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Lack of Evidence for the Effect of Oxytocin on Placebo Analgesia and Nocebo Hyperalgesia

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A placebo response refers to the improvement in a patient's clinical symptoms when administered with an inert substance, whereas a nocebo response is the worsening of clinical symptoms [1]. The neuropeptide oxytocin is implicated in social trust, stress/anxiety, and learning processes [2] that also contribute to placebo and nocebo responses. This leads to an intriguing hypothesis that pharmacological manipulation of oxytocin may modulate placebo/nocebo. It has been proposed that oxytocin may enhance social trust [3], a key element of the patient-doctor relationship, which may further boost placebo effects. Oxytocin has also been shown to be involved in reducing stress and anxiety [4], which might contribute significantly to the magnitude of nocebo.

To date, 4 studies have investigated the effect of oxytocin on placebo analgesia using a verbal suggestion paradigm. One study reported that a 40-international unit (IU) dose of intranasal oxytocin enhanced placebo analgesia in males [5]. Another study, however, found that 24 IU of oxytocin did not enhance placebo analgesia in either sex [6]. In a more recent study, it was found that 24 IU of oxytocin did not enhance placebo analgesia in females [7]. Most recently, Skvortsova et al. [8] found that 40 IU of oxytocin did not influence placebo analgesia and nocebo hyperalgesia responses in males. The links between oxytocin and placebo/nocebo remain to be established.

Here, we systematically tested the oxytocin effect on both placebo and nocebo responses using different experimental paradigms and different dosages. We recruited 318 healthy volunteers in 2 studies (online supplementary Material, see www.karger.com/doi/10.1159/000504967). Participants were randomly assigned to 4 groups (oxytocin 24 or 40 IU, saline 24 or 40 IU) in a double-blind trial for both studies (Fig. 1). Firstly, we calibrated participants' pain thresholds to find the electrical intensity parameters that would elicit low pain at ≈3 rating, high pain at ≈7 rating, and moderate pain at ≈5 rating on a numeric rating scale ranging from 1 to 9 for each participant. Participants were then administered intranasal oxytocin (40 or 24 IU) or saline.

After 40 min of the intranasal administration, in study 1, participants performed the conditioning procedure, with 2 abstract cues associated with either a high or low electrical pain for 20 times each. In the testing stage, the high cue, low cue, and a new cue were all paired with identical moderate pain for 20 times each. In study 2, a verbal suggestion plus conditioning paradigm was used. Three identical inert ointments were applied to 3 sites on each participant's forearm, with the sites randomized across participants. A female experimenter described ointments as creams that increase pain (nocebo), reduce pain (placebo), and have no effect on pain (control), respectively. After 10 min, the 3 sites were stimulated for 10 times with high, moderate, and low shocks, corresponding to their instructed functions, to strengthen the effect of verbal suggestions. In the subsequent testing stage, all 3 sites were paired with moderate shocks for 20 times each.

Participants rated how much pain they felt after each shock, using the same 9-point numeric rating scale. The placebo and nocebo effects were assessed as participants rated the low-cue (study 1) or placebo site (study 2)-associated shock as less painful and high-cue/nocebo site-associated shock as more painful compared to the new-cue/control site-associated shock during the test stage.

Data from 12 participants were excluded because of poor pain discrimination during calibration or doubt of ointments' effects, leaving 306 participants for analysis (age, 18–26 years). A 2 (group: oxytocin/saline) × 2 (dosage: 24/40 IU) factorial ANOVA on placebo effect revealed no main effects and interaction effect in both studies (study 1: group, $F(1, 156) = 0.16$, $p = 0.692$; dosage, $F(1, 156) = 0.06$, $p = 0.814$; group × dosage, $F(1, 156) = 0.30$, $p = 0.588$; study 2: group, $F(1, 142) = 0.17$, $p = 0.682$; dosage, $F(1, 142) = 0.03$, $p = 0.860$; group × dosage, $F(1, 142) = 0.09$, $p = 0.764$). A similar ANOVA on nocebo effect also revealed no main effects and interaction effect (study 1: group, $F(1, 156) = 0.29$, $p = 0.589$; dosage, $F(1, 156) = 0.65$, $p = 0.420$; group × dosage, $F(1, 156) = 1.12$, $p = 0.292$; study 2: group, $F(1, 142) < 0.001$, $p = 0.99$; dosage, $F(1, 142) = 0.57$, $p = 0.452$; group × dosage, $F(1, 142) = 0.98$, $p = 0.324$).

To further examine whether sex modulates the effect of oxytocin on placebo and nocebo effects, we conducted a 2 (group: oxytocin/saline) × 2 (dosage: 24/40 IU) × 2 (sex: female/male) factorial ANOVA on placebo and nocebo responses, respectively. Results showed no sex-related effects (p values >0.1), possibly due to the small number of participants in each subgroup for each gender. In addition, we examined pain ratings in the conditioning stage and found no significant differences across 4 arms in both studies (p values >0.1), suggesting that the strength of conditioning was well-balanced across groups.

