Oxytocin moderates the association between testosterone-cortisol ratio and trustworthiness: A randomized placebo-controlled study

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ABSTRACT

Oxytocin has been proposed to enhance feelings of trust, however, these findings have been difficult to replicate. Environmental or hormonal factors might influence this association. We studied whether oxytocin moderates the association between the testosterone-cortisol ratio, which is associated with risk taking behavior and aggression, and trustworthiness, while controlling for the general level of trust. A randomized double-blind placebo-controlled study with 53 healthy males was performed in which 32IU oxytocin (n = 27) or placebo (n = 26) was administered intranasally. Participants subsequently played the Trust Game in which they were allocated to the role of trustee. In the third phase of the Trust Game, we found a positive association between the testosterone-cortisol-ratio and the proportion of the amount that is returned to the investor (P = <0.01). However, administration of oxytocin reduced reciprocity in those with a high testosterone-cortisol ratio after reciprocity restoration (a significant interaction effect between administration of oxytocin and the testosterone-cortisol ratio in the third phase of the Trust Game, P = 0.015). The third phase of the Trust Game represents the restoration of reciprocity and trustworthiness, after this is violated in the second phase. Therefore, our data suggest that oxytocin might hinder the restoration of trustworthiness and diminish risk-taking behavior when trust is violated, especially in those who are hormonally prone to risk-taking behavior by a high testosterone-cortisol ratio.

1. Introduction

Oxytocin is a neuuropeptide secreted by the posterior pituitary that has been suggested to enhance trustworthiness [1]. It reduces fear responses and enhances the ability to give trust to others, even in situations characterized by the risk of betrayal as measured with the Risk Game [2]. However, subsequent studies were unable to replicate these findings [3-6]. Oxytocin seems not to alter trustworthiness, but rather makes us more likely to conform to the opinions of others [5]. Moreover, it is even found that oxytocin can hinder trust in those who are sensitive to rejection (e.g. patients with a borderline personality disorder, Bartz et al., 2011). According to the social salience theory, these differences might be caused by various biological and environmental factors that interact with oxytocin [7]. For instance, the influence that oxytocin has on behavior may depend on the interaction with other hormones than oxytocin alone. Cortisol and testosterone are two hormones that are, besides oxytocin, associated with trusting behaviors and might therefore interact with oxytocin. However, the terminology that is used to refer to trusting behaviors and closely related terms such as trustworthiness and risk-taking behavior is sometimes confusing.

From the perspective of hormonal effects overlapping terms are used to refer to trust, trustworthiness and risk-taking behavior. Furthermore, risk taking behavior and trust are known to co-vary; perceptions of trust indicate lower levels of risk [8]. Trust is the willingness to accept risk
due to perceived high predictability of positive outcome. Trustworthiness reflects the trustee’s propensity to fulfill those positive expectations. Trustworthiness requires recognizing another individual’s expectations and feeling a sense of responsibility to fulfill those expectations [9]. Risk-taking behavior is the extent to which someone makes choices that puts him/her in a vulnerable situation [10]. Oxytocin is often associated with trust and both perceived [11] and displayed [1] as trustworthy, while it tends to reduce risk-taking behaviors under stressful circumstances, which can be seen as a more defensive reaction [12]. Testosterone decreases trust [13], while it enhances risk-taking behavior [67]. Furthermore, the influence that testosterone has on risk-taking behavior and trust also depends on cortisol levels, because high saliva cortisol levels are associated with less risky behavior and trust, even in individuals with high saliva testosterone levels [14,15]. Therefore, it is likely that testosterone, cortisol and oxytocin influence each other when it comes to trust, trustworthiness and risk-taking behavior.

