



Intranasal administration of oxytocin decreases task-related aggressive responses in healthy young males

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ABSTRACT

Aggression and distrust are often challenging problems in mental health treatment. Converging evidence reveals that oxytocin increases trust in social interactions and decreases fear of social betrayal. However, oxytocin has also been associated with protective behavior and, as such, might increase defensive aggressive reactions.

In this randomized double-blind, placebo-controlled study, the effects of intranasal oxytocin (32IU) on task-related aggressive responses were measured using the Point Subtraction Aggression Paradigm (PSAP). Fifty-seven healthy males were enrolled and randomized to oxytocin ($N = 30$) or placebo ($n = 27$). Salivary oxytocin, cortisol and testosterone were measured serially prior to the intervention, and then before and after the PSAP, to evaluate the effects of oxytocin administration on hormonal functioning in relation to aggression. In addition, oxytocin was measured in urine collected directly after the experimental task, reflecting the 2 h period after oxytocin or placebo administration.

The proportion of aggressive responses to the PSAP was significantly lower in participants receiving oxytocin versus placebo ($\beta = -0.46$, $P = 0.01$). No significant effect of oxytocin was found regarding defensive reactions. Urinary oxytocin was negatively associated with the proportion of aggressive responses to the PSAP in both the oxytocin and the placebo group ($\beta = -0.02$, $P < 0.01$), suggesting that higher levels of urinary oxytocin corresponded with reduced aggressive responding. Our results indicate that oxytocin administration reduces aggressive behavior in healthy young men. Moreover, increased endogenous urinary oxytocin is associated with less aggressive responding. Taken together, these findings suggest that oxytocin signaling has a causal influence on aggressive behavior.

1. Introduction

Aggression is a frequently reported problem in forensic psychiatric settings and often cited as a prominent limiting factor for referral to rehabilitation programs (Daffern et al., 2007). Unfortunately, the currently available therapeutic options for reducing aggression are limited, not always effective and based largely on reactive and impulsive aggression (Coccaro and Siever, 2002). An alternative or complementary therapeutic approach might be to facilitate pro-social behavior. Oxytocin is a hormone widely known for its peripheral effects on the milk-ejection reflex during lactation and induction of uterine contractions during parturition (Gimpl et al., 2001). However, convergent evidence has shown that oxytocin also has central functions in the brain,

increasing pro-social behavior such as cooperation and generosity (Zak et al., 2007), enhancing feelings of trust and empathy (Striepen et al., 2011), and reducing social stress (Kubzansky et al., 2012). These pro-social effects have inspired a growing interest in the potential therapeutic efficacy of oxytocin for reducing aggression.

Animal research has shown that oxytocin, when administered intracerebrally or intranasally, reduces aggressive behavior in mice (Calcagnoli et al., 2013; Calcagnoli et al., 2015a, 2015b). When injected in the amygdala, it promotes social exploration and reduced offensive behavior (Calcagnoli et al., 2015a, 2015b). Conversely, increased aggressive behavior is seen in mice lacking oxytocin receptors (Dhakar et al., 2012).

Studying aggressive behavior in humans is more challenging than in

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animal models because aggressive behavior depends on diverse biological, emotional, environmental and cultural factors (Bartz et al., 2011). Oxytocin increases aggression when the social environment has to be protected but reduces aggression when there is no social threat (Shamay-Tsoory and Abu-Akel, 2015). A lifetime history of aggression was reported to be negatively correlated with cerebrospinal fluid oxytocin levels (Lee et al., 2009a). However, oxytocin can also induce defensive reactions. Intranasally administered oxytocin induced protective behavior towards vulnerable group members in an intergroup conflict game (De Dreu et al., 2012). Moreover, oxytocin has been associated with protective behavior in mothers (Mah et al., 2014). Using an aggression paradigm (Point Subtraction Aggression Paradigm, PSAP), oxytocin did not have a direct effect on the number of aggressive responses in women (Campbell and Hausmann, 2013). However, in their placebo condition, Campbell and Hausmann found a higher attack-earn ratio in woman with a high state anxiety, whereas in the oxytocin condition no difference was found between the participants with high and low state anxiety levels. Also, in healthy males, no direct effect of oxytocin on aggressive responses to the PSAP was found (Alcorn et al., 2015), although Alcorn et al. found a positive correlation with antisocial social personality traits and aggressive responding after administration of oxytocin. Participants who had high trait levels of interpersonal manipulation and anger and received intranasal oxytocin gave more aggressive responses than those who scored low on these trait levels. This implicates that administration of oxytocin in men with antisocial personality traits might enhance aggressive responding. Ne'eman et al. found an increase in aggressive responses (Ne'eman et al., 2016) after intranasal administration of 24IU oxytocin in a laboratory setting. These findings underline the hypothesis that oxytocin does not induce pro-social behavior per se, but that oxytocin rather causes an increase of social salience and that the effect depends on contextual factors. Administration of oxytocin (24IU) caused activation of the nucleus accumbens during a task in which cooperation was rewarded, whereas activation of the amygdala was found during a task in which aggressive behavior was rewarded (Lambert et al., 2017). This implies that the effect of oxytocin on the brain depends on social cues. Oxytocin may have a blunting effect on offensive aggression due to a higher empathic concern for the victim (Krueger et al., 2012), less fear for betrayal (De Dreu, 2012) and more trustworthiness (Zak et al., 2005). However, when the individual's personal integrity is violated, it might cause defensive aggressive reactions.