In summary, using a randomized, double-blind design, we found no evidence for oxytocin effects on placebo and nocebo across paradigms and dosages in a large sample. Our findings challenge previous findings that placebo responses can be enhanced by the application of intranasal oxytocin and further show the lack of oxytocin effect on nocebo responses in the context of pain. Despite the prominent role of oxytocin in promoting trust and learning (but see [9]), we suggest that it is premature to precipitously use oxytocin to harness the placebo effect and downplay the nocebo effect until the

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Experiment 1

Sample = 160	Oxytocin	Saline
24 IU	28F, 18M	24F, 17M
40 IU	19F, 18M	20F, 16M

Experiment 2

Sample = 146	Oxytocin	Saline
24 IU	19F, 19M	23F, 16M
40 IU	19F, 11M	23F, 16M

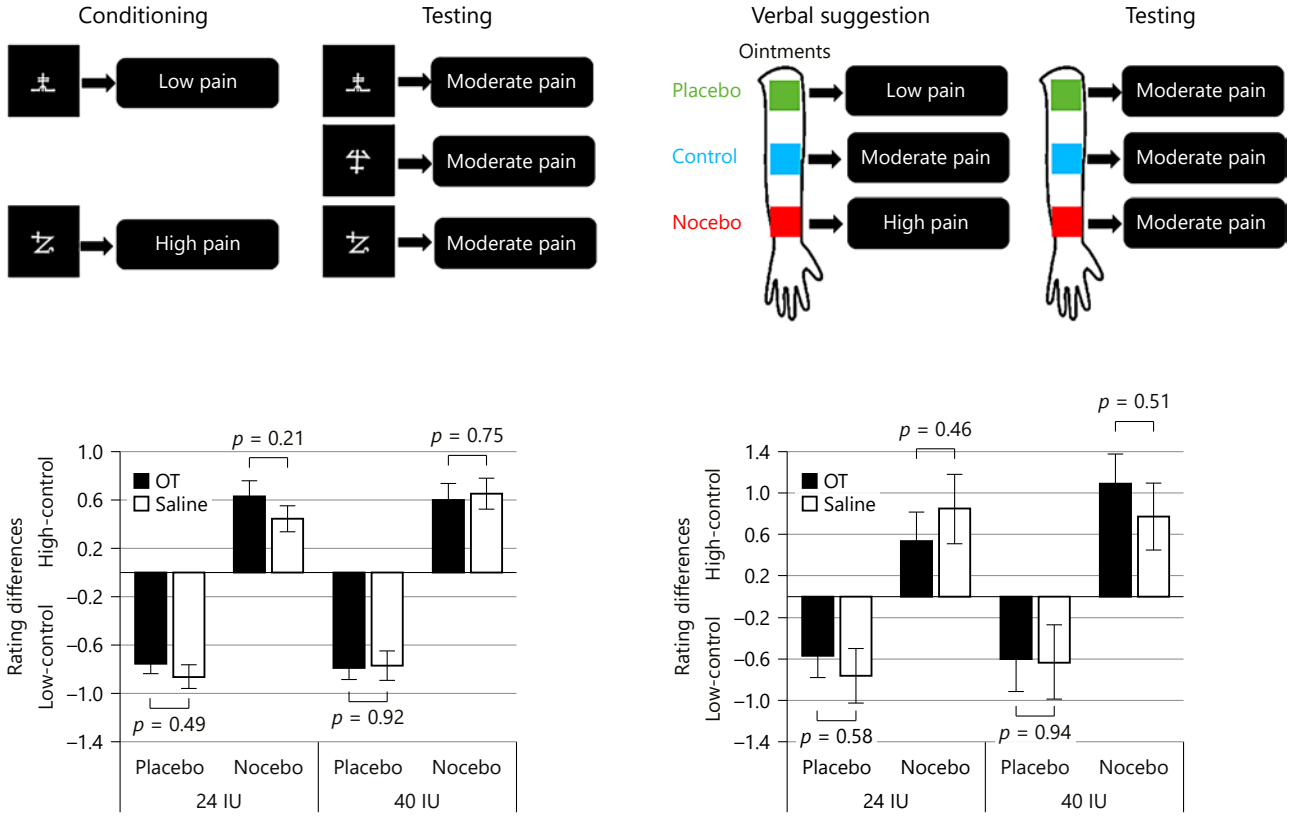

Fig. 1. Experimental protocols and results. Analgesic placebo effects were assessed as control minus low-cue rating differences, and hyperalgesic nocebo effects were assessed as high-cue minus control cue rating differences. *p* values indicate independent *t* test results by comparing oxytocin and saline groups. Error bars represent standard errors. F, female; M, male; IU, international unit.

therapeutic efficacy of oxytocin is rigorously assessed at various methods of placebo/nocebo effect induction and different doses for both sexes. Both placebo and nocebo have a huge impact on clinical outcomes in a broad variety of medical conditions [1]. The potential for clinicians to use oxytocin to influence responses to medicines and psychological interventions is highly tempting [10]. However, our findings indicate a lack of evidence for the effect of oxytocin on placebo analgesia and nocebo hyperalgesia.