It is hypothesized that the effect of oxytocin depends on cortisol and testosterone levels and that there might be an interaction between these hormones [16]. Testosterone is an important determinant of aggression, social dominance behavior and risk taking [17]; [66]; [18,19]. However, in the absence of competitive challenges, testosterone seems to enhance trustworthiness [20,21]. Additionally, it has also been found that testosterone increases social vigilance [13,20] by reducing the top-down inhibitory control of the amygdala, resulting in more intense amygdalar responses to signals of untrustworthiness [22]. Testosterone levels decrease after empathy induction, while oxytocin levels increase, although large inter-individual differences are found in these studies [23]. However, previous associations between testosterone and risk taking or trustworthiness have been difficult to replicate [24,25]. Also, a stressful environment is known to have a negative effect on cooperative behaviors, while oxytocin might have a buffering effect on this association [26], although it is also found that stress might contribute to more trustworthy reactions, independently of risk aversion and risk-taking behavior [27]. Cortisol is the end product of the hypothalamic-pituitary-adrenal (HPA) axis and is activated during stress reactions [28]. Considering the highly overlapping functioning of testosterone and cortisol, it is proposed that these hormones should be considered as one composing factor, which led to the formulation of the ‘dual-hormone hypothesis’.

According to the ‘dual-hormone hypothesis’, the influence that testosterone has on behavior such as dominance is disproportionately effectuated in individuals with low saliva cortisol levels, whereas high levels of saliva cortisol can ‘block’ such associations [29–32]. A heightened testosterone-cortisol ratio is associated with physiological stress [33], with anger [34] aggression [35,36], social dominance [32] and risk-taking behavior [15,37]. People with high testosterone-cortisol ratios tend to have less loss of aversion [38]. On the other hand, a low testosterone-cortisol ratio is associated with more pro-environmental behavior [39]. Overall, a high testosterone-cortisol ratio seems to be associated with competitive behavior and can rapidly change during stressfull circumstances, which implicates that there might be an alternative route to alter these levels as the activation via the hypothalamic-pituitary-adrenal (HPA) axis or the hypothalamic-pituitary-gonadal (HPG) axis is relatively slow [29]. Presumably the central effect of the testosterone-cortisol ratio is a consequence of suppression of cortical and subcortical neural communications that control social behavior [40].

Taken together, these data suggest that the positive effect of oxytocin on trustworthiness might be neutralized by a high testosterone-cortisol ratio. However, when a trusting relation is violated, a high testosterone-cortisol ratio might contribute to restoring trustworthiness, which might actually be a variation on risk-taking behavior, while oxytocin might contribute to a more defensive and risk averse reaction. Based on this, we hypothesized that there is an interaction between the testosterone-cortisol ratio and oxytocin on reciprocity on the trust game and thereby on trustworthiness, in which oxytocin might nullify the positive effect that the testosterone-cortisol ratio might have on trustworthiness.

To further substantiate these associations, we performed a randomized, double-blind placebo-controlled study, in which oxytocin or placebo was administered intranasally to a group of healthy young men who then performed a laboratory task to measure levels of trust, The Trust Game [41]. In this version of the Trust Game, 12 rounds of investments are made by a fictive investor which is tripled and sent to the participant, who can return an amount of money, which is seen as a measure of reciprocity. Three phases are distinguished: a phase in which reciprocity is established (first 5 rounds), a phase in which reciprocity is violated (second 6 rounds) and a phase in which reciprocity is restored (third 3 rounds). Reciprocity on the Trust Game is seen as a measure for trustworthiness [42]. As both participants in the Trust Game are totally unknown to each other, we conceive the effect of the testosterone-cortisol ratio on the Trust Game in the first place an effect of trustworthiness, competitive behavior and risk-taking behavior rather than dominance or status-seeking behavior. We controlled for baseline levels of trust, using the Trust Inventory [43]. Baseline saliva testosterone and cortisol levels were obtained prior to oxytocin or placebo administration.

2. Methods

2.1. Participants

Participants were recruited by means of local advertisements. In this study, only male participants were included. Subjects were screened for eligibility by phone to exclude subjects younger than 18 years, older than 35 years, or having any current psychiatric, neurologic or somatic disorder. In addition, participants with a past history of substance abuse or dependence, allergic rhinitis, smoking more than ten cigarettes a day, or trauma resulting in an acute stress disorder were also excluded. Analyses included 53 participants (n = 27 oxytocin, n = 26 placebo).

