Short Communication

Title: Changes in endogenous oxytocin levels after intranasal oxytocin treatment in adult men with autism: an exploratory study with long-term follow-up.

Running title: Treatment-induced changes in salivary oxytocin levels

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Key words

Oxytocin; autism spectrum disorder; endogenous salivary levels; intranasal administration; social responsiveness; repetitive behavior; attachment

Number of words

- Main text (introduction, methods, results & discussion)	2451
- Abstract:	228

Number of Figures:	1
Number of Tables:	1

Contents of supplemental material:

Supplementary Methods

Supplementary Figures:	1
Supplementary Tables:	2

Abstract

Intranasal administration of the neuropeptide oxytocin (OT) is increasingly explored as a potential treatment for targeting the core symptoms of autism spectrum disorder (ASD). Previously, interactions of exogenously administered OT with its endogenous production have been demonstrated following single-dose administrations. However, the impact of repeated, long-term OT use on endogenous salivary OT levels is unknown.

In this double-blind, randomized, placebo-controlled study with between-subject design, 34 adult men with ASD were either assigned to a four-week treatment of once-daily intranasal OT administrations (24 IU) or placebo. Salivary OT samples were obtained before and after the treatment period as well as at two follow-up sessions, four weeks and one year after cessation of the treatment.

Receiving OT intranasally but not placebo reliably increased endogenous salivary levels of OT immediately post-treatment and at the follow-up session four weeks post treatment, indicating an interaction between exogenously administered OT and its endogenous production. Notably, increases in salivary OT at the four-week follow-up session were most pronounced in individuals with larger behavioral improvements in ASD social symptoms. These results suggest that OT's positive effects on social behaviors may lead to a self-perpetuating elevation of OT levels through a feed-forward triggering of its own release.

Together, the current investigation provides initial evidence that repeated intranasal administration of OT can induce long-lasting changes in endogenous salivary OT levels, presumably through a positive spiral of OT release.

Introduction

Oxytocin (OT) is an endogenous neuropeptide, produced predominantly at the level of the hypothalamus, that acts as an important neuromodulator for mediating complex social behaviors; including interpersonal bonding, attachment and cooperative behavior (Bakermans-Kranenburg and van IJzendoorn, 2013; Bartz et al., 2011; Jurek and Neumann, 2018). During the past decade, intranasal administration of OT has been increasingly explored as a potential treatment for alleviating core social problems characteristic of autism spectrum disorder (ASD).

Initial single-dose administration studies in individuals with ASD consistently demonstrated behavioral improvements on various tasks assessing repetitive behavior, affective speech comprehension, emotion recognition and social decision making (see review (Ooi et al., 2017)). Also several multiple-dose administration studies have shown beneficial effects on ASD-symptoms. In children with ASD, two prior trials demonstrated significant improvements in the social domain after four or five weeks of intranasal OT treatment in 3-6 year-old children (Yatawara et al., 2015) or 6-12 year-old children (Parker et al., 2017). No significant improvements on core ASD-symptoms were demonstrated however, after an eight-week treatment in adolescent boys with ASD (Guastella et al., 2015). In adults with ASD, improvements in social functioning (Kosaka et al., 2016; Watanabe et al., 2015) and repetitive behaviors (Anagnostou et al., 2012; Bernaerts et al., 2020; Yamasue et al., 2018) have been reported after courses of four, six or twelve weeks of daily OT administrations.

Overall, the half-life of exogenously administered OT has been demonstrated to be approximately 3–6 min in plasma and approximately 20 min in cerebrospinal fluid (Jurek and Neumann, 2018). Notably however, elevated salivary levels of OT were shown to persist till up to 7-hours post-administration, indicating a triggering of endogenous production by the exogenously administered OT (van IJzendoorn et al., 2012). To date however, it remains unclear whether and how repeated use of exogenously administered OT over a longer period of time impacts endogenous productions of OT.

The current study explored whether a four-week OT-treatment of once daily intranasal administrations induced changes in endogenous levels of salivary OT in young adult men with ASD. Changes in salivary OT production were assessed immediately after the four-week treatment (but at least 24-hours after the final administration); and at two follow-up sessions, four weeks and one year after cessation of the treatment.