In cases of violence, there is likely an interaction between oxytocin, cortisol and testosterone (Fragkaki et al., 2018). Oxytocin administration leads to a blunted cortisol response as a reaction to social stress (Cardoso et al., 2013; Ditzen et al., 2009; Heinrichs et al., 2003; Linnen et al., 2012). In people with emotional dysregulation, oxytocin seems to have a notably positive effect on aggressive behavior by attenuation of the cortisol response (Quirin et al., 2011; Simeon et al., 2011). This effect, however, might be more pronounced in women than in men (Flanagan et al., 2018). Testosterone levels may also influence the relationship between oxytocin and aggression. In combination with low levels of cortisol, indicating low levels of stress in healthy subjects, testosterone can promote aggressive behavior in high dominant men (Carré and Archer, 2018). Furthermore, administration of oxytocin might function by altering testosterone levels and thus influence social behavior (Weisman et al., 2014). Previous studies found negative correlations between a lifetime history of aggression and cerebrospinal oxytocin levels (Lee et al., 2009), but the direct effect of oxytocin on aggressive behavior is not clear and seems to depend on different factors such as personality traits and anxiety level (Alcorn et al., 2015; Campbell and Hausmann, 2013; Ne'eman et al., 2016). However, in these studies a relative low dosage of intranasal oxytocin (24IU) was used.

To clarify the relationships between oxytocin, regulation of hormones and aggression, we performed a randomized, double-blind placebo-controlled study, in which we administered oxytocin or a placebo

intranasally to healthy young men. The primary outcome measure was proportion of aggressive responses on the Point Subtraction Aggression Paradigm (PSAP), a widely used task in which participants are provoked to harm a (fictive) opponent by stealing money from him (Carré and McCormick, 2008; Cherek, 1981). We hypothesized that intranasal oxytocin would result in a reduced proportion of aggressive responses during the PSAP. Because of the potential effect of cortisol and testosterone on the relationship between oxytocin and aggression, we measured these hormones in saliva prior to the intervention, and then before and after the PSAP. In addition, we measured oxytocin in urine immediately following the PSAP.

2. Methods

This study was approved by the Medical Ethical Committee of the Erasmus Medical Center Rotterdam and conducted conform the Declaration of Helsinki and the European Medicines Agency Guidelines for Good Clinical Practice.

2.1. Participants

Participants were included if they were in the age range between 18 and 35 years and did not have any medical condition. All were recruited via local advertisements. To exclude possible effects of the menstrual cycle on oxytocin levels, only males were included in this study (Salonia et al., 2005). Another reason to focus on males is that the effects of oxytocin on aggression are strongly modulated by estrogen (Campbell, 2008). Exclusion criteria were: a history of psychopathology or psychiatric disorders, relevant somatic disorders, past or current drugs or alcohol dependency, clinically significant allergic rhinitis, and heavy smoking (> 10 cigarettes per day). Potential participants were screened by phone regarding the inclusion and exclusion criteria. Sixty-nine participants were included and randomized into 2 groups. Thirty-four participants received oxytocin and thirty-five placebo. Participants were excluded from further analyses when they were not convinced to have played against another human being.

2.2. Procedure

Participants were first screened by filling in a standardized checklist. When coming to the lab, they were subsequently asked for any current or history of psychiatric illness or substance abuse, medication use, somatic disorders or treatment by any medical professional. If they responded positive on one of these questions, they were further screened by a medical doctor to check whether they met the exclusion criteria. Participants who passed the phone screening came twice to the laboratory. During their first visit, they completed different questionnaires regarding trust (the Trust Inventory, Couch et al., 1996), relationships (the Relationship Questionnaire, Bartholomew and Horowitz, 1991; the Experiences in Close Relationships, Brennan et al., 1998), aggression (the Buss Durkee Hostility Index, Buss and Perry, 1992; the Reactive Proactive Questionnaire, Raine et al., 2006; the Triarchic Psychopathy Measure, Patrick et al., 2009) and personality (the Neuroticism-Extroversion-Openness -Five Factor Inventory, Costa and McCrae, 1998). During this visit, exclusion criteria and health status were checked again by the researcher. On their second visit, the participants came to the laboratory at 01:00 p.m.; they self-administered intranasal oxytocin or placebo, after which they were asked to wait. During this period, the participants were allowed to read, but not to do any physical or psychological intensive exercises. After this, they were shortly introduced to their (fictional) opponent, but no further communication between the participant and the opponent was allowed. Seventy-five minutes after the oxytocin administration the PSAP task was performed. Saliva samples for assay of oxytocin, cortisol and testosterone levels were taken at 3 time points (at arrival, directly before the start of the PSAP and directly after finishing the PSAP). Urine was

