



Salivary oxytocin after oxytocin administration: Examining the moderating role of childhood trauma

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ABSTRACT

Although oxytocin administration influences behavior, its effects on peripheral oxytocin concentrations are mixed and derived from studies on healthy subjects. Additionally, trauma attenuates the behavioral effects of oxytocin, but it is unknown whether it also influences its effect on peripheral circulation. This study examined whether salivary oxytocin increased after oxytocin administration and whether trauma attenuated this effect. We conducted a randomized, double-blind, placebo-controlled, within-subjects study in 100 male adolescents living in residential youth care facilities. Participants self-administered intranasally 24 IU of oxytocin and placebo (one week later) and provided a saliva sample before and 15 min after administration. Salivary oxytocin increased significantly after oxytocin administration, but this effect might be inflated by exogenous oxytocin reaching the throat. Trauma did not moderate this effect. Our findings suggest that trauma did not attenuate the effect of oxytocin administration on salivary oxytocin, but more robust methodologies are recommended to draw more solid conclusions.

1. Introduction

During the last two decades, there has been a remarkable interest in oxytocin and its potential effects on social-affective behaviors. Although cumulative evidence has supported a beneficial effect of oxytocin administration on several social-affective behaviors and psychiatric symptoms (Bartz, Zaki, Bolger, & Ochsner, 2011; De Dreu & Kret, 2016; Hofmann, Fang, & Brager, 2015; Leppanen, Ng, Tchanturia, & Treasure, 2017), the underlying mechanisms and biological processes of how exogenous oxytocin reaches the central nervous system remain obscure. Oxytocin is a neuropeptide synthesized mainly in the paraventricular nucleus (PVN) and the supraoptic nucleus (SON) of the hypothalamus. It is transported to the posterior pituitary gland and then released into the bloodstream or the brain (Landgraf & Neumann, 2004). However, it can also be synthesized in additional hypothalamic and extrahypothalamic brain areas as well as peripheral tissues and organs, such as the heart and ovary (Lee, Macbeth, Pagani, & Young, 2009; Nishimori et al., 2008). Oxytocin acts peripherally as a pituitary hormone and centrally as neuromodulator in the brain. Peripherally, it is involved in several functions, such as reproductive functions, energy metabolism, eating behavior, bone and fat mass, as well as the cardiovascular system (Amri & Pisani, 2016; Japundzic-Zigon, Lozic, Sarenac, & Murphy, 2019; Landgraf & Neumann, 2004; Lawson,

Olszewski, Weller, & Blevins, 2019). Centrally, it affects many brain regions involved in social-affective processing, such as the nucleus accumbens in the ventral striatum, the hippocampus, and the amygdala (Landgraf & Neumann, 2004).

In an effort to better understand the biological processes of oxytocin, two lines of research have dominated the scientific field regarding oxytocin concentrations. Firstly, researchers examine and compare oxytocin concentrations in cerebrospinal fluid (CSF), blood, saliva, and urine. Contrary to expectations, a review demonstrated that these measurements do not correlate highly with each other and methodological shortcomings raise crucial questions about their reliability (McCullough, Churchland, & Mendez, 2013). This suggests that the comparison among studies using different methods of assay is problematic and unreliable. In addition, a meta-analysis revealed that central oxytocin levels (measured in CSF and extracellular fluid) did not correlate with peripheral oxytocin levels (measured in blood) in animal and human studies at baseline conditions (Valstad et al., 2017). These findings underscore that peripheral oxytocin does not provide sufficient information about central oxytocin at rest and cannot be considered a suitable proxy of central oxytocin secretion.

Secondly, researchers investigate the effect of intranasal oxytocin administration (OT-IN) on central and peripheral levels as well as brain activity to determine how oxytocin reaches and influences the central

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nervous system. It has been postulated that OT-IN circumvents the brain-blood barrier and reaches brain oxytocin receptors via olfactory and trigeminal nerve fibers, leading to an increase in central oxytocin (Veening & Olivier, 2013). However, skepticism of this mechanism has also been posited, supporting that increases in central oxytocin are very limited after OT-IN and the exact transnasal route is still obscure (Hurlemann & Scheele, 2016; Leng & Ludwig, 2016; Quintana, Alvares, Hickie, & Guastella, 2015). A meta-analysis of existing data revealed a correlation between central and peripheral oxytocin levels after OT-IN, supporting a direct nose-to-brain transport of oxytocin via olfactory and trigeminal nerve fibers (Valstad et al., 2017). Recent evidence from animal research found that nasal oxytocin administration led to increases in oxytocin concentrations in the brain and plasma of mice, supporting the passage from nasal administration to the brain (Smith, Korgan, & Young, 2019). Lastly, further meta-analytic evidence showed that OT-IN increased neural activity in the social and emotional brain networks in healthy and clinical populations, supporting its neural effects in the brain (Wang, Yan, Li, & Ma, 2017).

