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Vasopressin Boosts Placebo Analgesic Effects in Women: A Randomized Trial

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Abstract

Background—Social cues and interpersonal interactions strongly contribute to evoke placebo effects that are pervasive in medicine and depend upon the activation of endogenous modulatory systems. Here we explore the possibility to boost placebo effects by targeting pharmacologically the vasopressin system, characterized by a sexually dimorphic response and involved in the regulation of human and nonhuman social behaviors.

Methods—We enrolled 109 healthy participants and studied the effects of intranasal administration of Avp1a and Avp1b arginine vasopressin receptor agonists against 1) no-treatment, 2) oxytocin, and 3) saline, in a randomized, placebo-controlled, double-blind, parallel design trial using a well-established model of placebo analgesia while controlling for sex differences.

Results—Vasopressin agonists boosted placebo effects in women but had no effect in men. The effects of vasopressin on expectancy-induced analgesia were significantly larger than those observed in the no-treatment ($p<0.004$), oxytocin ($p<0.001$) and saline ($p<0.015$) groups. Moreover, women with lower dispositional anxiety and cortisol levels showed the largest vasopressin-induced modulation of placebo effects, suggesting a moderating interplay between pre-existing psychological factors and cortisol changes.

Conclusions—This is the first study that demonstrates that arginine vasopressin boosts placebo effects and that the effect of vasopressin depends upon a significant sex by treatment interaction. These findings are novel and might open up new avenues for clinically relevant research due to the

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Conflict of Interest Disclosures

All other authors reported no biomedical financial interests or potential conflicts of interest.

Author contributions

Colloca had full access to all the collected data in the study, took responsibility for the integrity accuracy of the data collection and analysis. Colloca planned and supervised the conduction of the experiments, analyzed the data and wrote the manuscript. Pine, Ernst, Miller and Grillon critically revised the final version for intellectual content.

therapeutic potentials of vasopressin and oxytocin as well as the possibility to systematically control for influences of placebo responses in clinical trials.

Keywords

Expectancy; Anxiety; Sexual dimorphism; Stress; Verbal suggestions; Pain

INTRODUCTION

Social and contextual cues around the individual, such as words, attitudes, and professionals' behaviors contribute to evoking symptomatic benefits including placebo effects (1–3). Compelling evidence has shown that placebo effects produce subjective, physiological (4–7) and functional changes (8–10). These changes are related to the activation of endogenous brain modulatory systems (8–10) and the release of endogenous opioids (5–7, 11–13), cannabinoids (8, 14), and dopamine (9). Recently, it has been demonstrated that oxytocin agonists when given intranasally, enhance placebo analgesia in men (15). The brain distribution of oxytocin receptors overlaps with those of arginine vasopressin and the two peptide hormones are critically involved in regulating social behaviors (16, 17). However, so far nothing is known about the role of the vasopressin system in placebo effects.

Here we explore for the first time the modulatory effects of nonselective arginine vasopressin Avp1a and Avp1b receptor agonists on placebo effects. Avp1a and Avp1b vasopressin receptors are largely expressed within the central nervous system and regulate social and stress behaviors across different species in a sex-specific manner (17–22). In nonhumans, vasopressin promotes aggression through actions in the septum, anterior hypothalamus, and central gray in males, and modulates affiliative behaviors through actions in the septum and ventral pallidum in females (18). In humans, vasopressin regulates conciliatory behaviors (23, 24) and social communication (25, 26) prompting women to display 'tend-and-befriend' response patterns toward other women, and 'fight-or-flight' responses in men (26).

On the basis of these considerations, we used a model of verbally-induced placebo effects and examined the modulatory role of nonselective Avp1a and Avp1b vasopressin receptor agonists in men and women against no treatment, oxytocin, and saline in a randomized, placebo-controlled, double-blind, parallel design trial. We administered oxytocin at a dose of 24 IU, the standard dose used in cognitive studies (27) that should have no intrinsic analgesic effects (27, 28). We expected to observe dimorphic influences with vasopressin facilitating placebo effects in women and oxytocin inducing small as previously observed (15), or no effects in men.