collected over the period between the intranasal administration and finishing the PSAP (2 h period). Participants were asked to empty their bladder before the start of the intranasal administration to ensure that only the urine produced during the task was collected. Fig. 2 shows the timeline of the procedures. Blood pressure and pulse were measured manually before the administration of oxytocin, before the start of the PSAP and directly after the PSAP. The participants were asked to refrain from any physical exercise before coming to the laboratory and not to drink (other than water), eat or smoke one hour before arrival. During the experiment they were only allowed to drink water. The participants received €30 after completing the study.

2.3. Oxytocin or placebo administration

The study vials were provided in sequentially numbered containers according to the randomization list. Study vials looked similar and were covered with aluminum foil. They were labelled according to the Good Manufacturing Procedure guidelines (e.g. World Health Organization, 2011). The participants self-administered either 32IU of oxytocin (Syntocinon) intranasally (4 puffs in each nostril) or the placebo (normal saline, 4 puffs in each nostril). 1 IU is equivalent to 2 µg oxytocin. In rats, changes in aggressive behavior are dose-dependent (Calcagnoli et al., 2013). Also in humans with an autism spectrum disorder, higher dosages seem to be more effective on the global clinical impression (Kosaka et al., 2016). Therefore, we choose to use a relative high dosage. Intranasal oxytocin administration has been shown to have few side-effects (MacDonald et al., 2011) at the dose level used in this study. After one spray, participants were asked to breath regularly after which they sprayed the other nostril. Then participants had to wait for one minute before administering the next spray to be sure that the oxytocin was absorbed properly.

2.4. The point subtraction aggression paradigm (PSAP)

During the PSAP task, participants were told that they had to earn as many points as possible, which were exchanged for money afterwards (Carré et al., 2009). At the start, the participant and his (fictional) opponent had the same amount of money. There were 3 optional responses when performing the PSAP task: (1) earning points, (2) stealing a point from the opponent, or (3) the prevention of points being stolen from you for a short time. The participants were told that stealing points from the opponent, however, did not increase the amount of their own points. The hypothesis was that stealing money without any profit is a measure of aggression because this represents an intent to harm. In relation to the effects of oxytocin, the third option is interesting, because this option can be seen as a specific defensive action. In order to provoke an aggressive reaction, money was subtracted from the amount that the participant had earned randomly. This was done via the computer program, with selected intervals between 6 and 120 s. When participants pressed 10 times button (3), an extra interval of 10 s was introduced. Pressing 10 times button (2) led to a decrease of one point for the fictive opponent and pressing 100 times button (1) led to an increase of one point for the participant. The PSAP task is developed by Cherek et al., based on his early work in pigeons (Cherek and Pickens, 1970; Cherek et al., 1973) and developed further for the use in humans (Cherek, 1981). The original version took 60 min to complete, although shorter versions gave comparable results (Golomb et al., 2007). We used the 40-minute task version as used by Geniole et al. (Carré et al., 2009; Geniole et al., 2011) because after intranasal administration oxytocin is only present in the bloodstream for a restricted period of time, although van IJzendoorn et al. found elevated levels 7 h after administration (van IJzendoorn et al., 2012). This PSAP task version consisted of 3 blocks of 10 min, with a 3-min interval in between. The number of points of all 3 types of responses (earning, aggressive and defensive responding) were used and corrected for the total amount of responses. As the reliability of the PSAP as a measurement of

aggression depends on whether or not the participants believed to be playing against another person, the participants were questioned at the end of the experiment how they felt their opponent had played the game and whether they believed their opponent to be real. Participants who did not believe that they had played against a real opponent were excluded from further analysis.

The PSAP has been validated in a number of studies. Female parolees with a violent history reacted more aggressively to the PSAP than parolees with a non-violent history (Cherek and Lane, 1999). Furthermore, aggressive behaviour to the PSAP is moderately correlated with various self-report measures of aggression (Gerra et al., 2001, 2007). In a randomized controlled trial, in which testosterone was administered during a 6-week period to healthy males, a significant increase of aggressive responses on the PSAP was found (Pope et al., 2000).

2.5. Hormonal measurements

Directly before the administration of oxytocin or placebo, the participants were asked to empty their bladder. About two hours after the intranasal administration of oxytocin or placebo, participants were asked to collect urine in a sterile container. Saliva samples were taken before the oxytocin or placebo administration and directly before and after the PSAP task. Saliva was collected with a 'salivette' (Sarstedt Salivette devices) for determination of cortisol and testosterone levels. Saliva for oxytocin analyses was collected by passive drooling of at least 1.5 ml into a sterile tube. Directly after collection, the urine and saliva samples were put on ice, and, after the experiment was completed, the samples were stored at -80 °C until assay.

