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Article

Salivary Oxytocin Has Nonlinear Relationships with Trust and Reciprocity

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Abstract: Oxytocin has been proposed to regulate human trust. Previous experiments supported this claim by demonstrating that exogenous and endogenous oxytocin is associated with trust (how much trust people place in strangers) and reciprocity (how much people reciprocate when trusted). However, recent replication attempts have been unsuccessful in demonstrating the trust-enhancing effect of oxytocin, and there is limited evidence on whether oxytocin is associated with reciprocity. This study aimed to replicate the previously found nonlinear relationships between the endogenous oxytocin concentration and both trust and reciprocity by utilizing a monetarily incentivized trust game. In a college sample, we found that salivary oxytocin levels showed (i) an inverted U-shaped relationship with trust in men and (ii) a U-shaped relationship with reciprocity in women. The current results confirm the previous finding that endogenous oxytocin levels have nonlinear relationships with trust and reciprocity. Further research on the role of oxytocin secretion in trust and reciprocity is warranted.

Keywords: oxytocin; trust; reciprocity; trust game

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1. Introduction

It is widely accepted that oxytocin, a peptide hormone produced in the hypothalamus, has an essential role in mating and reproduction in human and nonhuman species [1,2]. Over the past few decades, researchers have begun to investigate whether oxytocin plays a crucial role in psychology outside these domains. In humans, experimental evidence suggests that oxytocin also promotes trust (i.e., how much trust people place in strangers) and reciprocity (i.e., how much people reciprocally cooperate when trusted) toward others [3–5]. For instance, experimental studies examining the effects of oxytocin administration demonstrated that, compared to placebo controls, those who were administered oxytocin became more trusting: They entrusted more money to strangers [6] and judged unfamiliar others as more trustworthy [7]. Moreover, endogenous oxytocin secretion has also been shown to be associated with trust and reciprocity [8].

However, recent replication attempts have been unsuccessful in validating the trust-enhancing effect of oxytocin. A meta-analysis of oxytocin administration experiments failed to show a robust effect of oxytocin on trust [9], and endogenous oxytocin levels were shown to have no association with behavioral or attitudinal trust [5,10,11]. Similarly, there has been limited evidence on whether oxytocin is associated with reciprocal behaviors [5,8,12]. Therefore, the current literature requires researchers to carefully replicate and re-examine the previously reported associations between oxytocin and trust, as well as reciprocity.

The goal of this study was twofold: to test whether the results of previous research on the associations between oxytocin and trust and reciprocity could be replicated. We chose to replicate the study in which plasma oxytocin showed U-shaped, nonlinear associations with trust and reciprocity, as measured by a trust game (Zhong et al. [8]). We tested the association between oxytocin, trust, and reciprocity using methods comparable to those in the comparison study [8], except that we measured salivary oxytocin rather than plasma. We decided to measure oxytocin from saliva for two reasons: (i) compared to plasma sampling, saliva sampling is far less invasive and thus less likely to induce stress, which itself can interfere with oxytocin levels [13,14], and (ii) for its convenience, saliva sampling is becoming increasingly popular in the field, and using it makes this study easily comparable to other studies for future replication [15].

2. Results

2.1. Trust

On average, participants sent 48.8% (*SD* = 32.6%, range = 0–100%) of the endowment to their partner (DV 1: Trust; Figure 1a). Because trust was not normally distributed ($W = 0.91$, $p < 0.0001$), nonparametric tests were performed in the following analyses. First, a one-sample Wilcoxon Signed-Rank test indicated that the level of trust obtained in this study was significantly lower than that of the comparison study (Zhong et al. [8]) (55.5%, $V = 2766$, $p = 0.008$). Second, the Mann–Whitney U test showed no gender differences in trust (men: $M = 54.5\%$, $SD = 33.6\%$; women: $M = 42.5\%$, $SD = 30.6\%$; $z = 1.83$, $p = 0.067$). Third, a correlation analysis using Spearman's rank correlation coefficient showed that trust was not correlated with age ($r_{s\ (121)} = -.10$, $p = 0.293$).