Other studies focused specifically on the effect of intranasal oxytocin on peripheral circulation (particularly plasma and saliva) in healthy subjects. Cumulative evidence has suggested an increase in plasma and salivary oxytocin after OT-IN, indicating an effect of OT-IN on peripheral oxytocin (Andari et al., 2010; Daughters et al., 2015; Gossen et al., 2012; Quintana et al., 2015, 2018; Striepens et al., 2013; Van IJzendoorn, Bhandari, Van der Veen, Grewen, & Bakermans-Kranenburg, 2012; Weisman, Zagoory-Sharon, & Feldman, 2012). However, the timing of the peak increase and the longevity of this effect still remain unclear. One study found that salivary oxytocin increased after OT-IN, peaked after 1 h, and remained elevated 7 h after administration (Van IJzendoorn et al., 2012). Two other studies showed a peak increase 30 min after administration and elevated levels between 108–150 min post administration (Daughters et al., 2015; Gossen et al., 2012). However, there is also evidence that peak oxytocin levels in saliva can occur as rapidly as 10 and 15 min after administration (Quintana et al., 2018; Weisman et al., 2012).

Crucially, it has been suggested that this initial peak after 15 min may occur due to intranasal oxytocin dripping down from the nasal cavity to the throat (Daughters et al., 2015; Quintana et al., 2018; Weisman et al., 2012). However, substances from the nasal cavity to the throat are cleared after 12–15 min and are transferred to the oesophagus and the stomach via the swallow reflex (Marttin, Schipper, Verhoef, & Merkus, 1998). To elaborate on this issue, a study examined saliva concentrations after oxytocin administration intranasally and intravenously over the course of 2 h (Quintana et al., 2018). The findings showed that salivary oxytocin was higher after intranasal administration compared to intravenous administration and the authors suggested that this difference was due to exogenous oxytocin transferred from the nasal cavity into the oropharynx. The intranasal route might thus interfere with salivary oxytocin concentrations via the dropdown effect.

Overall, the effect of OT-IN on peripheral oxytocin and the longevity of this effect remain complex and the majority of existing data has resulted from studies in healthy subjects. Lastly, the implementation of the experimental procedures across studies should be taken into account. The method of assay and the participants' individual (genetic and environmental) differences may affect the findings. It should be emphasized that providing a saliva sample, although not intrusive, requires participant effort and may take up to 10–15 min to collect sufficient samples. Additionally, this timeframe varies greatly among participants. When we ask the participants to start filling a saliva tube at a specific time point post administration, some participants may need 5 min and others 15 min to complete the task. Therefore, the actual sample collected does not reflect the oxytocin levels at the time point of onset, but rather the levels during the entire time the participants were providing the sample. This crucial distinction should be taken into consideration when interpreting the findings.

Another important factor that may influence oxytocin levels and the response to OT-IN is psychopathology and individual differences among the subjects. Extensive research has explored the potential role of peripheral oxytocin levels as a biomarker of several psychiatric disorders, but the findings so far do not support this hypothesis (Rutigliano et al., 2016). However, a crucial factor that has shown a more consistent association with oxytocin is history of trauma (see for reviews Donadon, Martin-Santos, & Osorio, 2018; Fragkaki, Cima, & Granic, 2018). Particularly, previous studies have demonstrated lower peripheral oxytocin levels in healthy populations with history of trauma (Heim et al., 2009; Opacka-Juffry & Mohiyeddini, 2012; Riem et al., 2017), in women with borderline personality disorder (Bertsch, Schmidinger, Neumann, & Herpertz, 2013), revictimized individuals (Chatzittofis, Nordstrom, Uvnas-Moberg, Asberg, & Jokinen, 2014), traumatized adolescents with conduct disorder (Levy et al., 2015), and males with post-traumatic stress disorder (Frijling et al., 2015). It has been supported that trauma can modify oxytocin synthesis and oxytocin receptor binding and these changes may mediate the relationship between early negative experiences and social behaviors (Veenema, 2012).