2.6. Assay of salivary cortisol and testosterone

Saliva samples were collected using the Sarstedt cortisol salivette devices. The participants chewed on a synthetic swab on the specific time points. The samples were stored at -20 °C until they were analyzed. The free cortisol levels in saliva were analyzed with a commercially available ELISA kit (Demeditec Diagnostics, Kiel, Germany). Limit of detection is 0.276 nmol/l. The inter- and intra-assay coefficients of variation are < 10% and < 7%, respectively. The free testosterone levels in saliva were analyzed with a commercially available ELISA kit (DRG Diagnostics, Marburg, Germany). The limit of detection is 34.7 pmol/l. The inter- and intra-assay coefficient of variation is < 10% and < 15%, respectively.

2.7. Assay of urinary oxytocin

Levels of oxytocin were determined using the Oxytocin EIA kit (Enzo Life Sciences Inc., USA), a competitive immunoassay for the quantitative determination of oxytocin. We validated this method for use with urine samples in 20 healthy volunteers. The quantification of undiluted urine and a 1:1 dilution with assay diluent yielded a strong correlation ($r^2 = 0.98$). Furthermore, the quantification of undiluted urine samples with a 1:1 dilution with known standards also demonstrated an equally high correlation ($r^2 = 0.98$). All outcomes were within the measurement range, for which the mean differences between the repeat measurements was 8.9%. The sensitivity of the methods was 11.7 pg/mL. The intra-assay coefficient of variation was 9.1% at the level of 21.4 pg/mL. Urinary creatinine concentrations were measured using the Jaffé method. Oxytocin levels were normalized to creatinine. In 6 samples, creatinine levels could not be obtained because of technical problems in the laboratory, and they were therefore excluded from the analysis. Urinary oxytocin levels have been previously shown to have a linear correlation with plasma levels (Amico et al., 1987; Romero et al., 2014).

2.8. Assay of salivary oxytocin

Determination of salivary oxytocin was performed using a 96-plate commercial oxytocin-ELISA kit (ENZO, NY, USA). Measurements were performed in duplicate. Samples were diluted 1:5 in the assay buffer and treated according to kit's instructions. The assay's reported intra-assay and inter-assay coefficients of variability are 12–19.1% and 5.2–14.5%, respectively. The intra-assay and inter-assay coefficient we received were < 13.2% and 16.1%, respectively.

2.9. Statistics

Descriptive statistics were calculated to summarize characteristics and main outcomes of participants. Generalized linear models were used to examine differences in aggressive responses to the PSAP between the oxytocin and placebo group. In addition, generalized linear models were also used to study the association between urinary oxytocin levels and aggressive responses to the PSAP and to study the effect of administration of oxytocin on the cortisol and testosterone levels. We fitted quasi-Poisson models to take into account that aggressive responses were counted set against defensive responses, which could result in overdispersion and heteroscedasticity in the regression analysis. To control for the overall activity of a participants, the responses were calculated as a proportion of the total number of hits. The total number of hits (grand mean centered) was included as covariate interpreted as a proxy for the overall strategy that respondents adopted. We used residual plots and regression diagnostics to evaluate model fit. Bootstrapping with 10,000 resamples was used to estimate regression coefficients and 95% confidence intervals. All analyses were performed using IBM SPSS Statistics version 24.

3. Results

Descriptive statistics of the placebo and the oxytocin group are shown in Table 1. No adverse events were reported after oxytocin or placebo administration. Twelve participants (17% of all participants) did not believe to play against another person and were excluded from further analyses. Therefore, thirty participants in the oxytocin groups and 27 in the placebo group were available for statistical analyses (a flow chart of the inclusion process is shown in Fig. 1).

Table 1

Descriptives of the placebo and oxytocin group. Mean values with standard deviations of the mean are shown. BDHI = the Buss Durkee Hostility Index; RPQ = Reactive-Proactive Aggression Questionnaire; NEO = the Neuroticism-Extroversion-Openness Five Factor Inventory; ECR = Experiences in Close Relationships; TI = Trust Inventory.