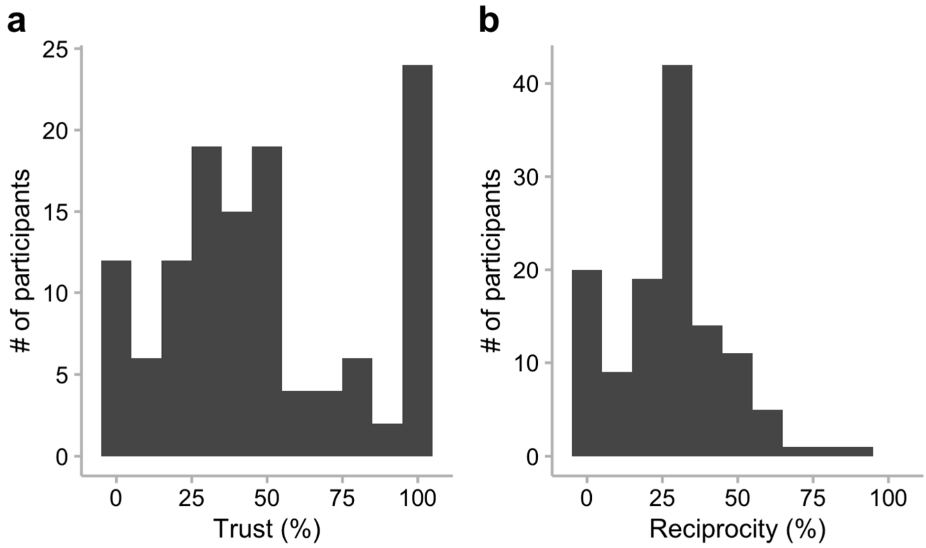

Figure 1. Histograms of (**a**) trust and (**b**) reciprocity.

2.2. Reciprocity

The mean return rate of money given by the first player (DV 2: Reciprocity; Figure 1b) was 27.9% (*SD* = 18.2%, range = 0–91%). Because reciprocity was not normally distributed ($W = 0.95$, $p = 0.0003$), nonparametric tests were used in the following analyses. The level of reciprocity reported in this study was significantly lower than that found by Zhong et al. [8] (33.3%, $V = 2381$, $p = 0.0003$). Reciprocity was not correlated with age ($r_{s\ (121)} = -0.12$, $p = 0.176$), and no gender difference was found (men: $M = 28.5\%$, $SD = 20.2\%$; women: $M = 27.2\%$, $SD = 15.9\%$; $z = 0.17$ $p = 0.863$).

2.3. Salivary Oxytocin Levels

The mean salivary oxytocin level was 55.8 pg/mL (*SD* = 24.1). Because salivary oxytocin levels were not normally distributed ($W = 0.82$, $p < 0.0001$), we log-transformed them. Salivary oxytocin did not correlate with age ($r_{(121)} = -0.03$, $p = 0.764$) or differ by gender (men: $M = 4.0$, $SD = 0.4$; women: $M = 3.9$, $SD = 0.4$; $t_{(121)} = 0.49$, $p = 0.626$) (before log-transformation: men: $M = 56.8$, $SD = 27.4$; women: $M = 54.7$, $SD = 19.9$).

2.4. Relationship between Salivary Oxytocin Levels and Trust

The linear regression analysis for trust showed no effect of oxytocin ($b = 3.38$, $SE = 7.38$, $p = 0.648$). The analysis by gender also showed no effect of oxytocin (men: $b = -4.64$, $SE = 11.66$, $p = 0.692$; women: $b = 9.47$, $SE = 9.33$, $p = 0.315$). However, a nonlinear regression analysis showed a nonlinear effect of oxytocin on trust in men (oxytocin: $b = 8.47$, $SE = 9.75$, $p = 0.389$; oxytocin squared: $b = -36.61$, $SE = 9.28$, $p = 0.0002$) but no effect of oxytocin overall or in women (see Table 1). Figure 2a shows an inverted U-shaped association between trust and oxytocin levels in men.

2.5. Relationship between Salivary Oxytocin Levels and Reciprocity

The linear regression analysis for reciprocity showed no overall effect of oxytocin ($b = -1.23$, $SE = 4.01$, $p = 0.760$) or in men ($b = -9.00$, $SE = 6.33$, $p = 0.160$) or women ($b = 5.83$, $SE = 4.02$, $p = 0.153$). However, the nonlinear regression analysis showed linear and nonlinear effects of oxytocin on reciprocity in women (oxytocin: $b = 9.55$, $SE = 4.65$, $p = 0.045$; oxytocin squared: $b = 12.34$, $SE = 5.43$, $p = 0.027$) but no effect of oxytocin overall or in men (see Table 2). A U-shaped association between reciprocity and oxytocin levels in women is shown in Figure 3b.

Table 1. Results of linear and nonlinear regressions of oxytocin concentrations on trust.

