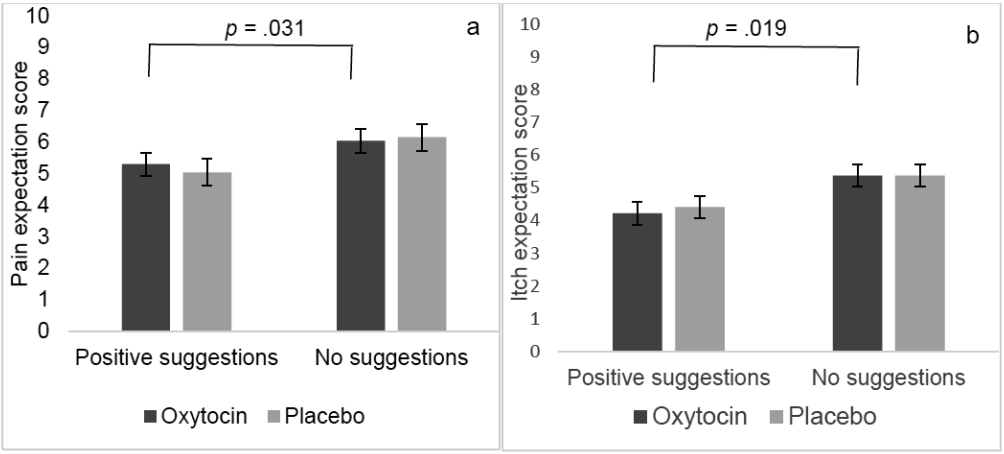

Figure 2. Post intervention pain (a) and itch (b) expectation scores for all groups. Error bars indicate standard errors.

The effect of verbal suggestions and oxytocin on pain outcomes

The pain intensity scores of CPT administration are presented in Figure 3a. There was a significant main effect of positive verbal suggestions on pain ($F(1, 103) = 5.48$, $p = .021$, $\eta_p^2 = .05$): participants who received positive verbal suggestions reported less pain in response to the post-intervention CPT in comparison with participants who did not, while controlling for baseline CPT levels. However, neither the main effect of the spray ($F(1, 103) = 0.78$, $p = .38$, $\eta_p^2 = .01$) nor the interaction between the spray and the positive verbal suggestions on pain scores ($F(1, 103) = 0.60$, $p = .44$, $\eta_p^2 = .01$) were significant. In addition, the results of the factorial ANCOVA indicated a significant main effect of suggestions on the unpleasantness ratings of the CPT ($F(1, 103) = 15.43$, $p = .002$, $\eta_p^2 = .09$): participants in the positive verbal suggestions group rated the post-intervention CPT as less unpleasant compared to the groups that received no suggestions, while controlling for baseline unpleasantness scores for the CPT. However, neither the main effect of the spray ($F(1, 103) < .001$, $p > .99$, $\eta_p^2 < .001$) nor the interaction between the spray and the positive verbal suggestions ($F(1, 103) = 1.55$, $p = .33$, $\eta_p^2 = .01$) were significant.

The effect of verbal suggestions and oxytocin on itch outcomes

The itch intensity scores of the four groups are presented in Figure 3b. No main effect of the positive verbal suggestions ($F(1, 104) = 0.93$, $p = .34$, $\eta_p^2 = .009$), the spray ($F(1, 104) = 1.11$, $p = .30$, $\eta_p^2 = .01$) or the interaction between the spray and the positive verbal suggestions ($F(1, 104) = 0.21$, $p = .65$, $\eta_p^2 < .001$) were found for itch. Three factorial 2x2 ANOVAs indicated also no significant main effects of the positive verbal suggestions and the spray on the size of skin redness, wheal size and skin temperature after the HI (see Table 2).

Perceived group allocation

The four groups did not differ in their perceived group allocation ($\chi^2(3, N = 108) = .49$, $p = .92$).