	Placebo (N = 27)	Oxytocin (N = 30)
Age (years)	21.6 (2.8)	21.9 (1.9)
Alcohol use (units/week)	7.9 (8.8)	8.5 (7.7)
Smoking (cigarettes/day)	0.2 (0.6)	0.3 (0.9)
Length (meters)	1.8 (0.1)	1.8 (0.1)
Weight (kg)	80.6 (11.5)	76.3 (8.6)
BDHI – overt aggression	7.3 (2.6)	6.7 (3.0)
BDHI – covert aggression	3.0 (1.9)	3.8 (3.1)
RPQ – proactive aggression	13.3 (1.4)	13.7 (2.3)
RPQ – reactive aggression	18.8 (3.1)	18.8 (3.0)
NEO – neuroticism	25.0 (7.6)	27.3 (7.6)
NEO – extraversion	45.2 (6.4)	45.0 (7.1)
NEO – openness	40.2 (6.1)	40.2 (6.6)
NEO – agreeableness	42.7 (7.2)	43.8 (6.0)
NEO – conscientiousness	42.6 (9.7)	45.5 (7.7)
ECR – avoidance	2.5 (1.1)	2.4 (1.1)
ECR – anxiety	3.2 (0.9)	3.0 (0.9)
TI – general trust	81.1 (10.5)	80.3 (12.1)
TI – partner trust	81.2 (9.1)	83.6 (11.1)

3.1. PSAP

Overall, participants receiving oxytocin gave less responses (earning points, aggressive responses and defensive responses together) during the PSAP than placebo (oxytocin: mean $\pm$ SEM, 187 ± 8.67 points; placebo: 205 ± 8.77 points; $\beta = -0.16$, CI [-0.25, -0.05], $P = 0.004$). Participants receiving oxytocin exhibited a significantly lower proportion of aggressive responses compared to those receiving placebo (oxytocin, 39 ± 5.94 points; placebo, 50 ± 5.93 points; $\beta = -0.46$, CI [-0.80, -0.15], $P = 0.01$) (Fig. 3). There is an estimated difference of 19 points on aggressive responses between the groups for respondents with an average number of total responses (raw median difference = 24). The proportion of defensive responses did not differ significantly between the two groups (74 ± 7.04 points in the placebo group versus 66 ± 6.90 points in the oxytocin group; $\beta = -0.21$, CI [-0.49, 0.06], $P = 0.15$).

3.2. Indirect effects of cortisol or testosterone

Oxytocin, cortisol and testosterone levels are shown in Table 2. Administration of oxytocin did not affect cortisol ($\beta = 1.14$, CI [-2.88, 5.15], $P = 0.58$) or testosterone ($\beta = 42.74$, CI [-41.60, 127.08], $P = 0.32$) levels. The effect of oxytocin on aggressive responses to the PSAP was not modified by testosterone ($\beta = 1.14$, CI [-2.99, 5.27], $P = 0.59$), cortisol ($\beta = 0.11$, CI [-0.08, 0.30], $P = 0.59$), nor the cortisol/testosterone ratio ($\beta = 0.00$, CI [0.00, 0.001], $P = 0.41$).

3.3. Controlling for the effect of age, blood pressure and lifetime history of aggression

The effect of intranasal administration of oxytocin on the proportion of aggressive responses on the PSAP was not significantly affected by age ($\beta = -0.03$, CI [-0.10, 0.05], $P = 0.51$), change in s blood pressure before and after intranasal administration of oxytocin (systolic $\beta = -0.02$, CI [-0.04, 0.00], $P = 0.08$ and diastolic $\beta = 0.01$, CI [-0.01, 0.03], $P = 0.34$) or lifetime history of aggression (BDHI total score; $\beta = 0.02$, CI [-0.02, 0.07], $P = 0.31$).

3.4. Urinary oxytocin levels

Urinary oxytocin was negatively associated with the proportion of aggressive responses during the PSAP ($\beta = -0.02$, CI [-0.04, -0.004], $P = 0.008$) (Fig. 4). We made an overall model to predict the aggressive responses on the PSAP in which both the administration of oxytocin and the urinary oxytocin levels were included. In this model, both the oxytocin administration ($\beta = -0.38$, CI -0.74, -0.08, $P = 0.03$), as well as the urinary oxytocin ($\beta = -0.016$, CI -0.03, 0.00, $P = 0.04$), contributed significantly to the reduction of aggressive responses. The relationship between urinary oxytocin and aggressive responding was similar between oxytocin and placebo groups ($\beta = -0.01$, CI -0.03, 0.03, $P = 0.64$), for which each 10 ng/mmol oxytocin increase of oxytocin was associated with an absolute reduction of 8 points in aggressive responding.

4. Discussion

We examined the effect of intranasal administration of 32IU oxytocin on aggressive responding in the PSAP laboratory paradigm, which revealed that intranasal oxytocin reduced aggressive responses without altering defensive responses. Prior literature on the effects of oxytocin on aggression are mixed, with reports of increased (Ne'eman et al., 2016), decreased (Campbell and Hausmann, 2013) and unaltered (Alcorn et al., 2015) aggression following 24IU intranasal oxytocin. To our knowledge, our study is the first to use the 32IU dose of intranasal oxytocin to evaluate aggressive behavior. Moreover, it is likely that social context and individual differences also moderate the effect of