Disclosure Statement

The authors have no conflicts of interest to declare.

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Author Contributions

Dr. R. Yu had full access to all of the data obtained in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

Study concept and design: R. Yu, C. Liu.

Acquisition of data: L. Chen.

Analysis and interpretation of data: C. Liu, Y. Huang, R. Yu.

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Supplementary Information

Lack of Evidence for the Effect of Oxytocin on Placebo Analgesia and Nocebo Hyperalgesia

Methods

Participants

Participants were recruited from the university. They reported no history of neurological or psychiatric disorders (including substance abuse and obesity). They were required to not drinking caffeine, alcohol, or nicotine within 2 hours before the experiment. No female participant reported pregnancy or used hormonal contraception in the last month. Prior to the experiment, participants provided demographic information and completed a set of questionnaires: Pain Catastrophizing Scale (1), Eysenck Personality Questionnaire-Neuroticism (2), Interpersonal Trust Scale (3), and State Anxiety Inventory (4). After the formal task, participants conducted the State Anxiety Inventory again and they were asked to guess whether they took OT or a placebo. **Table S1 and S2** show that there were no significant differences between the 4 groups of participants regarding demographic information and psychological traits.

The Institutional Review Board at South China Normal University approved all study procedures. The experiment was carried out in accordance with the approved guidelines. Participants gave written informed consent before beginning any study procedures. Protocols were pre-registered at Chinese Clinical Trial Registry (ChiCTR1800015647, http://www.chictr.org.cn/showproj.aspx?proj=22981). Please note the deviation of the final study from the trial preregistration in terms of sample size. The initial preregistration also did not provide important details, such as planned statistical analysis and main outcomes. The CONSORT Flow diagram and checklist are presented in **Figures S1 and S2**.

Materials

Electric stimulations were square pulses delivered to the right volar forearm by a Grass SD9 stimulator (Warwick, U.S.A.) with two 0.75cm diameter electrodes. The experiment was

conducted in a quiet room with a temperature of 24℃. All stimuli were presented using the Eprime 2.0 software (Version 2.0.8.22, http://www.pstnet.com).

Experimental procedures

Both studies consisted of three stages: (1) calibration, (2) intranasal administration, (3) conditioning paradigm (Experiment 1) or verbal suggestion reinforced paradigm (Experiment 2).

Stage 1: Calibration

Since individuals differ in their pain sensitivity, the threshold and tolerance to electric stimuli for each participant was calibrated at the beginning of the experiment. Calibrations were manipulated via ascending voltage of the electric currents with a fixed delivering duration of 80ms. Participants were asked to rate their pain intensity on a 9-point self-report Numeric Rating Scale (NRS, 1 = a little pain, 5 = moderate pain, and 9 = unbearable pain). After finding the physical voltage that participants rated around 3 (low pain), this parameter kept constant in further procedures. The next step was to find electrical parameters that would elicit low pain at ≈3 rating, high pain at ≈7 rating, and moderate pain at ≈5 rating on the NRS for each participant. With a previously determined constant voltage, we increased the stimulation time of electric currents, starting from 80ms to 800ms (increasing in sequence at multiples of 80ms), to increase participants' feeling of pain. Participants were given 2s to rate on the NRS by pressing the corresponding number buttons on the keyboard. Once the low, moderate, and high pain levels for each participant were determined, the participants were tested for rating response consistency. A random sequence of three low- and three high-intensity pain stimuli was administered. If the participants could reliably rate the high stimuli as more intense than the low stimuli, they proceeded to the next step of the experiment. The calibration lasted for 5 to 10 minutes.

Stage 2: Intranasal administration

In a randomized double-blind study, participants received either 24 IU oxytocin, 24 IU saline, 40 IU oxytocin, or 40 IU saline intranasally. 24 IU was administered with three puffs per nostril and 40 IU was administered with five puffs per nostril. After the drug administration, participants rested for 40 minutes and then proceeded to the next procedures.

Stage 3 for Experiment 1: Conditioning paradigm

In Experiment 1, participants performed the conditioning procedure (5). Two abstract images were used as cues during the conditioning stage. In total, there were 40 trials: one cue was coupled with a high pain level and the other cue was coupled with a low pain level for 20 trials each. The specific assignment of cues to a given trial type was fully counterbalanced across participants. Each trial started with the presentation of a fixation cross. The abstract cue was presented for 2s, followed by an interval of 2s, then the electric current stimulus was delivered to the right volar forearm. Participants rated how much pain they felt, using the same 9-point NRS. There was a 7-9s blank interval between trials to allow the feeling of pain dissipating. The conditioning sequence lasted for 15 minutes, with a break of 1 minute during the procedure. An experimental assistant was seated in a chair near the desk that the monitor was on, facing the side of the participant to make sure that participants were engaged in the experiment.