			95% CI			
Explanatory Variables	***b***	***SE***	**LL**	**UL**	***t***	***p***
All (N = 123)						
Model 1 ($R^2 = -0.007$, $p = 0.648$)						
Intercept	48.78	2.95	42.94	54.63	16.5	<0.0001
Oxytocin	3.38	7.38	−11.24	17.99	0.5	0.648
Model 2 ($R^2 = 0.003$, $p = 0.141$)						
Intercept	51.06	3.39	44.34	57.78	15.0	<0.0001
Oxytocin	3.60	6.43	−9.13	16.33	0.6	0.577
Oxytocin (square)	−15.67	8.11	−31.73	0.39	−1.9	0.056
Men (n = 64)						
Model 1 ($R^2 = -0.013$, $p = 0.692$)						
Intercept	54.61	4.23	46.15	63.07	12.9	<0.0001
Oxytocin	−4.64	11.66	−27.93	18.66	−0.4	0.692
Model 2 ($R^2 = 0.049$, $p < 0.0001$)						
Intercept	59.32	4.77	49.79	68.86	12.4	<0.0001
Oxytocin	8.47	9.75	−11.02	27.96	0.87	0.389
Oxytocin (square)	−36.61	9.28	−55.18	−18.05	−3.94	0.0002
Women (n = 59)						
Model 1 ($R^2 = -0.002$, $p = .315$)						
Intercept	42.71	4.02	34.67	50.75	10.64	<0.0001
Oxytocin	9.47	9.33	−9.22	28.15	1.02	0.315
Model 2 ($R^2 = -0.002$, $p = 0.365$)						
Intercept	40.07	4.47	31.12	49.02	8.97	<0.0001
Oxytocin	14.68	11.60	−8.57	37.92	1.27	0.211
Oxytocin (square)	17.30	12.87	−8.48	43.08	1.34	0.184

CI = confidence interval; LL = lower limit; UL = upper limit.

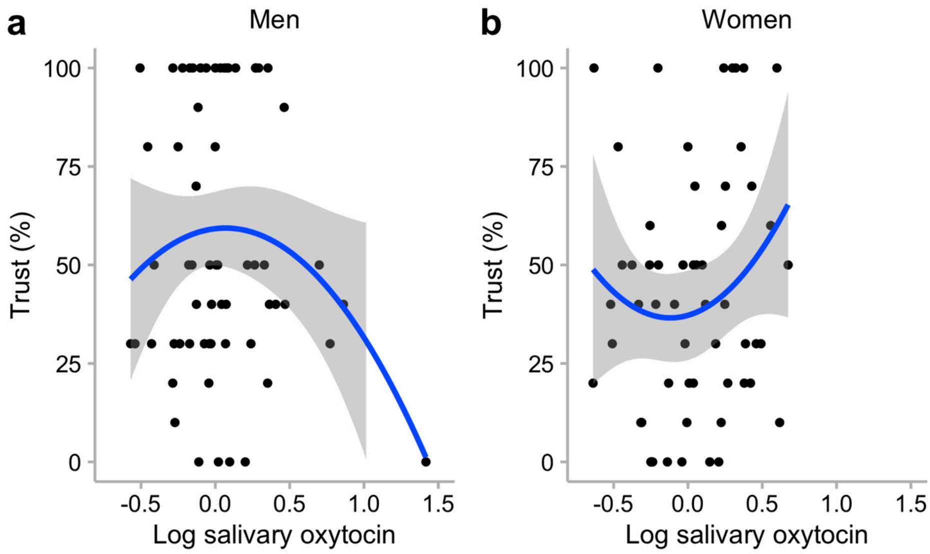

Figure 2. The relationship between salivary oxytocin (log-transformed) and trust in (**a**) men and (**b**) women.

Table 2. Results of linear and nonlinear regressions of oxytocin concentrations on reciprocity.