2.2. Nasal spray administration

Each spray of oxytocin provided a 4IU dose in normal saline. Participants sprayed 4 dosages in each nostril (0.1 mL per spray), with a total dose of 32IU per subject (0.8 mL). We selected a dosage of 32IU as doses in the range between 18 and 40IU are not associated with adverse outcomes [44]; the effect might be dose-dependent [45] with possible higher effectiveness in doses >24IU [46]. The placebo condition utilized the identical model of nasal spray device, volume per spray, number of administered sprays, and normal saline vehicle. Nasal spray devices were housed in identical blinded vials that could not be distinguished without unsealing the casing. Participants and researchers were blinded to the condition. An independent statistician provided a computer-generalized randomisation list prior to the start of the study. Each participant was randomly assigned to their condition based on a X-subject blocked design. The study-pharmacist was the only one in possession of the randomisation list until the study was officially finalized.

2.3. The trust game

In the trust game, there are two roles: ‘trustee’ and ‘investor’ [41]. We used a revised version of the Trust Game published by Ref. [47]. Participants were told that they would be randomly assigned as either trustee or investor. However, unknown to the participants, all were assigned as trustee, whereas the investor was a pre-programmed computerised set of 14 responses. The computerised investor begins with an initial amount of 10 monetary units (MU) in each round (Fig. 1). Part of this amount is sent to the trustee and the remainder is kept in the investor’s amount. The amount that is sent to the trustee is the
To correct for trait levels of trust in our statistical analyses, we used the scores on the generalized trust scale. The Trust Inventory [43] was used to measure trust to specific others and general trust. It consists of 32 items with 2 subscales: generalized trust and partner trust. To determine whether they were convinced of playing against another person, participants were systematically questioned about the game and their opponent to determine whether they were convinced of playing against another person.

2.4. Trust inventory

The Trust Inventory [43] was used to measure trust to specific others and general trust. It consists of 32 items with 2 subscales: generalized trust and partner trust. To correct for trait levels of trust in our statistical analyses, we used the scores on the generalized trust scale.

2.5. The neuroticism-extraversion-openness five factor inventory (NEO_FFI)

The NEO-FFI [48] was used to describe the personality traits of the study population. The NEO-FFI is an personality inventory to measure the five most important factors in personality: neuroticism, extraversion, openness to experience, agreeableness and conscientiousness.

2.6. Cortisol and testosterone measurements

Saliva samples were collected using Sarstedt cortisol salivettes. Samples were stored at −20 °C until they were analysed. Free salivary cortisol levels were quantified using a commercially available ELISA (Demeditec Diagnostics, Kiel, Germany). The limit of detection was 0.276 nmol/l. Inter- and intra-assay coefficients of variation were <10% and <7%, respectively. Free salivary testosterone levels were analysed with a commercially available ELISA (DRG Diagnostics, Marburg, Germany). The limit of detection was 34.7 pmol/l. The inter- and intra-assay coefficients of variation were <10% and <15%, respectively.

2.7. Procedure

This study was approved by the Medical Ethical Committee of the Erasmus Medical Center Rotterdam within the guidelines of the Declaration of Helsinki and the European Medicines Agency Guidelines for Good Clinical Practice. It was registered in the European trial register EudraCT (2012-002651-41, 40844) and Dutch trial register CCMO (NL40844.078.12). Individuals who passed the phone screening regarding the inclusion and exclusion criteria were invited to the laboratory. All participants provided written informed consent after the protocol was explained to them both orally and in writing. Participants were asked to refrain from any physical exercise before coming to the laboratory and not to drink (other than water), eat or smoke 1 h before arrival. Participants came to the laboratory at 13:00 where a medical doctor reconfirmed the inclusion- and exclusion criteria. After that, the baseline questionnaires (Trust Inventory, NEO-FFI) were completed and saliva samples for the assay of cortisol and testosterone levels were obtained. Next, the participants were introduced to their fictional opponent of the Trust Game, with whom they could briefly interact. Participants were instructed using a standardized explanation and one round of the Trust Game was played as a practice. Subsequently, the participants self-administered the blinded nasal spray (4 sprays in each nostril). After 45 min, the Trust Game commenced. During the waiting period, the participants were permitted to read quietly, but not consume anything other than water, nor engage in any physical or psychologically intensive activity. At the conclusion of the study session, participants were debriefed and screened for potential adverse events. All subjects received €30 for their participation.