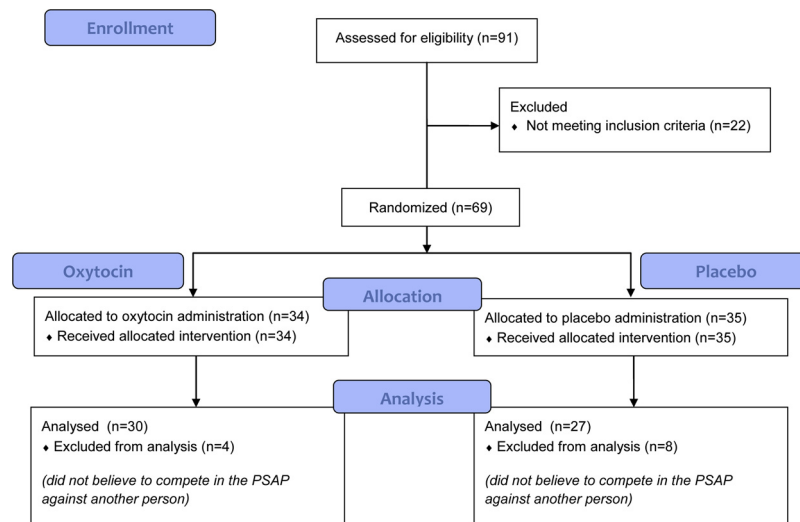


Fig. 1. Flow diagram for the inclusion of the study participants.

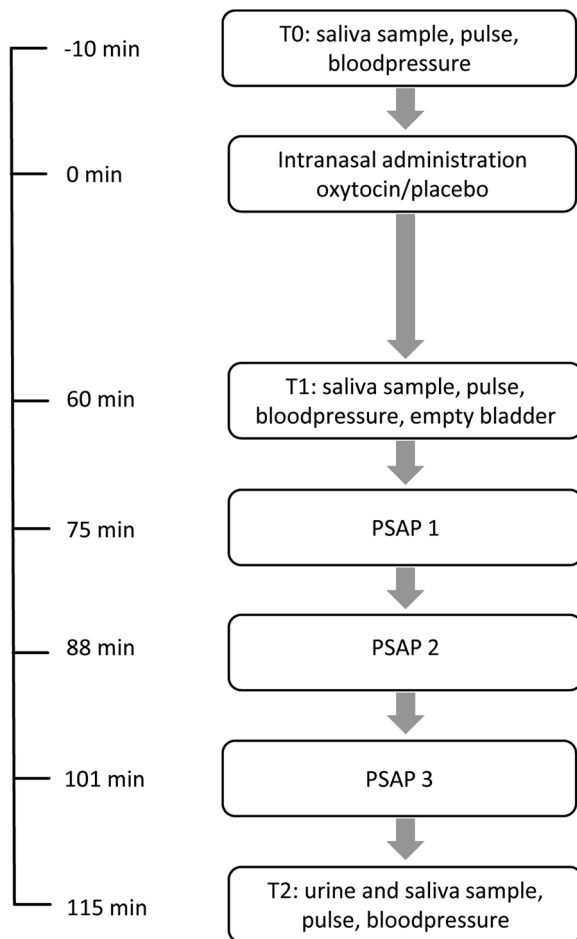


Fig. 2. Timeline of the experimental procedure.

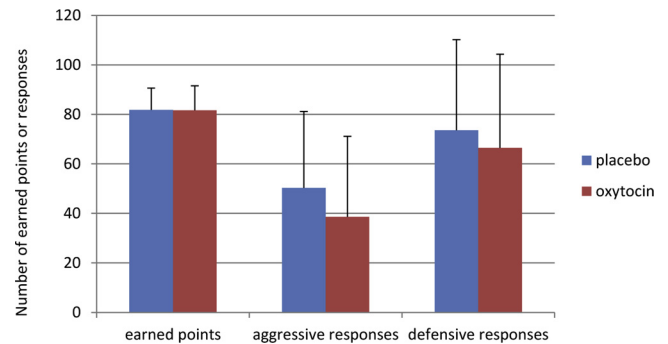


Fig. 3. Mean number of responses to the PSAP in the oxytocin and placebo group for the amount of earned points (Pressing 100 times button 1 is equal to one point), the number of aggressive responses (Pressing 10 times button 2 is equal to one aggressive response) and the number of defensive responses (pressing 10 times button 3 is equal to one defensive response). Compared to the placebo condition, the proportion of aggressive responses was significantly lower in the oxytocin condition ($\beta = -0.46$, $P = 0.01$). The proportion of earned points and defensive responses did not differ significantly between the two conditions.

Table 2

Mean (SD) values of the hormonal data before the intranasal administration of oxytocin or placebo (T0), one hour after the administration, before the PSAP (T1) and after the PSAP (T2) and blood pressure before intranasal administration (T0) and after the PSAP (T2) for the placebo and oxytocin group separately.