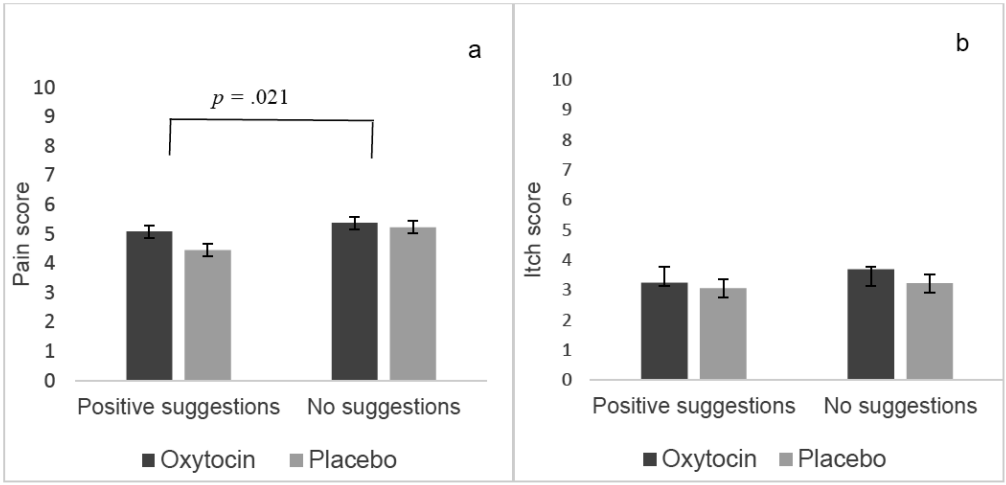

Figure 3. Subjective pain (a) and itch (b) intensities scores after the cold pressor test and histamine iontophoresis, respectively, for all groups. Error bars indicate standard errors.

Discussion

This is one of the first studies that investigated the influence of oxytocin and positive verbal suggestion on the placebo effects for both pain and itch. The study findings demonstrate that positive verbal suggestions were successful in inducing expectations of lower pain and itch, and subsequently were able to elicit small but significant placebo analgesia, but did not decrease itch sensitivity. However, oxytocin did not influence the placebo effects induced by positive verbal suggestions of pain or itch and also did not affect pain or itch sensitivity.

Firstly, we aimed to study whether positive verbal suggestions about nasal spray would change the expectations of participants about pain and itch and elicit placebo effect. The results demonstrated that the verbal suggestions successfully influenced expectations about pain and itch and elicited placebo analgesia. These findings are in line with studies that also showed that positive verbal suggestions can induce placebo analgesia (e.g., 9-11). We were however unable to demonstrate that these verbal suggestions can induce itch reduction, corresponding to previous research (13, 14, 32). The fact that participants

performed a baseline pain test but no baseline itch test, could explain the difference in the results between these two outcome measures. It might be that to exhibit a placebo effect, people need to experience the symptom before the suggestions of the improvement are given. This baseline exposure to the symptom might be a reference point that plays an essential role in the expectations of the participants regarding the effects of the treatment. In line with this hypothesis, Darragh and colleagues (15) found placebo effect for itch and wheal size only after participants experienced a baseline itch-inducing test.

Secondly, we studied whether the placebo effects induced by verbal suggestions can be enhanced by oxytocin. The results showed that oxytocin influenced neither the pain and itch expectations nor the placebo effect induced by positive verbal suggestions for pain or itch in women. It was previously hypothesized that oxytocin can influence positive treatment expectations and boost the placebo effect (16). Only two studies so far investigated oxytocin effects on the placebo effect with contradictory results. Our study is in line with the results of the study by Colloca and colleagues (23), who did not find the enhancing effect of oxytocin on placebo analgesia neither in men nor in women. Nevertheless, Kessner and colleagues (22), demonstrated that oxytocin can boost the placebo effect in men. There are several possible explanations of these divergent results. Kessner and colleagues (22) used a higher dosage of oxytocin (40 IU) than the study of Colloca and colleagues (23) or the present study where 24 IU was used. However, it is not clear whether a higher oxytocin dosage induces a stronger physiological or psychological effect. Van IJzendoorn and colleagues (38) demonstrated that there was no difference in the elevation of the endogenous oxytocin levels after the administration of 16 or 24 IU of oxytocin. Also, Cardoso and colleagues (18) showed that a 24 IU dose of intranasal oxytocin has a stronger effect on the cortisol response to vigorous exercise compared to a dose of 48 IU. A possible explanation for these findings is that by providing a larger amount of nasal spray, this will be swallowed and therefore cannot be absorbed (38). Alternatively, it has been proposed that higher doses of oxytocin bind to vasopressin receptors (39, 40), and that vasopressin instead of oxytocin enhances the placebo effect (23). Finally, gender differences in the effects of oxytocin might play an important role. It was shown that oxytocin has an opposite effect on brain activation patterns (41) and social interactions (42, 43) in men and women.