2.8. Statistical analysis

Participants who were not convinced of playing against another person were excluded from further analyses. Following the second phase 'betrayal' of the computerised investor, responses in the third phase were considered as the primary outcome measure for trustworthiness. Descriptive statistics were used to summarize demographic and clinical
characteristics. Generalized linear models were fitted to examine the relationship between oxytocin administration and responses in the Trust Game, as well as to evaluate a potential moderating effect of testosterone-cortisol ratio. Since the number of MU returned is proportional to the investment, we used a Poisson regression to model the outcome including an offset for the log-transformed maximum amount that could be transferred. The general trust scale of the Trust Inventory (grand mean centered) was used to correct for baseline levels of trust. Model fit was evaluated graphically and the deviance goodness-of-fit test was applied for model comparison. Type I error rate was set at the conventional 5% (two-sided). Analyses were implemented using SPSS (v24.0, IBM).

3. Results

Sixty-nine (n = 69) participants were included in the study. Sixteen (n = 16) participants were excluded because: a) they reported not believing they were playing against another person (n = 12), or b) hormonal data was unavailable (n = 4). Therefore, analyses included 53 participants (n = 27 oxytocin, n = 26 placebo). Participants scored relatively high on extraversion, openness, agreeableness and conscientiousness and relatively low on neuroticism, which is comparable with earlier research in healthy young males [49]. Descriptive characteristics and Trust Game mean response ratios (relative to investment) are shown in Table 1.

No difference was found between the oxytocin and placebo group on the proportion of MU returned to the investor in the first phase of the Trust Game (β = -0.077, 95% CI [-0.215 – 0.061] P = 0.275) and the second phase (β = -0.217, 95% CI [-0.594 – 0.160] P = 0.260), also not when the testosterone-cortisol ratio was added to the model (β = 0.153, 95% CI [-0.129 – 0.435] P = 0.288 and β = 0.223, 95% CI [-0.673 – 1.119] P = 0.626 in resp. the first and second phase). In the third phase, significantly less trustworthy reactions were found in the oxytocin group (β = -0.188, 95% CI [-0.367 to -0.010] P = 0.039) (Fig. 2).

Poisson regression modelling revealed a statistically significant difference between oxytocin and placebo in the association between testosterone-cortisol ratio and trustworthiness as measured by responses in the third phase of the Trust Game (Model fit χ² = 5.876, df = 1, P = 0.015). Fig. 3 displays the proportion of MU returned by the trustee to the computerised investor, controlling for differences in baseline measures of general trust. Within the placebo group, the proportion of MU returned was increased among respondents with a higher testosterone-cortisol-ratio (β = 0.016, 95% CI [0.004–0.027] P = 0.006), a relationship that was attenuated in the oxytocin-group (β = -0.013, 95% CI [0.026–0.001], P = 0.07).

Table 1

| Placebo (N = 26) | Oxytocin (N = 27) |
|-----------------|------------------|
| Age (years)     | 21.6 (2.9)       | 22.0 (1.9) |
| Length (meters) | 1.83 (0.08)      | 1.84 (0.07) |
| Weight (kg)     | 80.4 (11.7)      | 76.5 (8.9) |
| Cortisol (nmol/L) | 16.6 (7.9)      | 16.8 (8.3) |
| Testosterone (nmol/L) | 410.3 (151.5) | 387.2 (119.8) |
| Testosterone-cortisol ratio | 27.0 (9.9) | 28.3 (16.4) |
| Trust Inventory (general trust) | 81.1 (10.5) | 80.3 (12.3) |
| NEO neuroticism | 25.3 (7.0)       | 27.5 (7.9) |
| NEO extraversion | 45.2 (6.3)       | 45.2 (7.2) |
| NEO openness    | 40.1 (6.2)       | 40.6 (6.4) |
| NEO agreeableness | 41.7 (6.9)      | 43.9 (5.7) |
| NEO conscientiousness | 42.7 (8.7)   | 44.7 (8.3) |
| TG (first phase: round 1 t/m 5) | 28.9 (8.5) | 26.7 (7.3) |
| TG (second phase: round 6 t/m 11) | 10.0 (1.3) | 8.0 (6.1) |
| TG (third phase: round 12 t/m 14) | 18.9 (6.7) | 15.5 (4.8) |

4. Discussion

In this study, we found higher levels of reciprocity in the placebo group, but only in the third phase of the Trust Game, in which the cooperation is restored after ‘betrayal’ in the second phase of the task. Furthermore, in this phase of the Trust Game, we found a significant interaction between the testosterone-cortisol ratio and the administration of oxytocin on reciprocity. This effect of oxytocin on reciprocity in those with a high testosterone-cortisol ratio implicates that oxytocin might have an negative effect on trustworthiness in those with a high testosterone-cortisol ratio.