Previous analyses within the same sample of individuals with ASD (Bernaerts et al., 2020) have revealed that the four-week OT-treatment improved symptoms of autism, most notably in terms of repetitive behaviors (assessed with the Repetitive Behavior Scale – Revised:RBS-R); feelings of attachment avoidance (assessed with the State Adult Attachment Measure:SAAM) and, to a more variable extent, social functioning (assessed with the Social Responsiveness Scale for adults:SRS-A) (see **Supplementary Table 1-2** for a detailed description of the sample and behavioral effects (adopted from Bernaerts et al. 2020)). However, whether these behavioral effects are associated with inter-individual differences in treatment-induced changes in salivary OT is unknown. To shed more light on the biological basis of these clinical responses, a key aim of the current study was to explore whether the daily administrations of exogenous OT induced (long-lasting) changes in the endogenous production of salivary OT and importantly, whether higher post-treatment salivary OT levels are associated with (long-lasting) behavioral improvements.

Experimental Procedures

Study design. This study with double-blind, randomized, placebo-controlled, between-subject design investigated the (long-lasting) effects of multiple-dose OT-treatment on endogenous levels of salivary OT in young adult men with a clinical diagnosis of ASD (between 18-35 years of age). To do so, saliva samples were collected at baseline (T0); after four consecutive weeks of daily nasal spray administrations (T1); and at two follow-up sessions, four-weeks (T2) and one-year after cessation of the treatment (T3) (See **Supplementary Figure 1**, CONSORT Flow diagram for number of salivary samples collected and analyzed for each assessment session). Sample collections took place at Leuven University Hospital in the context of a larger study (registered at Eudract2014-000586-45 and clinicaltrial.gov: NCT02940574).

Participants. As shown in **Supplementary Table 1**, participants with ASD randomized to receive the OT (n=22) or placebo (PL) treatment (n=18) did not differ in terms of baseline symptom severity (Autism Diagnostic Observation Schedule), estimates of intelligence (6-subtest short-version of the Wechsler Adult Intelligence Scale-IV - Dutch version) and age. Information on sample size is provided in **Supplementary Methods**.

Nasal spray administration. All participants self-administered a once-daily dose of 24 IU (3 puffs/nostril) of OT (Syntocinon®,Sigma-tau) or PL (saline natrium-chloride solution) in the morning over four consecutive weeks (28-doses in total). Additional details on the procedures for recruitment (inclusion and exclusion criteria), participant characterization and nasal spray administration can be found in (Bernaerts et al., 2020).

Assessment of salivary oxytocin. Saliva samples were collected at each assessment session (baseline, T1, T2, T3) using the absorbent device technique (Saliva cotton swabs, size 10x10x5mm) and salivary OT levels were determined with the commercial enzyme immunoassay Oxytocin ELISA kit of EnzoLife Sciences (intra- and inter-assay coefficients of 13.3% and 20.9%) (**Supplementary Methods**). Measurements were performed on undiluted samples (100µl), and sample concentrations were calculated according to the relevant standard curve. Concentrations below the detection limit (15pg/ml) were set to a value half of the detection limit.

Statistical analysis. Data of three participants of the OT-group and one of the PL-group were excluded list-wise, due to missing baseline data. Additionally, data of one participant of the OT-group and one of the PL-group were identified as extreme outliers and were excluded from the analyses (six inter-quartile ranges (Q3-Q1) below or above the first (Q1), respectively third (Q3) quartile). Final analyses were performed on a total of 34 participants (OT:n=18; PL:n=16) (see Consort Flow diagram, **Supplementary Figure 1**). Primary analyses are reported using an intention-to-treat format with last-observations-carried-forward to replace missing data (**Figure 1A**) Secondary analyses are additionally reported on data without imputation of missing values (**Supplementary Figure 2**). To explore treatment-induced changes in salivary OT-levels, changes from baseline (T0) in OT-levels were calculated (separately for each assessment session: T1, T2, T3) and pre-to-post change scores were subjected to a mixed-effects model with the fixed factors 'treatment' (OT, PL) and 'assessment session' (T1, T2, T3) and the random factor 'subject'. Mixed-effects analyses are reported with and without correction for initial baseline values (inserted in the mixed model as continuous regressor). Single-sample t-tests are reported to assess within-group pre-to-post changes.