			95%			
Explanatory Variables	***b***	***SE***	**LL**	**UL**	***t***	***p***
All (*N* = 123)						
Model 1 ($R^2 = -0.008$, $p = 0.760$)						
Intercept	27.85	1.65	24.59	31.11	16.9	< 0.0001
Oxytocin	−1.23	4.01	−9.16	6.71	−0.3	0.760
Model 2 ($R^2 = -0.011$, $p = 0.622$)						
Intercept	28.54	1.89	24.79	32.28	15.1	< 0.0001
Oxytocin	−1.16	3.74	−8.56	6.25	−0.3	0.757
Oxytocin (square)	−4.69	4.80	−14.19	4.82	−1.0	0.331
Men (*n* = 64)						
Model 1 ($R^2 = 0.011$, $p = 0.160$)						
Intercept	28.60	2.54	23.53	33.67	11.3	< 0.0001
Oxytocin	−9.00	6.33	−21.65	3.65	−1.4	0.160
Model 2 ($R^2 = 0.009$, $p = 0.018$)						
Intercept	29.79	2.71	24.38	35.21	11.0	< 0.0001
Oxytocin	−5.68	7.16	−20.01	8.64	−0.8	0.431
Oxytocin (square)	−9.27	6.35	−21.97	3.43	−1.5	0.150
Women (*n* = 59)						
Model 1 ($R^2 = 0.004$, $p = 0.153$)						
Intercept	27.31	2.05	23.20	31.41	13.3	< 0.0001
Oxytocin	5.83	4.02	−2.22	13.88	1.5	0.153
Model 2 ($R^2 = 0.021$, $p = 0.070$)						
Intercept	25.43	2.62	20.18	30.67	9.7	< 0.0001
Oxytocin	9.55	4.65	0.23	18.86	2.1	0.045
Oxytocin (square)	12.34	5.43	1.45	23.22	2.3	0.027

CI = confidence interval; LL = lower limit; UL = upper limit.

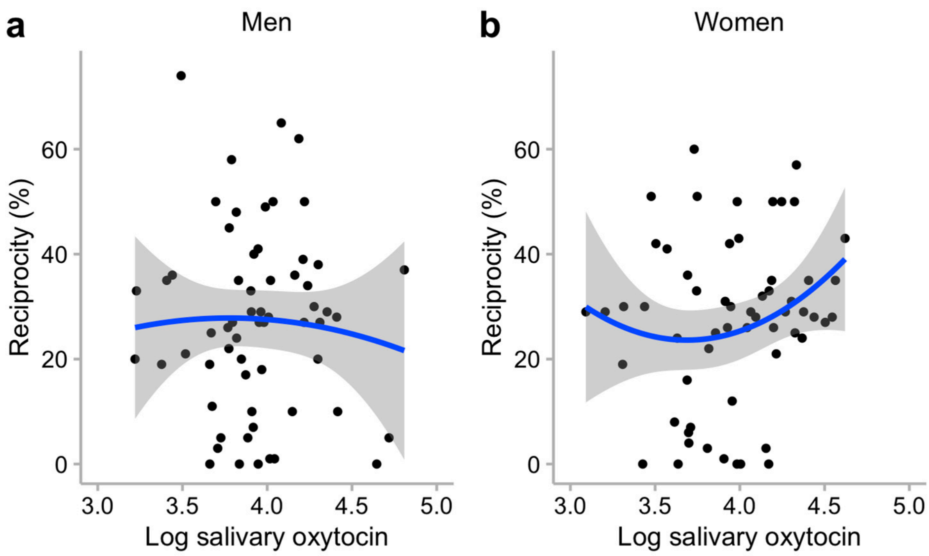

Figure 3. The relationship between salivary oxytocin (log-transformed) and reciprocity in (**a**) men and (**b**) women.

3. Discussion

The goal of the present study was twofold. First, we aimed to use oxytocin concentrations measured in saliva to re-examine the nonlinear association between trust and oxytocin, as shown in a previous study [8]. Our results replicated the nonlinear relationship between trust and oxytocin concentration in men. However, in the comparison study [8], the relationship was U-shaped, whereas we observed an inverted U-shaped relationship. Our study used the same methodology as the comparison study [8] (e.g., testing participants with similar demographic, socioeconomic, and cultural characteristics and applying the same statistical methods). Therefore, the inverse nonlinear relationship found in the current study may stem from the differences in the samples from which the oxytocin was measured: plasma (comparison study) vs. saliva (our study). So far, the origin of oxytocin in saliva remains unclear, and no correlation has been shown between salivary and plasma oxytocin levels after intravenous administration [16,17], indicating that oxytocin does not transfer from the blood to the saliva. Recent studies also found no or weak associations between baseline oxytocin levels in the blood and saliva [15–18]. Further studies are needed to determine how salivary and plasma oxytocin are associated and how they differ.

Our study demonstrated a nonlinear relationship between trust and oxytocin levels, which is consistent with previous studies [8,19]. These patterns may help us understand the mixed findings for the relationship between trust and oxytocin. The inverted U-shaped relationship in our study suggests that excessively high concentrations of oxytocin may also be associated with low trust, especially in men, which may explain why the intranasal administration of oxytocin, which raises central oxytocin levels [20], does not always enhance trust behaviors [9,21].