METHODS AND MATERIALS

Subjects

A total of 109 (54 women) healthy participants aged from 19 to 46 years (mean age 28.5; S.D. 7; Table 1) were enrolled to participate in this study at the Clinical Center, NIMH from June 2012 to January 2014. One participant discontinued his participation reporting feelings

of irritability after the drug administration and during the testing phase of the experiment. Healthy conditions of the participants were determined by a physical examination performed by a physician. A psychiatric interview was also conducted by a trained psychologist using the Structured Clinical Interview for the DSM-IV-TR (29), and participants were asked to self-report medication and nicotine use. Drug use was excluded by urine toxicology analysis. Exclusion criteria included history of psychotropic drug exposure; major medical or neurological illness; use of nicotine, illicit drug use or alcohol abuse within 1 year; lifetime history of alcohol or drug dependence; psychiatric disorders; current pregnancy or breast feeding; current pain and use of painkillers; abnormal high and low blood pressure values; and history of angioedema. Participants provided informed consent as approved by the NIMH Combined Neuroscience IRB to participate in a study including deceptive elements (30, 31). They were debriefed at the end of their study participation and offered to withdraw their data from the study. None of the participants chose to withdraw the data from the study. Participants were monetarily compensated for their time.

Experimental design and placebo manipulation

To test for modulatory effects of vasopressin on placebo effects, we adopted a model of expectancy-induced analgesia that by producing small changes, as previously observed (2, 32–34), would permit a better determination of any increases due to the action of the active drugs. Also, we used verbal suggestions rather than a conditioning paradigm to avoid ceiling effects related to learning mechanisms.

Electrical stimuli were square pulses delivered by a somatosensory stimulator (PsyLab, London, UK), with duration of 100 microseconds. Painful electrical stimuli were delivered to the dorsum of the hand to induce a moderate level of pain, titrated based on individuals' pain sensitivity. Pain sensitivity was assessed to define the intensity (in mA) of electrical shocks to induce moderate pain. An ascending series of stimuli (steps of 2.5 mA) was delivered starting at a sub-tactile threshold, until painful sensations were induced. Initially stimuli at a very low and usually imperceptible level were delivered. Next, the investigator increased the intensity of the stimuli in steps until participants reached a threshold, indicated by a level that they felt was "definitely painful, but tolerable". To minimize floor effects by ensuring that this level of stimulation was at least somewhat painful, when this threshold level was reached, the participant was asked to verbally report their pain on a scale of 1 to 10. If their reported pain was less than 6 out of ten, then the subject was asked if they felt comfortable trying a higher intensity, such that participants pain ratings at the end of calibration were at least 6 out of ten on a visual analogue scale.

After assessing pain sensitivity and tolerance, subjects were instructed to self-administer intranasal oxytocin, vasopressin or saline. A no-treatment group (nor drugs neither saline) was included to control for effects related to the mere administration of drugs. Participants provided with a treatment might assume that they will experience a difference in pain as result of the intranasal spray independently of any verbal instructions.

Forty minutes after the acute administration of one of the three agents or watchful waiting, the individual level of intensity necessary to induce moderate pain was re-tested. Then the expectancy manipulation took place (Figure 1). A sham electrode was pasted on the middle

finger of the non-dominant hand and participants were informed about the pain-relief procedure: ‘When the green light is on, there will be a stimulus sent to your middle finger so that you will feel either no pain or less pain. On the other hand, when you see the red light, then the stimulus to the finger is turned off so that you will feel pain’. Participants were also told that after the administration of blinded intranasal oxytocin, arginine vasopressin or saline (placebo) or nothing, we would conduct a test of their response to control pain and pain-relieving procedure, respectively. The instructions were given by a white-coat dressed female physician-scientist who entered the room and gently applied a sham electrode to the middle finger of the nondominant hand (also see complete set of instructions, Supplemental Materials). The physician explained that the green lights displayed on the computer screen would indicate the activation of the electrode and to reinforce expectations, each participant was given the opportunity to experience the effect of the red painful and green non-painful paired shocks once. Unbeknownst to the participant, two distinct levels of intensity were used to elicit non-painful sensation when the green light was displayed and a painful paresthesia when the red light was displayed as previously performed and described (2, 32, 34). In the testing phase, the intensity of the painful shocks was surreptitiously set at the individual high level defined during the pain sensitivity assessment and any difference in red- versus green-associated pain reports was operationally defined as ‘placebo analgesia’.