Further, step-wise multiple-regression models (with F-to-enter >1.0) were performed to explore whether inter-individual differences in treatment-induced changes in salivary OT levels (dependent variable) were related

to self-reported behavioral changes in terms of repetitive behaviours (RBS-R), attachment avoidance (SAAM) or social functioning (SRS-A) (independent variables) (see **Supplementary Table 2** for a detailed description of treatment-induced changes in these behavioral measures; adopted from Bernaerts et al. (2020)). Further, to assess robustness of identified relationships, a distribution of the correlation r-value test-statistic was fitted using Monte-Carlo (MC) bootstrapping (100 iterations). All statistics were executed with Statistica 13 (Tibco Software Inc.).

Results

A significant main effect of treatment was revealed ($F(1,32)=8.48$; $p=.0065$; $\eta^2=.21$) (with correction for initial baseline values; $F(1,31)=5.60$; $p=.024$; $\eta^2=.15$) indicating that across assessment sessions (T1, T2, T3), salivary OT-levels were significantly larger after OT- compared to PL-treatment (see **Figure 1A**). **Table 1** summarizes the between-group treatment effect sizes which were large at T1 (post-treatment), small-to-medium at T2 (four-week follow-up) and small to medium at T3 (one-year follow-up). No significant treatment-by-session interaction was identified ($F(2,64)=1.91$; $p=.16$; $\eta^2=.056$). Within-group analyses showed that in the OT-group, salivary OT-levels were significantly increased compared to baseline at the post-session (T1: $t(17)=3.02$; $p=.008$) and at the four-week follow-up session (T2:$t(17)=2.65$; $p=.014$), but not significantly at the one-year follow-up session (T3: $t(17)=1.85$; $p=.082$). Also in the PL-group, no significant pre-to-post changes in salivary OT were evident (**Table 1**). Secondary analyses on data without imputation of missing values yielded qualitatively similar effects for sessions T1 and T2 (i.e., large and small-to-medium effects), but only a negligible effect ($d<.20$) at the one-year follow-up session T3, i.e., yielding the latter effect inconclusive (**Supplementary Figure 2**).

Notably, stronger pre-to-post increases in salivary OT, evident in the OT-group four-weeks post-treatment (T2) were associated with more pronounced pre-to-post improvements in social functioning (SRS-A) ($\beta=-.64$; $t(15)=-3.15$; $p=.0066$) (MC bootstrapping: $r=-0.48$; 95% confidence interval (CI) (-.54;-0.45)) (**Figure 1B**). Also repetitive behaviors was retained in the step-wise multiple regression model (RBS-R) ($\beta=-.37$; $t(15)=-1.83$; $p=.086$); and together, these two variables explained 42.5% (R^2) of the variance ($F(2,15)=5.55$; $p=.016$). Notably, similar associations were evident between improved social functioning (SRS-A) immediately after treatment (T1) and higher levels of OT at the four-week follow-up session (T2) ($\beta=-.62$; $t(16)=-3.16$; $p=.0061$) (MC bootstrapping: $r=-0.51$; 95% CI (-.57;-0.46)), i.e., indicating that sustained higher levels of salivary OT at the four-week follow-up (T2) were predominantly evident in participants for whom the OT-treatment induced the largest improvements in social functioning immediately post-treatment (T1) (**Figure 1C**). Increases in salivary OT immediately post-treatment (T1) were however not significantly associated with simultaneous changes in behavior (at session T1) (no variables retained in the model: F-to-enter <1.0). Also no significant associations were evident in the PL-group (F-to-enter <1.0).

Discussion

The current study explored whether repeated use of exogenously administered OT over a four-week period impacts endogenous productions of OT in adult men with ASD. Reliable increases in endogenous levels of salivary OT were evident in participants receiving OT compared to PL, immediately post-treatment and also at the follow-up session, four weeks after cessation of the actual treatment, indicating an effect of the exogenously administered OT on the participant's endogenous oxytocinergic production. Notably, increases in salivary OT at the four-week follow-up session were most pronounced in individuals with larger behavioral improvements in ASD social symptoms.