During the last decade, the initial enthusiasm about the potential association between trust, trustworthiness and oxytocin was nuanced and it turned out that the finding that oxytocin enhances trust was difficult to replicate [6]. More recent studies suggest a nuanced view, in which oxytocin does not directly enhance trust and trustworthiness, but rather improves the accuracy of trustworthiness judgment of others [50] and promotes social learning [51]. Moreover, the effect of oxytocin on human behavior tends to depend on factors such as personality traits, situational factors and other hormones.

Because there is increasing evidence that other internal and external factors interact with the effect of oxytocin on behavior and thereby possibly causing the divergent results in different studies, we tested the effect of the testosterone-cortisol ratio on its association with oxytocin. Although it is known that oxytocin interacts with cortisol and testosterone, few prior studies have focused on the interplay between these hormones and their effects on social behavior [16]. We found that a higher testosterone-cortisol ratio was associated with more reciprocity during the Trust Game after betrayal. Intranasal administration of 32IU oxytocin significantly attenuated this association. Earlier studies showed that high reciprocity on the Trust Game by the trustee is associated with trustworthiness [41] although one might doubt whether the third phase of our version of the Trust Game resembles trustworthiness as other psychological factors such as taking revenge or fear for losing money interfere with this. Our results indicate that participants with a high testosterone-cortisol ratio show more reciprocity after betrayal but that this effect is neutralized by administration of oxytocin. This underlines the theory that oxytocin has an opposite effect to the testosterone-cortisol ratio regarding the effect on reciprocity, trustworthiness and risk-taking behavior.

The largest difference between the oxytocin and placebo group was found in the third phase of the Trust Game. This was the phase in which the computerised investor resumed a higher level of cooperation, following the second ‘betrayal’ phase in which the trustee received only small investments compared to the initial phase. Although it has been suggested that oxytocin may reduce betrayal aversion [2,52], females receiving oxytocin had significantly reduced trust repair following betrayal, but this effect was not found in males, such as included in our study [53]. Converging evidence shows that oxytocin does not appear to have solitary pro-social effects, but that the effect of oxytocin on human behavior is far more nuanced and also depends on environmental factors and personality traits (Bartz et al., 2011; [7]). It is proposed that oxytocin might increase the desire to inflict interpersonal punishment [65], possibly strengthened by the feeling to be betrayed by someone who was initially seen as someone who cooperated. This protective behavior by ‘punishment’ might be accentuated after oxytocin administration in this Trust Game. Oxytocin is known to contribute to creating and enforcing in-group norms of cooperation and sanctioning when cooperation is violated [54].

Trustworthiness and risk taking are highly interrelated concepts [55], in which trustworthiness can be seen as a pre-requisite for trust and risk-taking [10]. Risk aversion is predictive of more reserved responses during the Trust Game [56] and it is proposed that the Trust Game not solely measures levels of trust and trustworthiness, but also risk-taking behavior and risk aversion [55]. From this perspective, responses during the Trust Game can be seen as an index of risk taking.
betrayal. Together with a low stress level and thereby possibly less fear measure. Indeed does testosterone promote reciprocity [20], even after way, the transferred amount of money can rather be seen as a reciprocity and the way one mentalizes the behavior of the opponent. In this way, the transferred amount of money can rather be seen as a reciprocity measure. Indeed does testosterone promote reciprocity [20], even after betrayal. Together with a low stress level and thereby possibly less fear to lose money, reflected in low cortisol levels with less fear to lose money, this might result the willingness to transfer more money back to the investor.

The social component in the Trust Game is of great importance for the effect that oxytocin has on trusting behavior [52]: found that oxytocin has no effect when the social element is let out the experiment, while a more recent large replication study found no main effect and a possible small effect in those who had no social contact prior to the experiment [4]. This implied for us that the way that participants interact before the test might be of great importance. Because this is not standardized in most experiments, it might contribute to the different outcomes on the Trust Game.