Crucially, experimental studies have shown that the positive effect of OT-IN on social behaviors is attenuated in individuals with history of trauma, such as emotional neglect and harsh parenting (see for reviews Donadon et al., 2018; Fragkaki et al., 2018). It has been suggested that OT-IN does not have an effect on individuals with early negative experiences because they may be negatively biased in the interpretation of social stimuli (Bartz, Simeon et al., 2011; Zik & Roberts, 2015). However, other biological processes may also play a role. The negative effects of early trauma on the oxytocinergic system might impede the effect of OT-IN on the brain as well as on peripheral circulation. Evidence from animal studies has suggested that adverse experiences can alter oxytocin receptor expression in the hippocampus (Baker et al., 2017), which might explain the attenuated effect of oxytocin administration.

A recent study examined salivary oxytocin after OT-IN and the role of maltreatment and hippocampal volume in female undergraduate students (Riem, Van IJzendoorn, & Bakermans-Kranenburg, 2019). The results showed that maltreatment did not affect the oxytocin concentrations after administration. However, hippocampal volume played a moderating role as oxytocin concentrations after administration were higher in individuals with larger hippocampal volume. The authors proposed that hippocampal neuronal loss and epigenetic changes in oxytocin receptor expression due to early adversity may be associated with the attenuated effects of oxytocin administration in traumatized subjects (Riem et al., 2019). In contrast, other studies examining recent exposure to trauma showed that OT-IN had a positive effect on symptoms of post-traumatic stress disorder (PTSD) in individuals after trauma exposure (10 days to 6 months) (van Zuiden et al., 2017) and OT-IN reduced anxiety and fear processing in the amygdala in subjects with PTSD (Koch et al., 2016). It is thus still unclear whether and how adverse experiences influence the oxytocinergic system and the effects of oxytocin administration.

To contribute to this line of research, we investigated whether salivary oxytocin levels increased after OT-IN and whether this effect was moderated by childhood trauma in a randomized, double-blind, placebo-controlled, within-subjects, sequential study. We recruited male adolescents living in residential youth care institutions as high rates of childhood trauma are usually observed in this population (e.g., Dierkhising et al., 2013). The participants provided a saliva sample before oxytocin and placebo administration and another sample 15 min after administration. We hypothesized that salivary oxytocin would increase after OT-IN and this increase would be attenuated in individuals with history of trauma.

2. Materials and methods

2.1. Participants

The sample of this study is drawn from a larger project examining the effect of oxytocin administration on empathy and emotion recognition (Fragkaki & Cima, 2019). A detailed description of inclusion and exclusion criteria is published elsewhere (Fragkaki & Cima, 2019). The study included 100 male adolescents ($M_{age} = 16.51$, $SD_{age} = 0.96$) recruited from residential youth care facilities in the Netherlands. The participants were admitted to residential care for externalizing problems and delinquency, and/or adverse family environment. The majority of the participants were of Dutch origin ($n = 83$) and 96 participants followed a vocationally-oriented track in school. Eighty-seven participants exhibited conduct disorder (CD), oppositional-defiant disorder (ODD), attention-deficit hyperactivity disorder (ADHD), or resided in a closed group due to severe delinquent behavior. High rates of alcohol/substance abuse/dependence ($n = 71$), depression ($n = 33$), anxiety ($n = 35$), and manic/hypomanic symptoms ($n = 40$) were also reported, and 81 subjects had two or more comorbid disorders. Eight participants dropped out and one participant was excluded because he presented psychotic symptoms. The total sample size included in the analyses was 91 ($M_{age} = 16.50$, $SD_{age} = 0.93$).

2.2. Instruments

2.2.1. Clinical diagnosis

The Mini International Neuropsychiatric Interview for Children and Adolescents (MINI-KID; Sheehan et al., 1998) was administered to assess the presence of psychiatric disorders. MINI-KID is a fully structured and widely used screening interview for youth aged 6–17 years with a yes/no format. It has very good psychometric properties with substantial sensitivity and specificity, interrater and test-retest reliability, and concordant validity with other relevant instruments (Sheehan et al., 2010).