Considering the short half-life of exogenously administered OT (approximately 3–6 min in plasma and 20 min in cerebrospinal fluid (Jurek and Neumann, 2018)), the observed elevated levels after the OT-treatment are hypothesized to reflect a feed forward mechanism of the oxytocinergic system, such that treatment with exogenous OT may stimulate the feed-forward release of the endogenous peptide. Prior studies have demonstrated elevated OT-levels till up to 7-hours (van IJzendoorn et al., 2012) after a single-dose administration, indicating an interaction between exogenously administered OT and its endogenous production. In this context, De Dreu et al. (2012) put forward the notion of a positive spiral of OT-mediated and mutually reinforcing cooperation (De Dreu, 2012), suggesting that OT's positive effects on cooperative behaviors may lead to a self-perpetuating elevation of OT-levels through a feed-forward triggering of its own release. Within the current study, it was shown that higher levels of endogenous salivary OT at the four-week follow-up (T2) were predominantly observed in participants that previously self-reported the largest behavioral improvements in terms of social functioning four-weeks earlier (i.e., at T1, immediately after treatment) as well as in parallel, at the four-week follow-up (T2). These observations are therefore supportive of De Dreu's notion of a positive spiral of OT-release (De Dreu, 2012), suggesting that OT's positive impact on behavior (due to an increased availability of OT during the treatment), may lead to a self-perpetuating, feed-forward spiral of elevated levels of OT. While our design also included a long-term follow-up till one year post-treatment, it should be noted that due to a limited availability of samples (larger drop-out), findings of elevated OT levels were not conclusive for this follow-up measure (i.e., only negligible effects were evident on data without imputation of missing values).

While in the current study, only peripheral (salivary) levels of OT were assessed, moderate to strong correlations have been demonstrated between salivary and central (cerebrospinal fluid) OT levels (Martin et al., 2018). Also several studies in humans, non-human primates and rodents have demonstrated elevated OT levels in the central nervous system after exogenous administration of OT, presumably due to a triggering of its own production (Quintana et al., 2018). Nonetheless, the exact neurobiological pathway by which exogenously administered OT may impact its endogenous production remains unclear. Since only small amounts of synthetic OT can cross the blood-brain-barrier directly, OT is anticipated to reach the central nervous system primarily via passive diffusion through perineural clefts in the nasal epithelium, which provide a gap in the blood-brain-barrier (Martins et al., 2020). Alternatively, a higher availability of circulating OT also engages OT-receptors expressed throughout the body, and therefore may impact on brain function and behavior indirectly (Leng and Ludwig, 2016).

In the current study, salivary OT samples were assessed longitudinally from individuals with ASD, but no control sample was included. Hence, it remains unclear whether the ASD participants' baseline OT-levels were

lower compared typically developed individuals and/or whether the OT-treatment induced a 'normalization' of low baseline levels. Previous studies examining the relationship between endogenous OT-levels and ASD diagnosis have been inconclusive, with some studies showing overall low levels of OT in participants with ASD, compared to controls (Green et al., 2001; Zhang et al., 2016), while other studies showed no differential effect of diagnosis (Jacobson et al., 2014; Miller et al., 2013) or even higher OT-levels in adults with ASD (Jansen et al., 2006). More consistent associations have been identified beyond diagnosis, e.g., between endogenous OT-levels and maternal or paternal bonding behaviors, infant social engagement and attachment-related thoughts (Alaerts et al., 2019; Feldman et al., 2007; Gordon et al., 2008). For example, previous work showed strong positive associations between salivary OT and expressions of secure attachment, but not with scales assessing core autism symptom domains in adult men with ASD (Alaerts et al., 2019). Thus, although the human OT-system has been consistently linked to the expression of complex social and affiliative behaviors, consistent evidence is lacking for a direct link between ASD-diagnosis and aberrant oxytocinergic functioning.

While the current study provides important new insights into the effects of repeated use of OT on endogenous salivary OT-levels, some limitations need to be considered. First, we note the small sample size of included patients with ASD as a limitation of the current study as well as the lack of a control group of neurotypical individuals and the inclusion of only male participants, thereby limiting generalizability. Further, it currently remains unclear how peripheral, salivary OT measures (as performed in the current study) are functionally related to central OT activity, and therefore, future research should be directed at unraveling the exact mechanistic routes by which exogenously administered OT impacts the central oxytocinergic system.