	Placebo (N = 27)	Oxytocin (N = 30)
Oxytocin T2, urine (ng/mmol)	30.6 (9.8)	34.6 (9.6)
Oxytocin T0, saliva (pg/ml)	32.5 (29.3)	32.6 (29.9)
Oxytocin T1, saliva (pg/ml)	31.6 (33.9)	15,067.7 (31,966.0)
Oxytocin, T2, saliva (pg/ml)	28.5 (23.2)	8777.8 (23,391.4)
Cortisol T0, saliva (nmol/L)	16.4 (7.9)	16.8 (8.3)
Cortisol T1, saliva (nmol/L)	13.3 (8.1)	12.8 (6.5)
Cortisol T2, saliva (nmol/L)	10.7 (5.7)	9.2 (4.4)
Testosterone T0, saliva (pmol/L)	419.9 (146.4)	387.2 (119.8)
Testosterone T1, saliva (pmol/L)	413.1 (159.6)	376.7 (139.2)
Testosterone T2, saliva (pmol/L)	399.6 (144.2)	370.9 (103.3)
Testosterone/cortisol ratio T0	27.2 (10.1)	28.3 (16.4)
Testosterone/cortisol ratio T1	35.1 (13.7)	34.5 (17.2)
Testosterone/cortisol ratio T2	42.3 (17.2)	48.2 (24.8)
Systolic blood pressure T0	130 (10.4)	128 (10.3)
Systolic blood pressure T2	126 (12.3)	127 (11.3)
Diastolic blood pressure T0	81 (7.3)	78 (8.0)
Diastolic blood pressure T2	80 (9.9)	80 (8.5)

oxytocin on social behavior (Bartz et al., 2011).

Our study intended to evaluate the effect of exogenously oxytocin on aggression by minimizing the effects of the social context as much as possible. For this reason, participants met their "opponent" before the PSAP only briefly and without verbal communication. Despite that, 17% of the included participants did not actually believe to be playing against another person. Declerck et al. demonstrated that social interaction before a social task can significantly influence the effect of

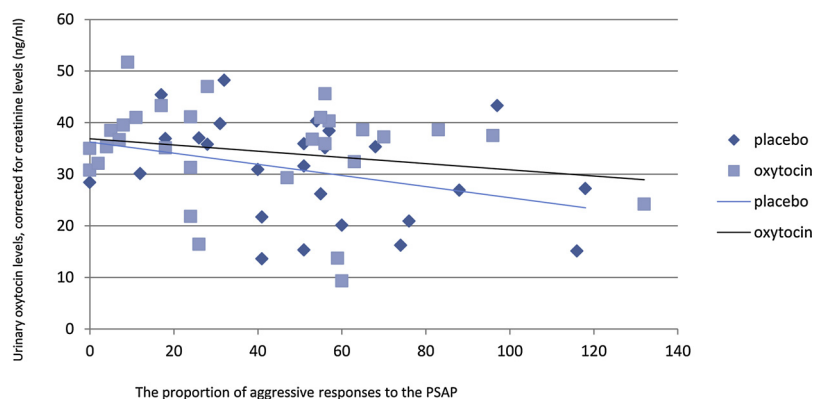


Fig. 4. Association between urinary oxytocin levels and the proportion of aggressive responses to the PSAP for the placebo and oxytocin group, separately.

oxytocin (Declerck et al., 2010). In this study, participants were randomized into two groups: one that interacted for 30 min prior to an interactive trust task, and one that played against an anonymous partner. The effect of oxytocin on trust was significantly higher in the group that had social information about their opponents. The social salience hypothesis postulates that oxytocin might reduce aggressive behavior in a positive supportive social context but has no effect or even an opposite effect when the context is unpredictable or threatening (Ne'eman et al., 2016; Shamay-Tsoory and Abu-Akel, 2015). This suggests that the narrative given to participants prior to the PSAP might influence task-related responses.

We utilized a dosage of 32IU intranasal oxytocin, which has been well demonstrated to have a favorable safety profile (Macdonald and Macdonald, 2010), consistent with our findings. Notably, however, all previous studies utilizing intranasal oxytocin for evaluation of aggressive behavior employed a lower 24IU dose. The difference in dosage might well explain the modest and inconsistent effects of these earlier studies. However, van IJzendoorn et al. showed that a higher dose does not necessarily lead to higher oxytocin levels (van IJzendoorn et al., 2012). We began the PSAP task 75 min following intranasal administration. Gossen et al. (2012) demonstrated that within 90 min after intranasal administration of 26IU oxytocin, plasma levels had returned to baseline. However, we found that 115 min after intranasal administration (post-PSAP), salivary oxytocin levels had already peaked but remained significantly higher in participants receiving oxytocin compared to placebo, consistent with the results of Huffmeijer et al. (2012) and van IJzendoorn et al. (2012). Moreover, cerebrospinal fluid levels of oxytocin rise much slower than plasma levels. Paloyelis et al. (2016) found increased cerebral flow 25–78 min after administration with a peak level after 39–51 min. Striepens et al. (2013) found that it took up to 75 min after intranasal administration of 24IU oxytocin to reach a significant rise of oxytocin levels in the cerebrospinal fluid and they found no correlation with plasma levels of oxytocin. This might explain why earlier studies that performed the PSAP 45 min after administration oxytocin found a more modest or nuanced effect (Ne'eman et al., 2016; Campbell and Hausmann, 2013). Alcorn et al. (2015) performed the PSAP at 30, 90 and 150 min after intranasal administration of oxytocin. However, they did not present the separate analyses for these time points in their paper, although based on the raw data, the difference between the two groups seems to be larger after 150 min. The pharmacokinetics and pharmacodynamics of neurobiologically functional intracerebral delivery of oxytocin following intranasal administration remains poorly understood (Leng and Ludwig, 2016). Furthermore, although cortisol and testosterone levels have been shown to be modified by oxytocin administration (Cardoso et al., 2013; Weisman et al., 2014), we did not observe a significant moderating relationship. However, the effect of oxytocin on cortisol and testosterone levels is often studied in response to conflict situations (Flanagan et al., 2018; Ditzen et al., 2009), during a social stress task