Possibly, oxytocin enhances placebo analgesia more in men than women (22) although different findings have been found as well (23). Various factors might possibly underlie gender differences such as for example, varying levels of oxytocin and other hormones in women during the cycle (44), gender difference in the baseline levels of gonadal hormones that were shown to modulate oxytocin receptor expression (45), differences in the brain structure of men and women (46). Future research should focus on gender differences in the effects of oxytocin on outcomes such as trust, anxiety, treatment expectations and the placebo response.

As an additional finding, there was also no evidence that oxytocin influences pain or itch ratings directly. The literature about the analgesic effects of oxytocin is contradictory while the effects of oxytocin on itch has not been studied so far. It remains unclear what underlies these contradictory findings. Some studies found that oxytocin can decrease experimentally induced pain (24-27), while others did not (22, 23, 28, 29, 30). A recent study by Tracy and colleagues (30) found pain-enhancing effects of oxytocin in women with chronic pain but no effects of oxytocin on pain sensitivity in healthy men and women. Most studies that found significant effects of oxytocin included male participants (24, 25, 27), however, there are also studies in men that found no analgesic results of oxytocin (22, 23, 30). The research about the effects of oxytocin on the pain sensitivity in women is scarce and it is not possible to state whether sex differences might play role in this relationship.

This study has several unique features. Our research design allows investigating the separate effects of verbal suggestions and oxytocin on pain and itch sensitivity and their interaction. Moreover, it was the first study to research the effect of oxytocin on pain and itch expectations. Besides these strong points, this study also has several limitations. First, as only women were included in this study, it is not possible to generalize the results to men. Second, we observed a placebo effect for pain but not for itch. Since no placebo effects for itch were found, this makes it impossible to generalize the results of the lack of oxytocin effects to itch. Moreover, even though the placebo effect for pain was found to be statistically significant, this effect was quite small and possibly would not be perceived by patients in clinical settings. Future studies should focus on ways to strengthen placebo effects by, for example, combining positive

verbal suggestions with conditioning. In addition, as the post-intervention expectations were measured after the baseline CPT, our pain and itch expectation measures might be not identical. It is recommended that future research either includes two separate sessions or uses other methods of itch induction that can be repeated twice. Additionally, in contrast to studies in which placebo effects were induced by giving positive verbal suggestions about a placebo cream (22) or a sham electrode (23), we gave positive verbal suggestions about the nasal spray itself. This procedure does not require an extra placebo treatment and might be clearer for the participants. However, future studies need to compare different placebo applications in order to find out which of the methods are more effective. Furthermore, the administration of a nasal spray with a particular taste and smell might have elicited a placebo response in both groups regardless of the verbal suggestions. Another limitation is that menstrual cycle phase was not measured in this study. Cycle phase can have an effect on the experience of pain (47). However, as we controlled for the baseline pain sensitivity that was measured at the same day, we expect these possible effects of the menstrual cycle to be negligible. Also, only one dosage of oxytocin was administered in this study. Future studies should preferably compare the influence of different dosages of oxytocin on the placebo effect. Finally, a general limitation of oxytocin research is the lack of knowledge of the underlying mechanisms of the intranasal oxytocin effects. There is an ongoing debate in the literature about how the intranasal oxytocin reaches the brain and via what pathways, central or peripheral, it influences behavior (48).

To conclude, we found that positive verbal suggestions can modify the expectations regarding pain and itch and induce small but significant placebo analgesia, however, oxytocin does not seem to influence expectations or the placebo effect for pain in healthy women. Future studies should investigate the effects of oxytocin on placebo effect at various methods of placebo effect induction and different doses for both genders. This will advance our knowledge of the role of oxytocin in placebo effect and the possible ways to maximize the placebo effect in clinical settings.

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