Importantly, procedures were standardized for all participants. To keep the conditions as similar as possible the no treatment group received the instructions after a watchful waiting time of 42 minutes. The groups receiving the intranasal treatment were also told that vasopressin, oxytocin or saline would be administered to study how any potential influences with the ‘pain-relief’ procedure. The intensity of the painful shocks was surreptitiously set at the same level and any difference in red versus green-associated pain reports was operationally defined as ‘placebo analgesia’.

Primary and secondary outcomes

The primary outcome was pain modulation measured using a Visual Analogue Scale ranging from 0=no pain to 10=maximum tolerable pain. Pain analgesia was operationally defined as the difference between red- and green-associated pain reports. The secondary outcome was salivary cortisol. We included as exploratory endpoint, the assessment of gonadal hormones. Participants were also required to complete the State-Trait Anxiety Inventory (35). No changes in state anxiety were observed as result of the treatment and this outcome is not further discussed.

Randomization and drug administration

This study was designed as a randomized, placebo-controlled, double-blind, and parallel trial. Participants were enrolled by the Principal Investigator after an independent team of clinicians and psychologists had confirmed eligibility to the study. The random allocation sequence was independently generated by the NIH Pharmaceutical Development Service (PDS) and described in Supplemental Materials. The Principal investigator called PDS the day prior to each experiment to determine if the participant was randomized to “no treatment”. Participants were first stratified for sex (Flow Diagram, Supplemental Materials)

and then randomized to no-treatment, intranasal oxytocin (24 IU), saline (0.4 mL) or vasopressin (40 IU) group, respectively.

Investigators, staff, and participants were blinded to the treatment options. Each of the three agents (oxytocin, vasopressin or saline) was administered by means of a nasal spray. Participants were instructed by a nurse to self-administer the nasal spray as follows: one spray in each nostril alternating sides, 30 seconds apart for a total of two sprays per nostril.

Saliva and blood collection

Experiments were performed in the morning (8–11 am). Upon arrival screened participants provided a baseline saliva sample for cortisol determination. An additional sample of saliva was taken 65 minutes after the drug administration (Figure 1).

The collection and determination of cortisol were performed following a standardized saliva collection protocol (Salimetrics kit) in 2-ml cryovial test tubes, which were refrigerated at -80°C within one hour of collection and subsequently analyzed using a highly sensitive enzyme immunoassay according to the Salimetrics manufacturer's recommended protocol (Institute for Interdisciplinary Salivary Bioscience Research, Temple, AZ).

We also controlled for menstrual phase and circulating levels of gonadal hormones. Serum levels of estradiol and progesterone were measured for women from a blood sample taken the day of the experiment before the experimental session took place. Samples were centrifuged at 4°C within 20 min of blood draw. Serum was collected and frozen at -80°C until assay. Serum levels of progesterone and E2 were analyzed by high performance liquid chromatography. Calibration curves were obtained to quantify the concentration of each steroid against its internal standard using linear regression with $1/\times$ weighting (NMS Labs, Willow Grove, PA).

Statistical analysis

Given that we adopted a well-validated paradigm, the sample size was calculated by using means, standard deviations and Cohen's coefficient (δ) of previously studies (2, 32, 34), making the assumption that verbally-induced effects should be enhanced under vasopressin to reach a magnitude comparable to that observed under conditioning. For testing placebo analgesia, with a directional hypothesis, we determined that the minimum total required sample size is $n=10$. Due to sexually dimorphic influences, we increased the accrual to reach a minimum of 15 women and 15 men for the oxytocin and vasopressin groups, and 10 women and 10 men for the saline and no-treatment (natural history) groups, respectively. The normal distribution of data was tested with the Kolmogorov-Smirnov test. Because we found no significant difference between our data set and a normal distribution, a parametric statistical approach was used.