2.2.2. Childhood trauma

Childhood traumatic experiences were assessed with the Childhood Trauma Questionnaire – Short Form (CTQ; Bernstein et al., 2003). It consists of 25 items and has five subscales: physical/sexual/emotional abuse and physical/emotional neglect. The items are rated on a 5-point scale indicating the frequency of the traumatic experiences ($1 = \text{never true}$, $5 = \text{very often true}$). The sum score of all the items was calculated in this study and higher scores indicated higher frequency of childhood trauma. CTQ has very good psychometric properties in community, psychiatric, and forensic populations (Cima, Smeets, & Jelicic, 2008; Thombs, Bernstein, Lobbstaal, & Arntz, 2009). The Cronbach's α in this study was 0.93.

2.2.3. Oxytocin administration

Participants self-administered intranasally a single dose of 24 IU of oxytocin (Syntocinon) and placebo (sodium chloride) in accordance with the guidelines by Guastella et al. (2013). The bottles were identical to ensure double-blinding. The dose of 24 IU is the most frequently used dose in previous experimental studies and OT-IN has no severe adverse side effects when delivered in doses 18–40 IU in research settings (MacDonald et al., 2011), although mild side effects have also been reported in children (DeMayo, Song, Hickie, & Guastella, 2017).

2.2.4. Oxytocin levels

Participants provided two saliva samples using a saliva tube; one before oxytocin/placebo administration and one 15 min after administration. For each sample, participants were asked to provide 5 mL of passive drool. As mentioned in the introduction, there is a debate about the optimal timeframe to detect the peak increase of salivary oxytocin after OT-IN. However, the collection of the saliva sample is a time-

consuming task that might take up to 15 min to complete and feasibility concerns should be considered. Taking that into consideration and in order to continue to the experimental tasks 30 min post administration, we selected the second sample to start being collected 15 min post administration. Samples were stored at -40°C until analysis. Oxytocin was measured in 1 mL saliva by ELISA (Enzo Life Sciences) after solid phase extraction described by Szeto et al. (2011) and reconstitution in 250 μL assay buffer. The total coefficients of variation (CVs) were 7.1 % at 57.3 pg/mL and intra assay CV was 16 % in the range 10–400 pg/mL.

2.3. Procedure

The study had a randomized, double-blind, placebo-controlled, within-subjects, sequential design with three sessions; one screening session and two experimental sessions. During the screening, psychopathology, personality characteristics, and history of childhood trauma were assessed. Participants were instructed to abstain from caffeine and nicotine 2 h prior to the next experimental sessions. During the experimental sessions, participants performed behavioral tasks, provided a saliva sample, and then self-administer intranasally oxytocin or placebo. The order of the substance allocation was randomized with block randomization (blocks of 10) performed by computer randomization. The clinical research coordinator generated the randomization codes and directly communicated to the experimenters the group to which the participant was assigned at each session and the order of the stimuli. Only the clinical research coordinator had access to the assignment schedule to ensure allocation concealment. Fifteen minutes after the administration, the participants provided another saliva sample and 30 min after the administration they performed again the experimental tasks. The results on the behavioral effects of OT-IN are published elsewhere (Fragkaki & Cima, 2019). In the next experimental session (one week later), participants underwent the same procedure but the other substance was administered. Participants provided written informed consent and received financial compensation for their participation. The study was granted medical ethical approval by the Central Committee on Research Involving Human Subjects and the Dutch Ministry of Health. The study has been registered to the EU Clinical Trials Registry (EudraCT: 2016-000367-16).

2.4. Analytic strategy

Mixed effects models were conducted using R to account for the nested structure of the data (R Core Team, 2017). The data were nested at two levels: 1) time level (four measurements before and after administration for each condition), and 2) participant level. The intraclass correlation (ICC) for the participant level was 0.78 and the ICC for the time level was 0.74, suggesting high variability in both levels. To build our final model we compared the following two models; one model included the random intercept of the participants and the second model included the addition of the random slope of time. The results showed that the inclusion of the random slope of time did not significantly improve the model ($p > .05$) and the AIC and BIC values were higher in this model. Therefore, only the random intercept of the participants was included in the reported analyses.