(Linnen et al., 2012; Heinrichs et al., 2003) or father-infant interaction (Weisman et al., 2014). In line with the social salience hypothesis (Shamay-Tsoory and Abu-Akel, 2015), the effect of oxytocin on hormones like cortisol and testosterone might therefore depend on social cues and individual differences.

The mechanism by which oxytocin exerts its effects on brain function remains unknown, as oxytocin does not appear to cross the blood-brain barrier (Churchland and Winkelman, 2012). Measuring oxytocin remains a considerable technical challenge, due to its short half-life (plasma $t_{1/2}$ ~20 min). We performed oxytocin measurements in both saliva and urine. In principle, urinary measurements provide a time-integrated metric of oxytocin exposure. Consequently, urinary oxytocin levels are often poorly correlated with the more temporally dynamic salivary and plasma levels (Amico et al., 1987; Feldman et al., 2011). Accordingly, we found only a very small difference in urinary oxytocin levels between the experimental and placebo group, while salivary levels revealed a considerably larger effect. Urine is pooled over time and the measurement of oxytocin in urine might therefore be more stable and less affected by changes such as oxytocin administration than saliva. Oxytocin levels in urine are influenced by renal glomerular filtration rate (GFR), which can be influenced by a wide variety of variables (Reyes et al., 2014). Moreover, oxytocin itself appears to decrease GFR. Therefore, we have corrected all urinary measurements for creatinine as a standardized correlate of GFR.

Urinary oxytocin levels collected after the PSAP were negatively associated with the proportion of aggressive responses, indicating that more aggressive participants had lower oxytocin levels. This is in line with earlier research by Lee et al., who found a negative correlation between lifetime history of aggression and cerebrospinal levels of oxytocin (Lee et al., 2009), and is consistent with the hypothesis that oxytocin lowers aggressive behavior.

4.1. Strengths and limitations

Our trial design had a number of strengths. We tried to maximize the credibility of the PSAP by a protocolized introduction to the task. Moreover, we obtained credibility ratings after completion of the task and excluded participants if they doubted to play against a real opponent. Our study was performed in a homogenous sample of healthy young adult males with generally low levels of trait aggression.

Among the limitations are that our results are sex-specific since we recruited only men, and therefore our findings cannot be generalized to women without additional experimental confirmation. Compared to earlier studies, our cohort involved healthy young adult males with low baseline levels of aggression (Table 1). The hypothesis that oxytocin might function differently in persons known to exert aggressive and violent behavior is supported by the association of oxytocin receptor genotype with extreme, persistent and pervasive aggressive behaviors in children (Malik et al., 2012) and antisocial behavior in men (Waller

et al., 2016).

Furthermore, we used the PSAP as a well-validated instrument for measuring reactive aggression that has been shown to correlate with life history of aggression and task-related testosterone induction (Geniole et al., 2017). However, it remains unknown to what extent acute pharmacologically-mediated changes in PSAP aggressive behavior of healthy young adult males under standardized laboratory conditions is predictive of clinical efficacy in naturalistic settings. As noted by Walnum et al. (2016) small sample sizes are a recurrent problem in studies that use intranasal administration of oxytocin. Also our sample size is relatively small and due to a possible learning effect for the PSAP, we were not able to use repeated trials. Because of this, it was not possible to correct for a baseline level of aggression or perform a cross-over design, which would have strengthened the design of the study. Although participants were randomized to one of the two intervention groups, it cannot be ruled out that there might be differences in composition of the groups.

4.2. Conclusion

We conclude that intranasal administration of oxytocin reduces aggressive behavior in healthy young adult men. Moreover, higher levels of urinary oxytocin are associated with a lower rate of aggressive responses. Further confirmation will be required to determine the clinical potential of intranasal oxytocin on aggressive behavior.

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