The current investigation provides initial evidence that repeated use of OT can induce long-lasting changes in endogenous salivary OT-levels that outlast the period of actual administration until four-weeks after treatment. Our findings also hold important implications, by showing that long-lasting treatment effects at the behavioral level are paralleled by an interaction of exogenously administered OT with its endogenous production. However, considering the exploratory nature of the study, the obtained results should be considered preliminary and future research in larger; more representative samples are needed before therapeutic use of OT can be considered in clinical settings.

Figure Legend

Figure 1

Treatment-induced changes in salivary oxytocin levels.

Panel A visualizes treatment-induced pre-to-post changes in salivary oxytocin levels separately for each treatment group (oxytocin, placebo) and assessment session, i.e., at the post session (T1), and the four-week (T2) and one-year (T3) follow-up sessions. Vertical bars denote +/- standard errors.

Panel B visualizes the association between treatment-induced increases in salivary oxytocin at the four-week follow-up session (T2) and parallel behavioral improvements in social functioning at session T2 (Social responsiveness Scale-Adult version: SRS-A).

Panel C visualizes the association between treatment-induced increases in salivary oxytocin at the four-week follow-up session (T2) and behavioral improvements in social functioning (SRS-A) as reported four weeks earlier, i.e., at T1, immediately after treatment.

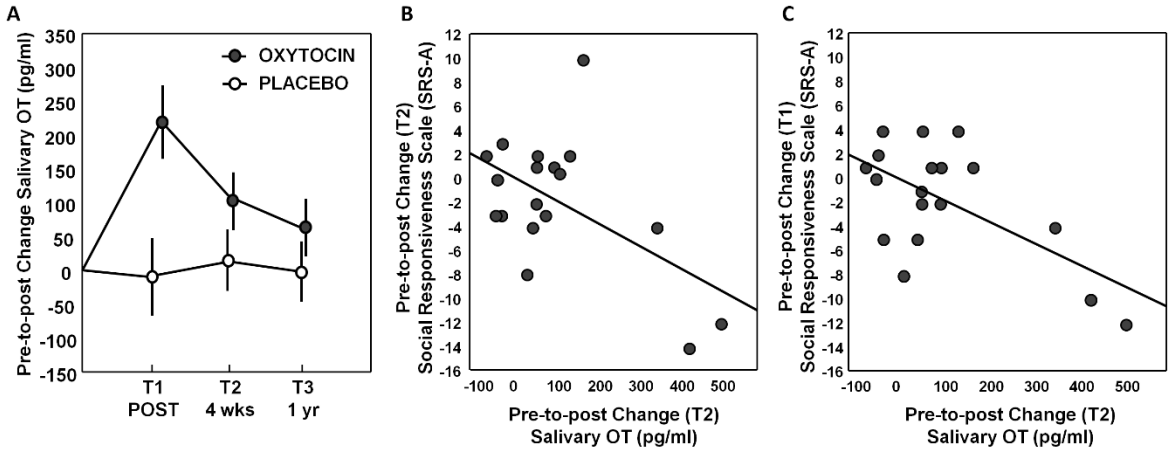

Table 1

Treatment-induced changes in salivary OT-levels. Mean pre-to-post change scores (in pg/ml) are listed separately for each treatment group (oxytocin, placebo) and assessment session (T1, T2, T3).

		Oxytocin				Placebo			Between-group difference
Salivary OT	N	Mean ± SD	*t*-value	*p*	N	Mean ± SD	*t*-value	*p*	Cohen's *d*
Post (T1)	18	218.66 ± 307.44	3.02	0.008	16	-9.34 ± 77.31	-0.48	0.636	1.19
Four-week follow-up (T2)	18	102.32 ± 164.11	2.65	0.017	16	15.16 ± 196.9	0.31	0.762	0.48
One-year follow-up (T3)	18	63.27 ± 145.35	1.85	0.082	16	-1.51 ± 210.99	-0.03	0.978	0.36

T- and *p*-values correspond to single-sample *t*-tests assessing within-group pre-to-post changes separately for the oxytocin and placebo group. Cohen's *d* effect sizes of between-group differences (pre-to-post change-score$_{OT}$ –pre-to-post change-score$_{PL}$)/pooled SD) are reported (small effect: 0.2; medium effect: 0.5; large effect: 0.8).

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Supplementary Information

Title: Changes in endogenous oxytocin levels after intranasal oxytocin treatment in adult men with autism: an exploratory study with long-term follow-up.