The values of salivary oxytocin were examined for outliers and standardized values above 2 were treated as outliers ($n = 15$). The outliers were excluded from further analyses in an effort to remove unreliable observations that might be extremely high due to the drop-down effect. A log distribution was better fitted to the data and thus generalized mixed effects models were applied, using the package 'lme4', function 'glmer', and identity link function 'log'. Trauma was grand mean centered and added as moderator and age was grand mean centered and added as a control variable. Optimizers with increased number of iterations were applied only in the model that includes the trauma variable to achieve convergence of the model. Ninety-one subjects were included in the analyses with 333 observations.

Table 1
Means and standard deviations of the study variables.

	<i>M(SD)</i>
Age	16.45 (0.93)
Trauma	45.99 (18.64)
Salivary oxytocin (pg/mL):	
Before placebo	11.73 (3.78)
After placebo	11.95 (7.54)
Before oxytocin	14.70 (12.35)
After oxytocin	4161.68 (7531.00)

Conditional R^2 was used as an index of effect size, because it captures the variance explained by both fixed and random factors, with the method proposed by Nakagawa and Schielzeth (2013). We run two models: Model 1 included the Condition x Time interaction to examine whether salivary oxytocin increased after oxytocin administration but not after placebo and Model 2 included the 3-way Trauma x Condition x Time interaction to examine whether childhood trauma moderated the effect of oxytocin administration on salivary oxytocin. Simple slope analyses were performed to examine the interactions. Regression analyses were performed to examine whether baseline salivary oxytocin levels (log transformed) were associated with trauma controlling for age.

3. Results

The means and standard deviations of the study variables are presented in Table 1.

Model 1 was a generalized mixed-effects model that examined the Condition x Time interaction and found that the 2-way interaction was significant, $b = -6.81$, $SE = 0.27$, $t(326) = -25.39$, $p < .001$, indicating that salivary oxytocin levels increased significantly after oxytocin administration compared to placebo. The full model explained 78 % of the variance, $R^2 = .78$. A simple slope analysis was conducted to examine the interaction. Only the slope in oxytocin condition was significant, $b = 6.93$, $p < .001$, indicating that salivary oxytocin increased after oxytocin administration but not after placebo administration (Fig. 1).

Second, a regression analysis showed that baseline oxytocin levels before administration were not associated with history of trauma, $b = 0$, $SE = 0$, $t(88) = 0.83$, $p = .41$.

Trauma was added to the generalized mixed-effects model and Model 2 found that the Trauma x Condition x Time interaction was not significant, $b = 0$, $SE = 0.02$, $t(322) = 0.23$, $p = .82$, indicating that the increase in salivary oxytocin levels after oxytocin administration was not influenced by history of trauma. Finally, neither age nor trauma had a main effect on salivary oxytocin levels ($p > .05$). The results of Model 1 and Model 2 are depicted in Table 2.

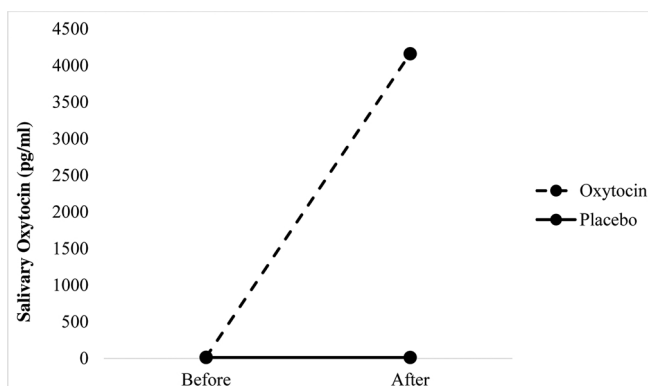


Fig. 1. Salivary oxytocin levels before and after oxytocin and placebo administration.

Table 2
Results of the generalized mixed models.