Second, we aimed to re-examine whether a nonlinear relationship existed between reciprocity and oxytocin concentration, as shown in the same comparison study [8]. Our results did not replicate the previous finding: Reciprocity was associated with salivary oxytocin concentration only in women. Moreover, unlike trust, a U-shaped association was found between oxytocin and reciprocity. As in our study, a reversed relationship based on gender has been observed in many studies dealing with oxytocin. For instance, the associations between (a) salivary oxytocin concentration and altruism [22] and (b) oxytocin receptor gene polymorphisms and amygdala volume [23] are reversed in men and women. These patterns may be explained by sexually differentiated oxytocin release, which is not yet fully understood [24].

Potentially, sex-specific adaptive problems may have selected for sexually dimorphic pathways of oxytocin in the brain regions that are related to social behaviors, including trust and reciprocity. For example, given that oxytocin facilitates childbirth, lactation, and maternal care in mammalian females [1,25], only in women may oxytocin coordinate social behaviors to establish bonds with partners and alloparents, which can help them meet the increased maternal energetic burden. It is possible that sex-dependent oxytocin effects nullify or even reverse the sex-independent effects of oxytocin to promote trust and reciprocity, resulting in differential patterns in men and women. To further elucidate the effects of oxytocin on prosocial behaviors, other well-established roles of oxytocin in mating and reproduction should be considered in future research [2].

As a replication, our study differed in several respects from the comparison study (Zhong et al. [8]). First, our sample size (N = 123) was smaller than that of the comparison study (N = 1158). Nonetheless, our study replicated the non-linear associations found in the comparison study, which indicates that the previous significant associations were unlikely type I errors due to the large sample size. Second, unlike the comparison study, we measured oxytocin from saliva rather than plasma. Although this disparity made it hard to compare our results with those of the comparison study, we opted for salivary measurement to encourage future replications by other researchers: Salivary oxytocin measurement is less invasive, less stress-inducing, and more convenient than plasma measurement [13–15]. Third, while our sample population (Japanese undergraduates in Tokyo) may be demographically similar to that of the comparison study (Han Chinese undergraduates in Singapore), very few studies have analyzed differences in prosocial behaviors between these societies (e.g., [26]). Even though these populations are typically deemed to belong to the same culture (e.g., "Asia", "Confucian" [27]), differences among them should be carefully investigated in the future. Overall, the current replication provides support for further investigation into the nonlinear, potentially sex-dependent relationships between oxytocin and trust, as well as reciprocity.

Oxytocin was shown to be associated with trust almost 20 years ago [6]. To date, many studies have shown that this relationship is questionable [9,21], and the debate continues as to the exact nature of the relationship. This may be because trust is not a one-dimensional concept, and neither is reciprocity. Trust and reciprocity, as measured in trust games, are complex combinations of motives, including self-interest maximization, unfairness aversion, betrayal aversion, reciprocity, reputation concern, and motivations to initiate a cooperative relationship. Recent research has begun to conceptually distinguish the internal components of trust and examine which are associated with oxytocin [11]. To determine whether oxytocin plays a key role in regulating trust and reciprocity, future research should consider examining the relationships between oxytocin and different components of these concepts separately, rather than "trust" or "reciprocity" as a whole.

4. Materials and Methods

4.1. Participants

One hundred and twenty-six Japanese undergraduates (48% women, M_{age} = 19.8, SD_{age} = 1.1) at Tamagawa University in Tokyo participated in this study. Our participants were demographically similar to those in the comparison study [8]: Han Chinese undergraduates in Singapore. The gender ratios of our sample and those of the comparison study did not significantly differ ($X^2{}_{(1)}$ = 0.27, p = 0.603). Although the two samples significantly differed in age (the comparison study: M_{age} = 21.2, SD_{age} = 1.5; $t_{(1279)}$ = 13.2, p < 0.0001), they were both sampled from four-year colleges. The two samples also share similar socioeconomic and cultural backgrounds; they were both residents of large, urban, industrialized cities in Asia: Tokyo (our study) and Singapore (the comparison study).

We found that our participants were less trusting and less reciprocating than those in the comparison study [8] (see Sections 2.1 and 2.2), which is consistent with previous research [26]. However, it is unclear whether these differences in prosocial behaviors between our population (Japanese) and that of the comparison study (Han Chinese) are significant when compared with other regions of the world [27–29].