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Supplementary Methods

Sample size

In one prior randomized placebo-controlled clinical trial, a cross-over design was used to assess the neural and behavioral effects of multiple-dose OT-treatment (six weeks of twice-daily doses) in 20 adults with ASD and significant effects (large-size) were reported for a total of 17 participants who completed the OT/ PL cross-over treatment (Watanabe et al. 2015). Considering this prior cross-over study and the lack of other prior studies assessing chronic neural and behavioral effects using parallel designs, the current sample size was set at a comparable sample size.

Assessment of endogenous salivary oxytocin

Samples were collected using the absorbent device technique (saliva cotton swabs, size 10 x 10 x 5 mm). Prior to collection, subjects were oral resting (no eating, chewing gum, smoking, drinking) for approximately 45 min. Participants were asked to chew a cotton swab for approximately 60 sec until it was saturated with saliva. After collection, swabs were placed in a sterile conical tube and placed in a −20°C freezer for storage. Samples were centrifuged at 4°C for 15 min at 17.000 rpm and salivary OT levels were determined via the commercial enzyme immunoassay Oxytocin ELISA kit of Enzo Life Sciences, Inc. as used in previous studies (Feldman et al., 2010; Lebowitz et al., 2016; Tsuji et al., 2015; Weisman et al., 2012). Note that the sampling volume of the adopted saliva cotton swaps (10 x 10 x 5 mm) was smaller compared to other commercially available swabs (e.g. Salivette, Sarstedt 97/16.8 mm), as pilot research showed an enhanced detection of salivary OT in smaller total sampling volumes. All sample extraction and concentration procedures were conducted in accordance with the official kit manual. Measurements were performed on undiluted samples (100 µl), and the concentrations of samples were calculated according to the relevant standard curve. Concentrations below the detection limit (15 pg/ml) were set to a value half of the detection limit. The intra-assay and inter-assay coefficients of the assay are lower than 13.3% and 20.9%, respectively. While the exact pharmacokinetics of OT in saliva are not fully understood, there are several advantages to salivary collections, including that they are less invasive to acquire and present a more clean matrix compared to blood or plasma (i.e., oxytocin in plasma is more prone to binding to large plasma proteins, possibly limiting its detection) (MacLean et al., 2019).