There were three cues in test stage: the cue previously associated with high pain (high-pain cue), the cue previously associated with low pain (low-pain cue), and a new cue that participants had not seen before (neutral cue). Unknown to participants, the cues were all paired with identical moderate electric shocks for 20 trials each. The streamline of each trial was the same as in the conditioning stage. This testing stage lasted for 20 minutes, with a break of 1 minute during the procedure. In addition, similar to previous studies, another six "booster trials" were added to the test stage (6). Specifically, three high-pain cues and three low-pain cues were respectively paired with their original electric currents. As all electric current stimuli were at the same level of intensity in the test stage, the booster trials served to prevent habituation and extinction and to ensure participants remain vigilant. These booster trials were not included in the statistical analysis of analgesic and hyperalgesic effects.

Stage 3 for Experiment 2: Verbal suggestion reinforced paradigm

Experiment 2 followed a well-established placebo analgesia paradigm including both expectation and conditioning components (7). We informed participants that the aim of the study was to investigate the effect of ointments on pain perception. Three identical inert ointments were applied to three sites on each participant's forearm, with the sites randomized across participants. A female experimenter introduced ointments as creams that increase pain (nocebo, red label), reduce pain (placebo, green label), and have no effect on pain (control, blue label), respectively. Participants were then told to wait for 10 minutes for the creams to

take effect. Of note, we did not randomize cream colors across participants given that the color itself impacts on placebo/nocebo responses with red color associating with hazard and green color associating with safety.

Next, verbal suggestions were reinforced by a conditioning procedure to convince participants that creams applied are effective in reducing or increasing pain. Participants were told that they could be stimulated on three skin areas with moderate intensity of shock (i.e., pain level 5). Unknown to them, however, the shock intensity was lowered to pain level 3 during the placebo condition and was heightened to pain level 7 during the nocebo condition. This conditioning stage consisted of 3 sessions with 10 stimulations for each cream session, lasting for 10 minutes in total. Each session started with a word reminder such as high pain, low pain or control pain condition. After the reminder, each trial in the session started with a cross fixation with the font color consisted with the cream label for 2s. Then the electric current stimulus was delivered to the corresponding skin area that administered creams. Participants rated how much pain they felt after each shock, using the same 9-point NRS. There was a 7-9s blank interval between trials. After the reinforced conditioning, participants were asked to rate how much did them expect the ointments to increase pain / reduce pain on a rating scale ranging from 0 = 'no effect at all' to 4 = 'very effective'. Participants (n=7) who reported 'no effect at all' were excluded from the data analysis.

In the subsequent testing stage, all three sites were paired with moderate shocks for 20 times each. The testing stage lasted for 20 minutes with short breaks during the procedure. The procedure for each trial was the same as in the reinforced conditioning stage.

Power calculation

The main purpose of our study was to examine the effects of oxytocin and its dosage on placebo and nocebo responses. Based on the initial effect size of d = 0.495 reported by Kessner, Sprenger (8) that showed the effect of oxytocin on placebo analgesia in males, a sample size calculation using G*Power for a repeated-measures between-factors ANOVA with 4 groups (group: oxytocin/saline X dosage: 24 IU/40 IU) and 2 measurements (placebo/nocebo responses) revealed that 60 male participants in total (15 in each group) would be needed to obtain a power of 0.95 at an alpha level of 0.05 (9). To further explore the effects of oxytocin on females, we applied the same sample size of 15 in each group to

female participants. This led to a sample size of 30 male and female participants altogether in each group. Considering the exclusion of subjects, we recruited 40 subjects in each group.

Results

Successful induction of placebo and nocebo effects independent of treatment.

In experiment 1, a 2 (group: oxytocin/saline) X 2 (dosage: 24 IU/40 IU) X 3 (cue: high/control/low) repeated measures ANOVA showed that there was a significant effect of the cue on pain ratings during the test stage ($F(1, 156) = 233.78$, $p < 0.001$). Post hoc tests using Bonferroni corrections indicated that participants rated the stimuli following the high-pain cues (mean ± SE: 5.02 ± 0.10) more painful than stimuli following control cues (mean ± SE: 4.44 ± 0.09) and low-pain cues (mean ± SE: 3.65 ± 0.09) which indicated a nocebo effect, and low-pain cues stimuli as significantly less painful than control cues stimuli which indicated a placebo effect (all Ps < 0.001). There were no other significant main effects and interactions (all Ps > 0.05).

In experiment 2, a similar 2 X 2 X 3 repeated measures ANOVA showed that there was a significant effect of the cue on pain ratings during the test stage ($F(1, 142) = 40.33$, $p < 0.001$). Post hoc tests using Bonferroni corrections indicated that participants rated the stimuli following the high-pain cues (mean ± SE: 4.82 ± 0.15) more painful than stimuli following control cues (mean ± SE: 4.01 ± 0.11) and low-pain cues (mean ± SE: 3.37 ± 0.12), and low-pain cues stimuli as significantly less painful than control cues stimuli (all Ps < 0.001), indicating a successful induction of placebo and nocebo effects. Unexpectedly, there was a main effect of dosage ($F(1, 142) = 5.50$, $p = 0.020$), with 40 IU (mean ± SE: 4.26 ± 0.13) showing higher pain ratings than 24 IU (mean ± SE: 3.86 ± 0.12). There were no other significant main effects and interactions (all Ps > 0.05).