The participants completed a written informed consent form prior to participating in the study. The study was conducted in accordance with the Declaration of Helsinki, and the protocol was approved by the Ethics Committee of Tamagawa University (approval #: TRE18-030).

4.2. Trust Game

There are two roles in the trust game [30]: trustor and trustee. The trustor, who receives an endowment of Japanese Yen (JPY) 1000, decides how much of it (in increments of JPY 100) they wish to send to their counterpart, the trustee, who receives no endowment (as in the comparison study [8]). The trustee then receives the tripled amount of money and decides what percentage of it (in increments of 10%) they would like to return to the trustor (the money the trustee returns is not tripled).

Participants played the trust game twice, once as a trustor and once as a trustee. First, they were assigned to the trustor role and asked to decide how much of the endowment they would like to send to the trustee, another participant with whom they would be randomly paired later. Then, the participants were assigned to the trustee role and asked to decide how much money they wished to return for each possible amount that they might receive from the trustor: JPY 100, 200, 300, 400, 500, 600, 700, 800, 900, and 1000 (i.e., we used the strategy method). The participants were told that the trustor and the trustee with whom they would be paired would be different people. They earned, (a) as the trustor, however much out of JPY 1000 they did not send and, (b) as the trustee, however much they did not return out of the tripled amount of money they received from a randomly matched trustor.

We calculated (a) the percentage of JPY 1000 the participants sent as the trustor (DV 1: Trust) and (b) the average percentage of the tripled amount of money they returned as the trustee (DV 2: Reciprocity).

4.3. Salivary Oxytocin Concentration

Oxytocin concentrations were measured in the participants' saliva samples. We collected saliva from the participants at rest using the passive drool method with the Saliva Collection Aid (Salimetrics, LLC, Carlsbad, CA, USA). The participants provided at least 1.2 mL of saliva prior to the trust game. The samples were collected in cryovials and were immediately stored at −80 °C.

One milliliter of each sample was freeze-dried overnight (FD-1000; Tokyo Rikakikai Co., Ltd., Tokyo, Japan). The samples were then dissolved by adding an assay buffer. From the four-times concentrated samples, we conducted an ELISA (enzyme-linked immunosorbent assay) to assess the levels of oxytocin (Enzo Life Sciences, Inc., Farmingdale, NY, USA). The assay was conducted in duplicate. After the assay, we calculated the concentration using a standard curve via a microplate reader (Sunrise Rainbow RC-R; TECAN Group, Ltd., Zurich, Switzerland). The intra- and inter-assay coefficients of variation ($n = 4$) were 6.8% and 7.4%, respectively.

Oxytocin levels were measured according to the manufacturer's protocol, with the exception of the extraction step. Extraction was not performed to align with the methods of the comparison study [8].

4.4. Statistical Analysis

Three participants were excluded from the analysis because of missing data (e.g., they did not provide enough saliva, or their trust game data were lost due to technical errors); thus, we analyzed data from 123 participants (48% women, $M_{\mathrm{age}} = 19.8$, $SD_{\mathrm{age}} = 1.1$). The purpose of this study was to determine whether the results of the comparison study [8] could be replicated using salivary oxytocin. Therefore, we used the same analytical methods. We performed (a) a linear regression analysis with oxytocin added to the explanatory variables (Model 1) and (b) a nonlinear regression analysis with oxytocin and the square term of oxytocin added to the explanatory variables (Model 2). Robust standard errors were used, and all explanatory variables were centered before the analysis. All analyses were performed in R version 4.2.1 (R Core Team, Vienna, Austria) [31], using the R package "estimatr" for regression [32].

5. Conclusions

Although the direction of the relationship is different from that found in a previous study [8], our results further confirm that endogenous oxytocin levels have nonlinear relationships with trust and reciprocity, thereby emphasizing the need for further investigation.

Author Contributions: Conceptualization: F.K., M.W. and H.T.; methodology: Q.S. and H.T.; validation: S.A.; formal analysis: S.A., Q.S. and H.T.; investigation: M.W., K.K. and H.T.; resources: H.T.; data curation: K.K., Q.S. and H.T.; writing—original draft preparation: S.A. and H.T.; writing—review and editing: S.A., M.W., Q.S., H.T. and F.K.; visualization: S.A.; supervision: F.K.; project administration: M.W. and H.T.; funding acquisition: M.W. and H.T. All authors have read and agreed to the published version of the manuscript.

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Data Availability Statement: All relevant data are available at https://osf.io/ze4c5/?view_only=d326a9da5f7844deb32d551e6e7e824e (accessed on 8 February 2023).

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