	Variance	SD	<i>b</i>	SE	<i>T</i>	<i>P</i>
Model 1						
<i>Random effects:</i>						
Subject	507.4	22.52				
Residual	143.0	11.96				
<i>Fixed effects:</i>						
Age			0.28	0.21	1.30	0.19
Time			13.75	0.38	36.53	< .001
Condition			-0.07	0.22	-0.34	0.74
Time x Condition			-6.81	0.27	-25.39	< .001
Model 2						
<i>Random effects:</i>						
Subject	505.6	22.49				
Residual	142.8	11.95				
<i>Fixed effects:</i>						
Age			0.29	0.22	1.34	0.18
Trauma			0.01	0.02	0.38	0.71
Time			13.75	0.38	36.04	< .001
Condition			-0.07	0.22	-0.32	0.75
Trauma x Time			-0.01	0.02	-0.46	0.65
Trauma x Condition			-0.01	0.01	-0.39	0.70
Time x Condition			-6.82	0.27	-24.97	< .001
Trauma x Time x Condition			0	0.02	0.23	0.82

4. Discussion

The aim of this study was to examine the effect of oxytocin administration on peripheral salivary oxytocin and the moderating role of trauma. We found that salivary oxytocin levels increased after oxytocin administration but not after placebo. Crucially, the findings showed that trauma did not moderate the effect of oxytocin administration on peripheral salivary oxytocin levels.

Our finding on the increased salivary oxytocin after oxytocin administration is in line with previous studies with similar results and current meta-analytic evidence (Valstad et al., 2017). However, reservations about this effect have also been raised in the literature especially regarding the time point after administration and the drop-down effect. The participants in this study were asked to provide a saliva sample before oxytocin administration and a second sample 15 min after oxytocin administration. It is noteworthy that the time needed to provide the saliva sample ranged considerably among the participants. Some participants needed 3–5 min to provide the sample, whereas others needed 10–15 min. This variability highlights that the salivary oxytocin levels captured in this study reflect the levels in the timeframe between 15 and 30 min after administration.

This clarification is crucial, as previous studies have supported that salivary oxytocin levels might peak 15 min post-administration due to intranasal oxytocin dripping down from the nasal cavity to the throat (Daughters et al., 2015; Quintana et al., 2018; Weisman et al., 2012). The study by Quintana et al. (2018) showed that salivary oxytocin was higher overall after intranasal administration of 24 IU compared to intravenous administration over the course of 2 h and especially immediately after administration, supporting that intranasal oxytocin traveled to the oropharynx. Other evidence has suggested that substances from the nasal cavity to the throat are cleared after 12–15 min (Martin et al., 1998). It is thus possible that our participants who provided the saliva sample quickly might have had higher oxytocin concentrations due to the dropdown effect compared to the participants who needed more time to complete the task. Unfortunately, the exact time needed for the collection of the saliva sample for each participant was not documented and we cannot address this question. In addition, we observed much higher salivary oxytocin concentrations after administration compared to other studies (e.g., Riem et al., 2019) that also support the presence of the dropdown effect. Taken together and given

the very high concentrations observed after oxytocin administration, our findings showed that intranasal oxytocin administration increased salivary oxytocin during 15–30 min post-administration, but this increase was likely inflated by exogenous oxytocin reaching the throat. Therefore, the dropdown effect poses a crucial limitation in the investigation of salivary oxytocin after administration and obscures the true effect. It is imperative to develop and apply new rigorous methodologies to address this limitation in order to better understand how oxytocin administration influences peripheral circulation.

Our second finding showed that trauma did not moderate the effect of oxytocin administration on salivary oxytocin. Although some previous studies found lower peripheral levels in traumatized individuals (Bertsch et al., 2013; Chatzittofis et al., 2014; Heim et al., 2009; Levy et al., 2015; Opacka-Juffry & Mohiyeddini, 2012), in our study there was no association between trauma and oxytocin levels at baseline. This is in line with the findings by Riem et al. (2019) who also found no correlation between maltreatment and baseline oxytocin. Most importantly, trauma did not attenuate the increase in salivary oxytocin after administration. This finding is also in accordance with the study by Riem et al. (2019) who reported that maltreatment did not moderate the effect of OT-IN on salivary oxytocin in healthy females.

There is evidence that the effect of oxytocin administration on social-affective behaviors is attenuated in individuals with history of trauma (see for reviews Donadon et al., 2018; Fragkaki et al., 2018), but previous evidence did not simultaneously examine the oxytocin effect on behavior and on peripheral oxytocin levels. This study is part of a larger project that investigated the effect of oxytocin administration on empathy and emotion recognition in residential youth (Fragkaki & Cima, 2019). In the larger project, the behavioral effects of oxytocin administration indicated that trauma did not influence the effect of oxytocin on empathy or emotion recognition. The current study adds to these findings and shows that trauma also did not moderate the effect of oxytocin administration on peripheral oxytocin. We thus provide a more comprehensive picture illustrating that in residential youth trauma did not moderate the effects of oxytocin administration neither on behavior nor on peripheral salivary oxytocin.