Altogether, these results demonstrated that the conditioning and verbal suggestions successfully induced placebo and nocebo effects independent of treatment.

Effects of oxytocin on the extinction of placebo and nocebo responses.

To test whether oxytocin affects the extinction process of placebo analgesia and nocebo hyperalgesia responses in the test stage, we divided the test stage into early and late sessions.

Using group (oxytocin versus saline) and dosage (24 IU versus 40 IU) as between-subject factors and time (early/late: first versus second half trials in the test) as within-subject factor, we tested how these factors affected placebo responses by treating control minus low cue rating difference as the dependent variable. The same analysis was conducted for nocebo responses with high minus control cue rating differences as the dependent variable.

In Experiment 1, results showed no main effect of time on placebo responses (time, $F(1, 156) = 3.15$, $p = 0.078$) as well as no interactions with other factors (time X group, $F(1, 156) = 0.02$, $p = 0.902$; time X dosage, $F(1, 156) = 0.55$, $p = 0.460$; time X group X dosage, $F(1, 156) = 0.24$, $p = 0.627$). The analysis on nocebo responses showed a significant effect of time (time, $F(1, 156) = 23.91$, $p < 0.001$), with the first half of trials (mean ± SE: 0.73 ± 0.07) revealing greater nocebo responses than the second half of trials (mean ± SE: 0.42 ± 0.06). There were no interactions between time and other factors (time X group, $F(1, 156) = 0.19$, $p = 0.666$; time X dosage, $F(1, 156) = 0.40$, $p = 0.527$; time X group X dosage, $F(1, 156) = 0.30$, $p = 0.587$).

In Experiment 2, results showed no main effect of time on placebo responses (time, $F(1, 142) = 0.001$, $p = 0.981$) and no interactions between time and other factors (time X group, $F(1, 142) = 0.96$, $p = 0.328$; time X dosage, $F(1, 142) = 0.01$, $p = 0.942$; time X group X dosage, $F(1, 142) = 2.83$, $p = 0.095$). The analysis on nocebo responses also showed no main effect of time (time, $F(1, 142) = 0.51$, $p = 0.478$), and no interactions between time and other factors (time X group, $F(1, 142) = 0.05$, $p = 0.819$; time X dosage, $F(1, 142) = 0.29$, $p = 0.590$; time X group X dosage, $F(1, 142) = 0.08$, $p = 0.784$).

Taken together, these results demonstrated that oxytocin did not influence the extinction of placebo analgesia and nocebo hyperalgesia effects, in consistent with Skvortsova et al.' findings (10).

Equivalence test and Bayesian hypothesis test to assess the null findings of oxytocin effects.

Given that all main effects and the interaction effect of the group X dosage ANOVA results were non-significant in two experiments, we followed these null hypothesis significance tests with equivalence testing and Bayesian hypothesis testing to assess the sensitivity of our null findings of oxytocin on placebo and nocebo responses compared to the saline treatment.

Equivalence testing was conducted in RStudio using the TOSTER package (11).To determine the smallest effect size of interest (SESOI) for setting equivalence bounds, we calculated the mean of SESOI based on a recent meta-analysis report that synthesized 32 intranasal oxytocin studies as well as a set of unpublished data (12). The calculated SESOI average was 0.58. Therefore, we set the lower and upper equivalence bounds to −0.58 and 0.58. In Experiment 1, equivalence tests in comparing oxytocin and saline treatment was significant on both placebo ($t(131.24) = 3.20$, $p < 0.001$) and nocebo responses ($t(155.6) = -3.04$, $p = 0.001$). Equivalence tests for Experiment 2 also revealed significant results (placebo: $t(143.79) = 3.09$, $p = 0.001$; nocebo: $t(143.99) = 3.20$, $p < 0.001$).

We also used Bayesian hypothesis testing in JASP (version 0.11.1.0) to assess the null effects of oxytocin on placebo and nocebo response (13). Bayesian testing is particularly beneficial for providing information on the relative degree of evidence that the data provide in favor of either the alternative or null hypotheses (14). In Bayesian testing, Bayes Factor is calculated to infer the ratio of the posterior odds of the alternative and null hypothesis to its prior odds (15). Bayesian hypothesis tests in comparing oxytocin and saline groups found no support for the alternative hypothesis in both experiments (Experiment 1: placebo $BF_{10} = 0.187$, nocebo $BF_{10} = 0.204$; Experiment 2: placebo $BF_{10} = 0.212$; nocebo $BF_{10} = 0.212$).

To sum up, findings from equivalence testing and Bayesian testing demonstrated that the oxytocin and saline treatment showed equivalent effect on placebo and nocebo responses.

Meta-analysis of oxytocin effects on placebo effects.