The primary outcome was the placebo analgesia operationally defined as the difference between red- and green-associated pain reports. We first ran an omnibus repeated measures ANOVA design, estimating the main effect of the manipulation (red versus green stimuli) and then we explored the relative effect of verbal suggestions in the no-treatment and placebo groups to confirm that the manipulation was effective in eliciting placebo analgesic

effects. Therefore, we focused on the treatment effects across the four experimental groups. To account for potential inter-individual pain differences across groups, we expressed placebo analgesia as [Red (control) – Green (test)] differences. A total of 6 differences (delta) were obtained from each single participant. These differences were analyzed using a mixed analysis of variance model with the 6 VAS delta set as dependent variable, Treatment and sex as fixed variables (36). Planned, follow-up Bonferroni-adjusted multiple testing comparisons were applied.

Paired t-test comparisons (2-tailed) were calculated for assessing pre-post cortisol changes in the vasopressin group. Due to inter-individual baseline variability in cortisol levels, pre and post-treatment levels were normalized and expressed as Jaccard index = (post–pre)/(post + pre). Placebo analgesic responses were correlated with salivary cortisol changes and anxiety trait scores using Pearson (1-tailed) regression approach.

In order to determine any outliers in each outcome, we calculated the 25% (Q1) and 75% (Q3) percentiles to define upper and lower boundaries and we plugged these values into the following Tukey formula:

$$Upper=Q3+(2.2*(Q3-Q1); Lower=Q1-(2.2*(Q3-Q1))$$

No outliers were found for VAS as well as stress responses (cortisol values and dispositional anxiety scores). Statistical analyses were performed with IBM SPSS version 21, with $p < 0.05$ considered significant and alpha set at 0.015.

RESULTS

Participants reported less pain in association with the green light across all the four experimental groups ($F_{1,106}=31.303$; $p < 0.001$). To verify that the verbal suggestions were effective in inducing placebo effects, pain changes in the no-treatment and placebo groups were analyzed using a repeated measures ANOVA approach with the 6 red- and 6 green-VAS scores. In the no-treatment group, there was a significant effect of the manipulation ($F_{1,18}=5.641$; $p < 0.029$; $\delta : 0.169$) with no sex differences ($F_{1,18}=0.884$; $p = 0.360$). Similarly, verbal suggestions induced a significant analgesic effect in the placebo group ($F_{1,22}=6.023$; $p < 0.022$; $\delta : 0.188$) with no sex influences ($F_{1,22}=0.042$; $p = 0.839$), confirming that the paradigms was effective in producing as anticipated small, still significant placebo effects.

Therefore, we analyzed the modulatory effect of vasopressin (and oxytocin) on placebo effects. To further ensure that any difference across the four experimental groups was unrelated to individual pain sensitivity and variability in baseline VAS scores, we expressed the pain reports as differences (red minus green scores) and we estimated the effect of treatment using a mixed analysis of the variance model. There was a significant main effect of treatment ($F_{3,104}=4.642$; $p < 0.003$), and sex was not significant ($F_{1,106}=0.971$; $p = 0.325$). Importantly, the interaction between treatment and sex effects was significant ($F_{3,104}= 3.059$; $p < 0.028$). Accordingly, we performed separate analyses for men and women. There was a significant main effect of treatment in women ($F_{3,50}=7.099$; $p < 0.01$; Fig. 2a), but not in men ($F_{3,50}=0.173$; $p = 0.92$, Fig. 2b). In women, post-hoc group comparisons indicated that vasopressin enhanced placebo effects significantly, relative to the no treatment ($p < 0.004$),

oxytocin ($p<0.001$) and saline ($p<0.015$) groups. In women, the effect size of vasopressin-induced analgesic responses was medium ($\delta=0.603$) versus a small and non-significant effect in men ($\delta=0.301$).

We controlled for pre- and post-treatment variations in pain tolerance, defined as the level of electricity (in mA) necessary to evoke a moderate pain scored as 6 on the VAS scale. Vasopressin-induced analgesic effects were independent of any intrinsic analgesic effect on pain tolerance in both women ($F_{3,50}=1.943$, $p=0.14$) and men ($F_{3,50}=0.359$, $p=0.78$).