Considering the high prevalence of trauma in residential youth, these findings suggest that the behavioral and peripheral effects of oxytocin administration might not be attenuated by trauma in this population. Interestingly, positive effects of oxytocin administration on symptoms of post-traumatic disorder after trauma exposure have also been reported (van Zuiden et al., 2017). Given the contradictory findings on this topic, it is imperative to further explore whether the behavioral and peripheral effects of OT-IN are influenced by trauma, taking into account different types and severity of adverse experiences not only in residential youth and but also in other clinical populations. Moreover, as mentioned above, a dropdown effect may have inflated the observed salivary oxytocin after administration. It is thus reasonable to question whether the dropdown effect could have masked the influence of trauma on salivary oxytocin. Taken together, two crucial points should be emphasized: a) the complexity and different types of traumatic experiences may influence the oxytocinergic system and the effect of oxytocin administration on salivary concentrations differently, and b) new methodologies should be applied to eliminate the dropdown effect.

Furthermore, the current findings should be interpreted in light of several limitations. First and foremost, the dropdown effect may have inflated the observed salivary oxytocin concentrations, as intranasal oxytocin reaches the throat via the nasal cavity. The study by Quintana et al. (2018) addressed this limitation by comparing intranasal with intravenous administration in a small sample. This methodology is promising from an experimental standpoint and can potentially clarify this issue in replication studies with larger samples. Similarly, collecting blood samples and comparing all the methods of assay is also recommended to better understand how exogenous oxytocin affects the periphery. However, these experimental designs are sometimes difficult

to implement in clinical samples and especially in youth due to ethical and feasibility issues. In our study, we could not include comparative measurements due to feasibility concerns and time limitations.

Second, we used the traditional nasal spray that has been criticized on drug absorption and clearance patterns. A new Exhalation Delivery System (EDS) device that has better drug absorption and clearance pattern has gained attention and has been used in a few studies (Quintana et al., 2015, 2018). Future studies are urged to try new devices that can deliver better drug absorption, clearance, and eventually achieve better accuracy and standardization. Thirdly, as previously mentioned, we used a saliva tube and asked the participants to provide 5 mL of saliva. This task is more demanding than a salivette swab and the participants needed 5–15 min to provide the sample. As a result, there was great variability in the time needed and eventually the timeframe captured for the saliva concentrations. We thus emphasize that the salivary oxytocin reported in this study reflects concentrations between 15 and 30 min post administration. Fourth, we included only males and thus no generalization to females can be applied. More importantly, this population is very complex with a wide range of psychopathology and different types and severity of trauma. Our findings showed that history of childhood trauma did not moderate the effect of oxytocin administration on salivary oxytocin, but others types of trauma and different levels of severity should also be explored to draw solid conclusions.

Overall, our study showed that salivary oxytocin increased after oxytocin administration in residential youth and this effect was not attenuated by history of trauma. This evidence combined with the lack of a moderating effect of trauma on behavioral oxytocin effects investigated in the same sample (Fragkaki & Cima, 2019) suggests that moderate levels of childhood trauma in residential youth do not interfere with the effect of oxytocin administration on behavior or peripheral oxytocin concentrations. However, these findings should be interpreted with caution, as it is unclear whether the dropdown effect might have masked a potential effect of trauma on salivary oxytocin. Future research should use new more effective tools and different comparative measurements to address current methodological limitations and further disentangle how oxytocin administration affects peripheral concentrations and most importantly whether this effect is associated with the behavioral effects of oxytocin.

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Ethical standards

The study was conducted in accordance with the Declaration of Helsinki and was granted medical ethical approval by the Central Committee on Research Involving Human Subjects and the Dutch Ministry of Health. The study has been registered to the EU Clinical Trials Registry (EudraCT: 2016-000367-16). Written informed consent was obtained from all the participants. Additional parental written informed consent was obtained for participants below the age of 16 according to the Dutch law. All the data were confidential and anonymous in accordance with the Dutch Personal Data Protection Act.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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