To improve estimates of the size of the effect, we combined the results from multiple studies and did a meta-analysis. Four studies and our two experiments were included **(Table S3)**. The results of the individual studies were transformed into common metric of the standardized difference (Cohen's d) between oxytocin and control conditions. The meta-analysis was conducted by using the random-effects model of the "metafor" R package (16). Our analysis of six studies showed that the combined effect size of oxytocin on placebo effect was small and not significantly different from zero (Cohen's d = 0.02, 95% CI [-0.14, 0.18], z = 0.25, p = 0.80 **(Figure S3)**. Influence measures showed that Kessner, Sprenger et. al.' study (8) with the largest effect size was identified as a potential outlier and influential case (17). Compared with the other five cases, this study incurred the largest change in the Cook's distance (0.62) and standardized residuals (2.16). Although meta-analyses possess more power to detect

effects than individual studies, our meta-analysis is based on a small number of individual studies and has limited power. The results of our exploratory data analysis should be interpreted with great care.

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Table S1. Participants' characteristics and experimental results (mean ± SE) in Experiment 1.

	24 IU		40 IU			
	Oxytocin (N=46)	**Saline (N=41)**	**Oxytocin (N=37)**	**Saline (N=36)**	***F/χ2***	***P***
Characteristics						
Age (years)	20.43±0.30	19.54±0.22	19.78±0.26	20.03±0.24	2.33	0.076
BMI	20.59±0.54	20.85±0.61	20.07±0.36	19.76±0.32	1.01	0.392
PCS	19.67±1.55	18.22±1.34	17.73±1.34	18.74±1.75	0.32	0.810
NEO	4.89±0.52	4.88±0.40	4.62±0.55	4.63±0.49	0.09	0.965
ITS	79.00±1.30	78.12±1.56	79.97±1.70	78.82±2.01	0.214	0.887
S-AI before	35.72±1.20	37.40±1.25	35.83±1.15	36.06±1.34	0.41	0.747
S-AI after	37.17±1.26	37.90±1.29	37.08±1.06	37.18±1.44	0.09	0.965
Guess oxytocin	23	18	9	16	6.10	0.107
Guess saline	23	23	28	20		
Shock intensity	2.19±0.13	2.64±0.25	2.44±0.11	2.31±0.15	1.36	0.258
Results						
Pain rating differences in conditioning stage						
High-low	3.62±0.19	3.46±0.16	3.52±0.20	3.79±0.21	0.55	0.652
Pain rating differences in testing stage						
Control-low	0.75±0.09	0.86±0.14	0.79±0.09	0.77±0.14	0.18	0.911
High-control	0.63±0.10	0.44±0.11	0.60±0.12	0.66±0.13	0.67	0.554

Abbreviations: BMI, body mass index; PCS, pain catastrophizing scale; NEO, Eysenck personality questionnaire-neuroticism; ITS, interpersonal trust scale; S-AI before, state anxiety inventory conducted before drug administration; S-AI after, state anxiety inventory conducted after the whole experimental procedures.

Table S2. Participants' characteristics and experimental results (mean ± SEM) in Experiment 2.

	24 IU		40 IU			
	Oxytocin (N=38)	**Saline (N=39)**	**Oxytocin (N=30)**	**Saline (N=39)**	***F/χ2***	***P***
Characteristics						
Age (years)	20.21±0.30	19.90±0.28	19.97±0.32	19.97±0.24	0.24	0.869
BMI	21.15±0.61	20.35±0.45	19.90±0.37	19.96±0.33	1.55	0.205
PCS	21.45±1.48	17.38±1.28	17.57±1.53	18.68±1.48	1.74	0.162
NEO	4.55±0.56	4.64±0.40	4.57±0.55	4.81±0.50	0.06	0.982
ITS	80.89±1.52	77.05±1.49	81.70±1.82	78.08±1.91	1.71	0.162
S-AI before	36.34±1.61	37.42±1.22	37.46±1.09	35.77±1.32	0.38	0.767
S-AI after	34.97±1.60	36.61±1.38	34.92±1.46	36.26±1.57	0.33	0.806
Guess oxytocin	20	11	11	19	5.89	0.117
Guess saline	18	28	19	20		
Shock intensity	1.92±0.10	1.97±0.10	2.34±0.12	2.15±0.14	2.492	0.063
Results						
Pain rating differences in conditioning stage						
Control-low	2.18±0.24	1.68±0.25	2.27±0.24	2.20±0.29	1.15	0.332
High-control	1.40±0.26	2.03±0.31	1.51±0.36	1.77±0.34	0.83	0.480
Pain rating differences in testing stage						
Control-low	0.57±0.21	0.76±0.28	0.60±0.31	0.63±0.29	0.10	0.959
High-control	0.53±0.26	0.84±0.33	1.09±0.36	0.77±0.32	0.48	0.694

Abbreviations: BMI, body mass index; PCS, pain catastrophizing scale; NEO, Eysenck personality questionnaire-neuroticism; ITS, interpersonal trust scale; S-AI before, state anxiety inventory conducted before drug administration; S-AI after, state anxiety inventory conducted after the whole experimental procedures.

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Table S3. A summary of studies examining the effect of oxytocin on placebo analgesia and nocebo hyperalgesia.