The enhancement of placebo effects induced by vasopressin in women was also not related to the menstrual phase. We dichotomized women in follicular (Fp) and luteal (Lp) menstrual phases (Tab. S2) and the menstrual phase did not influence the treatment effects in the vasopressin group ($F_{1,12}=1.311$; $p=0.275$) as well as across the four experimental groups ($F_{3,37}=0.218$; $p=0.883$). Moreover, vasopressin effects were independent of gonadal hormonal levels ($F_{1,13}=0.820$, $p=0.711$), indicating that these results may hint for sex differences in brain receptor distribution rather than upon circulating gonadal hormones.

We examined the relation between vasopressin-induced responses and cortisol levels. Baseline levels of cortisol did not differ between women and men ($t_{(1,28)}=-.0547$, $p=0.558$; Fig. 3a). After vasopressin administration, cortisol levels decreased significantly in women ($t_{(1,14)}=2.646$; $p<0.019$), but not in men ($t_{(1,14)}=1.279$; $p=0.222$). Women who had lower cortisol (baseline and vasopressin-induced changes expressed as Jaccard index) showed larger placebo responses ($r=-0.500$; $p<0.03$ Fig. 3b). Dispositional anxiety was also inversely correlated with placebo responses ($r=-0.469$; $p<0.04$; Fig. 3c). These effects were not found in men (correlation between placebo responses and cortisol, $r=-0.210$, $p=0.226$; placebo responses and anxiety, $r=0.152$, $p=0.294$, respectively). Together these results suggest an interesting modulatory interplay of vasopressin on placebo and stress responses in women.

Harms and unintended effects

Adverse events were assessed in a blinded way by a nurse using a structured checklist. We observed minor adverse events that included dizziness (less than 4% of all participants, 3 subjects in the oxytocin group, 1 in the vasopressin group), nasal congestion (23 % of participants in the vasopressin group), drowsiness (11% of all the participants, 4 subjects in the oxytocin group, 3 in the saline and 5 in the vasopressin group), anxiety (7% of all the participants, 1 subject in the oxytocin, 4 in the saline and 3 in the vasopressin group respectively) and self-reported propensity to act aggressively (4 men in the vasopressin group included the participant who discontinued the study participation).

DISCUSSION

This study is the first to explore in a blinded fashion the modulatory effect of vasopressin agonists on placebo effects while controlling for sexually dimorphic influences. Using a psychopharmacological approach we have demonstrated that nonselective Avp1a and Avp1b vasopressin receptor agonists produce placebo effects in women but not in men. The

modulatory action of vasopressin was highly significant when compared to the no-treatment, oxytocin and saline groups.

Exogenous synthetic vasopressin is rapidly absorbed through nasal mucous membranes reaches the peak effects within 30–50 min after administration and central system fluid levels remain elevated at least for 80 min (37). Methodologically, the inclusion of a no-treatment group rules out any change due to mere administration of an intranasal drug. Participants might behave differently or report an effect after receiving an intranasal spray because of expectations induced by the act of administering a treatment. The comparison between vasopressin and the saline group controls for expectation-driven effects (e.g. participants may expect to get a benefit from the drug administration). Finally, the oxytocin arm served as a positive control. It has been shown that oxytocin enhances placebo effects in men (15).

In our experimental conditions, a 24 IU of intranasal oxytocin did not enhance placebo effects in either sex. At least two factors may explain the difference in the oxytocin findings: 1. the 24 versus 40 IU dose suggesting that oxytocin effects are dose-dependent (38) and, 2. the sex of the experimenter -a woman in the present study, a man in Kessner et al. (15) - in line with oxytocin regulating distinct social behaviors (16) in men (39) and women (40–44) with potential in- and out-group behavioral dynamics (45, 46). Clarifying both these aspects represents an achievement worthy of future investigation. Moreover, Kessner et al. (15) and our study also differ methodologically for the inclusion of the no-treatment group, a condition that when applicable, helps rule out biases or expectation effects due to the act of receiving a treatment.