Our findings may be particularly relevant for people among whom difficulties with trustworthiness result in problems of daily life functioning, including those with borderline or antisocial personality disorder. Developmental deficits in trusting others and being trustworthy have been associated with aggressive behavior. Children that show aggressive behavior from an early age tend to be perceived as untrustworthy by their siblings [57]. Moreover, trustworthiness is related to maladaptive outcomes, including externalizing behavior [58]. These results suggest a causal role for trusting behavior, and particularly trustworthiness, in the development of aggressive behavior.

Though, in patients with a borderline personality disorder, the administration of oxytocin can have an opposite effect, as it hinders trust and cooperation [64]. Also, in forensic psychiatric patients, psychopathic traits are correlated with high urinary oxytocin levels (Mitchell et al., 2013). This is remarkable as psychopathic traits are associated with reduced trustworthiness in social decision-making [59]. These results implicate that the association between oxytocin and trustworthiness is more complex than originally thought and therefore provides further evidence for the social salience theory in which the effect of oxytocin depends on a variety of contextual aspects and individual characteristics. In this study, we propose that one of these biological factors is the testosterone-cortisol ratio, based on the significant association we found between the administration of oxytocin versus placebo and the testosterone-cortisol ratio on trustworthiness.

4.1. Limitations of the study

Our study has limitations that should be considered. First, oxytocin might have a more prominent effect in those with higher levels of anxiety, due to a differential benefit from the anxiolytic effect of oxytocin [60]. Oxytocin has been found to decrease salivary cortisol levels and it exerts an anxiolytic effect [16]. In our analyses, we did not control for the state anxiety level.

Second, we investigated a relatively small sample (N = 53). Two recent meta-analyses on the dual-hormone theory emphasized the need for larger samples [61,62]. According to the dual-hormone theory effects might be subtle and, therefore, require larger sample sizes for sufficient statistical power. Furthermore, the effects of oxytocin on behavior are highly debated as they are often based on underpowered studies [63]. Third, our sample consisted exclusively of young adult males and can therefore not be extrapolated to females. A previous study showed that the effect of oxytocin following betrayal on the Trust Game might be gender specific [53]. Fourth, we quantified baseline levels of trust using a questionnaire (Trust Inventory), independent of the Trust Game. It would have been preferable to perform the Trust Game multiple times to establish a robust baseline measure and employ a
cross-over design to obtain more statistical power. However, we decided against a cross-over study design due to the potential for test-retest learning effects. Furthermore, the effect of betrayal and the restoration of trust might be further clarified in a modified version of the Trust Game in which a condition is added where no change in investments is made. In this way, participants could be randomized in a group that is betrayed and a group that is not betrayed.

4.2. Strengths of the study

For the reliability of the Trust Game, it is important that participants believe to play against another person. Our findings suggest that the effect of oxytocin during the Trust Game selectively occurs within the script. We have not published these data previously. None of the authors declared a personal or financial interest that could be perceived as affecting the interpretation of the results.

Declaration of interest statement

personality disorder. Particularly interesting for patients where trust and trustworthiness those with a high testosterone-cortisol ratio. These findings might be Furthermore, administration of oxytocin reduces trustworthiness in testosterone-cortisol ratio is associated with trustworthiness in men. This research was funded by Fivoor, the Erasmus MC and the Koningsheide foundation (P2013/485). A. Henry, J.R. Sattizahn, G.J. Norman, S.L. Beilock, D. Maestripieri, Performance

5. Conclusion

In summary, our findings demonstrate that following betrayal, the testosterone-cortisol ratio is associated with trustworthiness in men. Furthermore, administration of oxytocin reduces trustworthiness in those with a high testosterone-cortisol ratio. These findings might be particularly interesting for patients where trust and trustworthiness form recurrent problems in their daily life and for those with deviant testosterone and cortisol levels, such as patients with an antisocial personality disorder.

Declaration of interest statement

This research was funded by Fivoor, the Erasmus MC and the Koningsheide foundation (P2013/485). All of the authors have carefully reviewed and approved the manuscript. We script. We have not published these data previously. None of the authors reports any competing financial interest or conflict with the research described in the manuscript.

Declaration of competing interest

None.

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