Study	Groups and sample size	Pain stimulation	Paradigms inducing placebo/nocebo responses	Findings
Kessner, 2013	1. 40 IU OT: n=37 male 2. Control: n=38 male	Heat stimuli	Verbal suggestions: Ointment that reduces pain (placebo) An inert ointment (control)	OT boosted placebo analgesia.
Colloca, 2016	1. 24 IU OT: n=17 female+17 male 2. Control: n=12 female+12 male	Electrical stimuli	Verbal suggestions: Green light indicates no pain/less pain (placebo) Red light indicates pain (control)	OT had no effect on placebo analgesia.
Skvortsova, 2018	1. 24 IU OT+suggestion: n=27 female 2. Control+suggestion: n=27 female 3. 24 IU OT: n=27 female 4. Control: n=27 female	Pain: cold pressor test Itch: histamine iontophoresis	Verbal suggestions: Oxytocin that decreases pain and itch Placebo that decrease pain and itch Oxytocin without suggestion Placebo without suggestion	OT had no effect on placebo analgesia.
Skvortsova, 2019	1. 40 IU OT: n=39 male 2. Control: n=37 male	Heat stimuli	Conditioning + verbal suggestions: Low-pain cue (placebo) High-pain cue (nocebo) Moderate-pain cue (control)	OT had no effect on placebo analgesia and nocebo hyperalgesia.
Our study: Experiment 1	1. 24 IU OT: 28 female+18 male 2. 24 IU control: 24 female+17 male 3. 40 IU OT: 19 female+18 male 4. 40 IU control: 20 female+16 male	Electrical stimuli	Conditioning: Low-pain cue (placebo) High-pain cue (nocebo)	OT had no effect on placebo analgesia and nocebo hyperalgesia.
Our study: Experiment 2	1. 24 IU OT: 19 female+19 male 2. 24 IU control: 23 female+16 male 3. 40 IU OT: 19 female+11 male 4. 40 IU control: 23 female +16 male	Electrical stimuli	Verbal suggestions: Ointment that decreases pain (placebo) Ointment that increases pain (nocebo) An inert ointment (control)	OT had no effect on placebo analgesia and nocebo hyperalgesia.

Figure S1. CONSORT Flow Diagram for Experiment 1.