We demonstrated for the first time that vasopressin might be involved in boosting placebo effects enhancing the relatively small responses that can be usually induced by a model of expectancy manipulation as shown in the no-treatment and placebo groups as well as in previous studies (2, 32–34). Notably, we observed a significant sex by treatment interaction which is consistent with animal and human studies. Central arginine vasopressin and vasotocin (the nonmammalian homologue) control social behaviors across different mammalian species with notable sexual and contextual differences (47–49). In female rats, vasopressin release in the medial bed nucleus of the stria terminalis (BSTM) promotes affiliative behaviors (50, 51), while in male rats, the release of vasopressin in the BSTM determines distinct patterns of aggression (52). The response of the BSTM vasopressin cells appears to be specific for social stimuli (52) and there is a lack of response in asocial species such as the Syrian hamster (53). Interestingly, receptor autography studies in the socially monogamous coppery titi monkey showed that *Avp1a* receptors are present in areas crucially involved in social behavior processing such as the cortex (cingulate, insular and occipital) as well as the central amygdala, nucleus accumbens, caudate, putamen, endopiriform nucleus, and hippocampus (54).

Human studies have pointed to distinct social patterns of behaviors and brain activation of reward and salience circuits in men and women (26). Intranasal vasopressin agonists affect emotional and cognitive evaluation of communication, including facial motor patterns, differentially in men and women (55). Men under the effects of vasopressin show decrease

of friendliness and approachability and increase of agonist facial motor patterns in response to faces of unfamiliar men. Conversely, in women, vasopressin stimulates affiliative facial motor responses patterns and perception of friendliness in response to the faces of unfamiliar women (26), thus indicating that vasopressin in humans influence emotional social communication. Vasopressin increased the probability to cooperate as second player in women but not in men following a partner defection, favoring conciliatory gesture and cooperation (24).

Our results are in line with the studies described above. Evolutionary theories have also indicated distinct patterns of social interactions as a result of group living pressures, with women displaying 'tend-and-befriend' response patterns toward other women, and 'fight-or-flight' responses in men (56). It is plausible to think that at least in our experimental conditions, vasopressin agonists acted as reinforcers of social aspects of placebo effects, prompting women to engage in alliance tendencies. Vasopressin most likely shaped the *meaning* of the instructions, resulting in an enhancement of expectancy-induced analgesic responses, emphasizing the crucial role of 'response to meaning' in forming placebo effects (57). Future brain imaging studies can identify which brain mechanisms are responsible of this modulation.

Importantly, the sexually dimorphic influences on vasopressin-induced potentiation of placebo effects are independent of female estradiol and progesterone levels. We monitored female participants for menstrual phases and circulating gonadal hormones. There was no significant interaction between placebo effects and circulating hormones, indicating that these results depend upon sex differences in brain receptor distribution rather than merely upon circulating gonadal hormones.

Vasopressin affects the hypothalamic–pituitary–adrenal (HPA) axis, inducing a sex-specific response in the secretion of cortisol (58). Distinct vasopressin-induced behaviors also have been observed in rats separated for low or high anxiety, suggesting that the nonapeptide effects on social behaviors are integrated with anxiety processing (59). We observed larger vasopressin-induced placebo effects in those women with lower cortisol levels and lower dispositional anxiety, indicating a possible relation among psychological factors (e.g. anxiety levels), cortisol secretion (baseline and vasopressin-induced changes) and placebo effects. This aspect linking together psychophysiology changes and behaviors represents a promising line of research to better understand placebo responsiveness (60).

Study limitations and generalizability

Limitations of this study include a lack of assessment of participants' beliefs about the treatment allocation and participants' expectancies about the analgesic effect of the procedure. Another limitation is represented by the fact that we did not monitor baseline and post-treatment peripheral changes in oxytocin and vasopressin release. Future studies should include noninvasive peripheral assessments (e.g., blood, urine, or saliva) of vasopressin and oxytocin as a proxy for central measures.

Our findings might have a direct impact on research aimed at exploring the therapeutic effects of vasopressin (and oxytocin) across different psychiatric diseases (61–68) for the

potential to systematically control for moderating influences of sex and ultimately, placebo effects in clinical trials. Moreover, knowledge about the underlying neurobiological mechanisms might allow clinicians therapeutically target placebo effects in clinical contexts. In translating these findings into personalized care for every patient, it is important to devise factors (e.g. sex, stress reactivity and, hormonal influences) that might shape placebo responsiveness and ultimately, treatment outcomes.