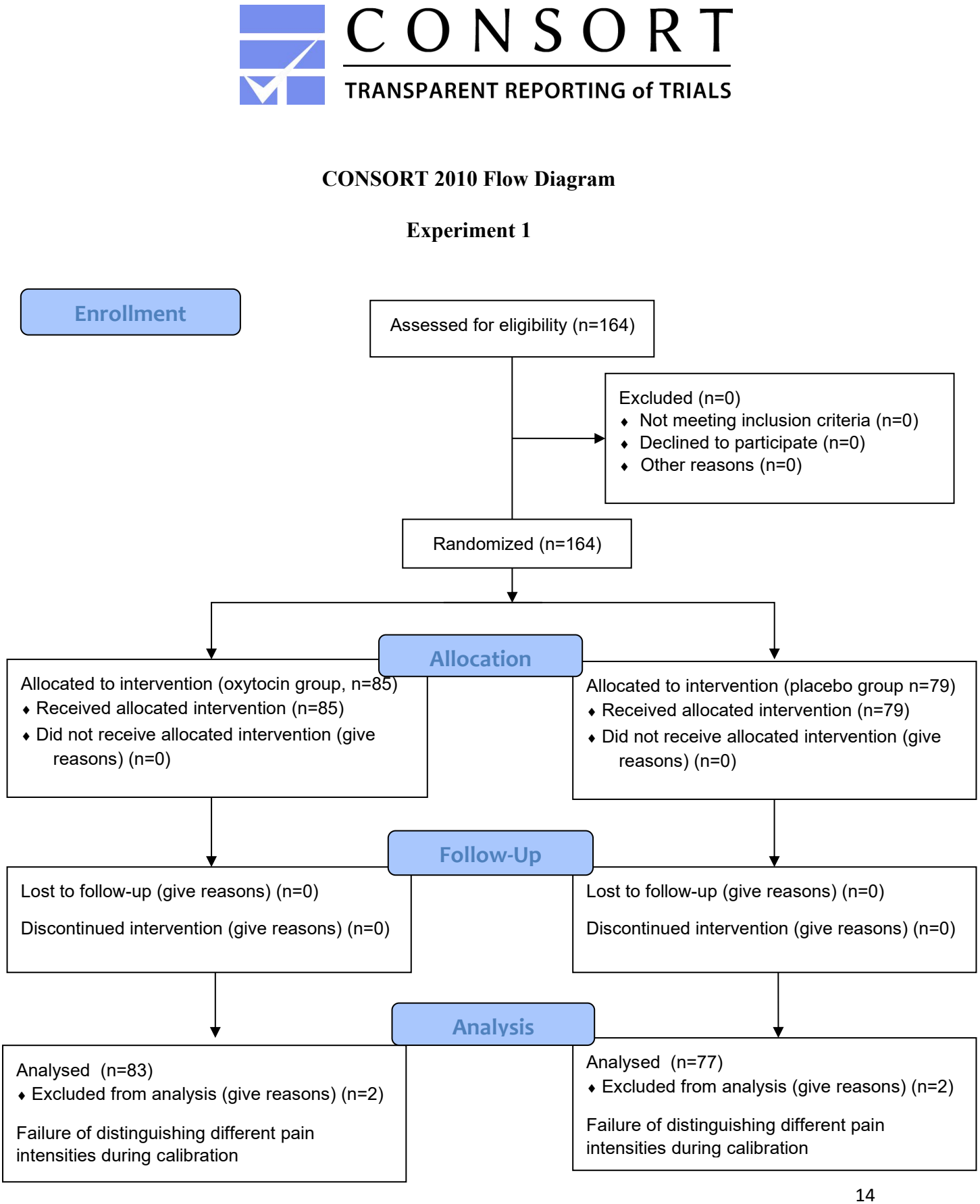

Figure S2. CONSORT Flow Diagram for Experiment 2.

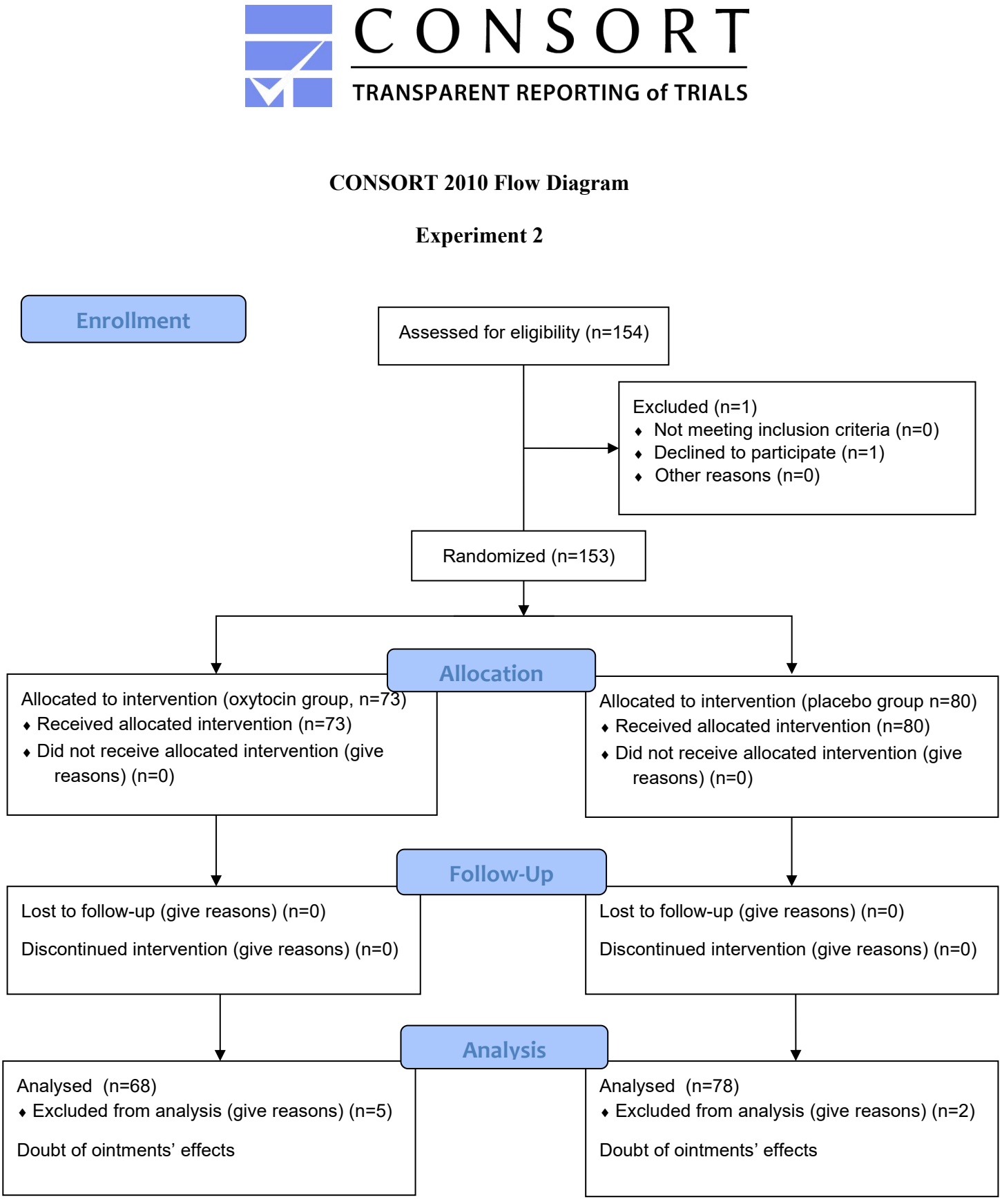

Figure S3. Forest plot of effect sizes for oxytocin on placebo effect studies. Square sizes represent study weights. Filled diamonds represent summary effect sizes.