We demonstrated for the first time that the arginine vasopressin produces placebo effects in women but not in men. Variations in individual placebo effects may be related to the vasopressin (and oxytocin) system functions and the social brain. Future research with selective Avp1a and Avp1b receptor antagonists might unravel intriguing additional aspects of how vasopressin circuits modulate placebo effects with relevant mechanistic and translational implications.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1. Study outline

Upon arrival of screened participants, a baseline saliva sample was collected for cortisol determination. Then pain sensitivity was assessed to define the intensity (in mA) of electrical shocks to induce moderate pain. Afterwards, participants were instructed to self-administer intranasal oxytocin, vasopressin or placebo. A no-treatment group (nor drugs neither saline) was included to control for effects related to the mere administration of drugs. Forty minutes after the acute administration of one of the three agents or watchful waiting, the individual level of intensity necessary to induce moderate pain was re-tested. Then the placebo manipulation took place. A sham electrode was pasted on the middle finger of the non-dominant hand and participants were informed about the pain-relief procedure. During the testing phase, red and green lights were displayed for 5–7 seconds and followed by the electric shock (0.3 second delay) set at the current (mA) inducing moderate pain and scored as 6 on a Visual Analogue Scale (VAS) ranging from 0 (no pain) to 10 (most tolerable pain). The inter-trial interval (ITI) was 12.5 seconds. A total of 12 shocks (6 red+ 6 green) were delivered.

t0= Baseline pain assessment. t1: Intranasal administration of vasopressin, oxytocin, saline or watchful waiting. t40: Post-drug pain sensitivity assessment. t42: Placebo manipulation. t45: Testing of placebo analgesia. t65: End of the placebo analgesia testing and saliva collection.

Figure 2. Impact of vasopressin on placebo effects

Arginine vasopressin increased placebo analgesia significantly relative to saline ($p < 0.015$), oxytocin ($p < 0.001$) and no-treatment ($p < 0.004$), in women (a) but not in men (b). Placebo responses are calculated as 'Red-green' associated pain reports and expressed as mean difference $\pm$ sem. AVP = arginine vasopressin; OXT = Oxytocin.

Figure 3. Relation between vasopressin-induced effects and stress responses

Baseline levels of cortisol were not different in women and men ($t_{(1,28)}=-.0547$, $p=0.558$). After vasopressin administration, cortisol levels decreased significantly in women ($t_{(1,14)}=2.646$; $p<0.019$), but not in men ($t_{(1,14)}=1.279$; $p=0.222$) (a). Lower cortisol levels correlated inversely with placebo analgesic effects ($r=-0.500$; $p<0.03$) (b). Similarly dispositional anxiety was inversely correlated with placebo analgesic responses ($r=-0.469$; $p<0.04$) (c), indicating an interesting interplay between vasopressin enhancement of placebo effects and the HPA axis. Data are presented as mean difference $\pm$

sem (a) and cortisol level changes were normalized and expressed as Jaccard index = (post –pre)/(post+pre) (b).

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Table 1

Participants' characteristics

	No treatm	Oxytocin	Saline	Vasopressin	Overall	F	p value
Sex (F, M)	10 F, 10 M	17 F, 17 M	12 F, 12 M	15 F, 15 M	54 F, 54 M		
Age, y	27.5 ± 5.4	30.5 ± 8.1	28.8 ± 8.2	27.6 ± 6.7	28.6 ± 7.4	1.448	0.233
BMI	24.2 ± 5.6	25.5 ± 4.5	25.5 ± 4.1	25.2 ± 5	25.2 ± 4.7	0.402	0.752
Baseline pain scores	7 ± 0.8	6.8 ± 1.1	6.9 ± 1.1	7 ± 0.8	6.9 ± 1	0.337	0.798
Trait Anxiety score	29 ± 7.5	28.8 ± 10.5	30 ± 6.5	28.6 ± 7.5	29 ± 8.2	0.301	0.825

There were no differences across groups for Age, Body Mass Index (BMI), and Trait Anxiety scores as assessed via STAI-2³⁵.
Data are expressed as Mean ± S.D.