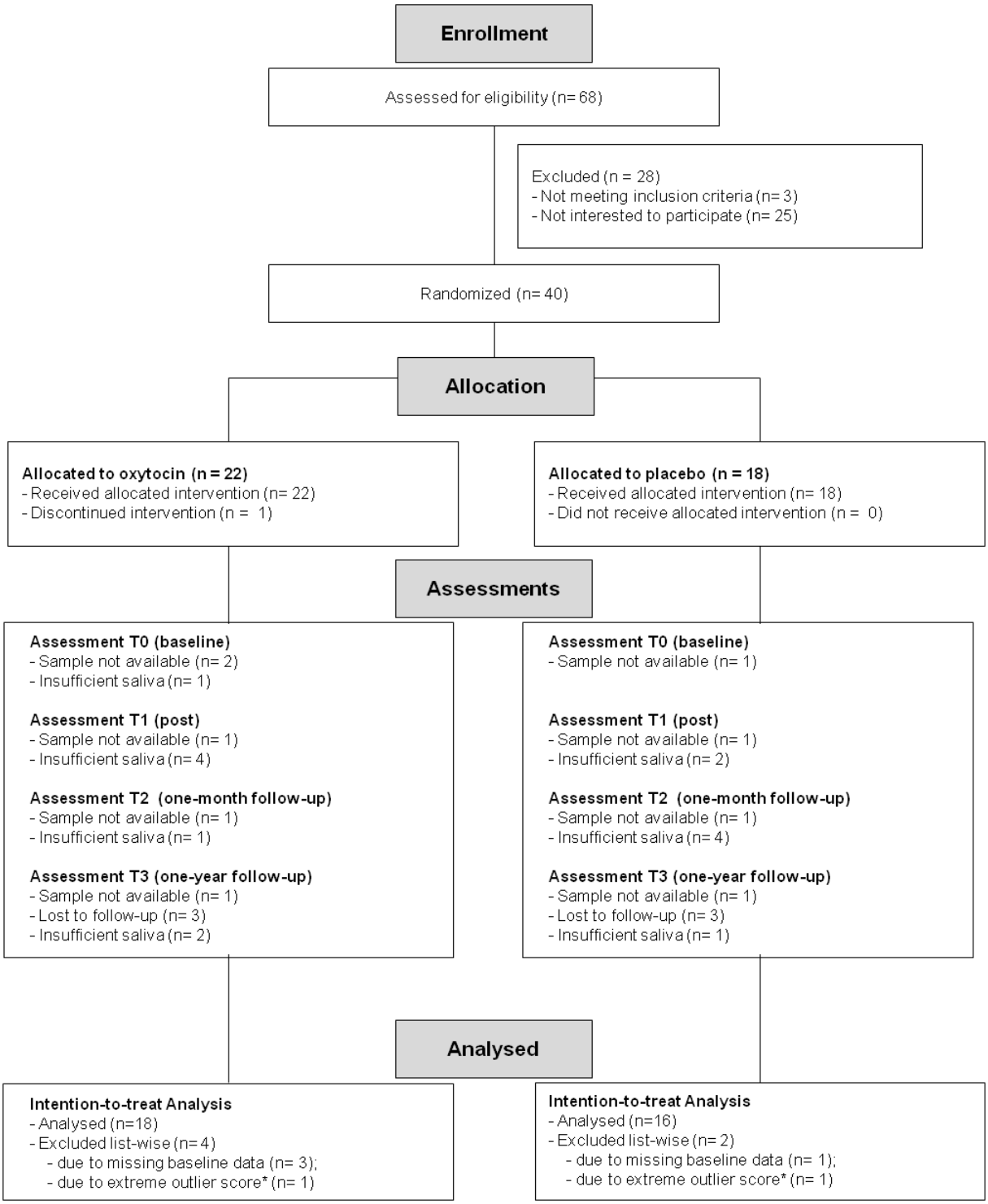
As listed in the **Consort Flow diagram** in **Supplementary Figure 1**, data of three participants of the OT group and one of the PL group were excluded list-wise, due to missing baseline data (n=1: insufficient saliva; n=3: sample not available). Additionally, data of one participant of the OT group and one of the PL group were identified as extreme outliers and were excluded from the analyses (six inter-quartile ranges (Q3-Q1) below or above the first (Q1), respectively third (Q3) quartile). At session T1, salivary OT data were not available for six participants (all,

insufficient saliva); at session T2, for five participants (all, insufficient saliva); and at session T3, for ten participants (n = 4: insufficient saliva; n=6: lost to follow-up, hence no sample available).

For the majority of participants, the saliva collections took place between 17-21h (mean 17:30h ± 2.65 SD). To assess potential variations in salivary OT levels due to diurnal variations, partial correlation analyses (Pearson) were performed between the time of collection and OT concentrations (corrected for 'assessment session': T0, T1, T2, T3). However, no significant relationships were revealed across groups ($r_{(136)}$= -.086; p= .32) or for each group separately (OT: $r_{(72)}$= -.028; p= .82) (PL: $r_{(64)}$= -.17; p= .18).

Supplementary Figure 1

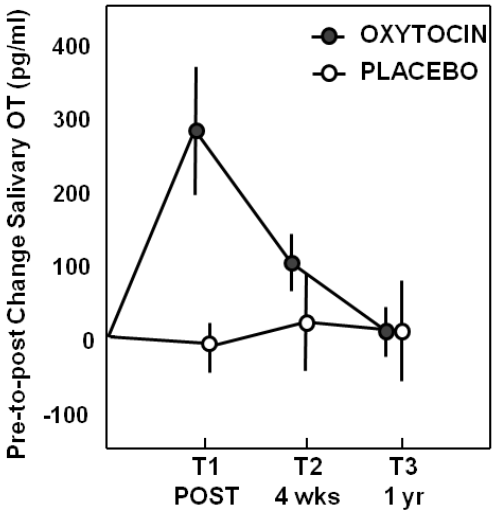
CONSORT Flow diagram. Salivary samples were collected at baseline (T0); after the four-week (oxytocin/ placebo) treatment (T1); and at two follow-up sessions, four weeks (T2) and one year after cessation of the treatment (T3). Data were analysed using an intention-to-treat format with last-observations-carried-forward to replace missing data. Data of three participants of the oxytocin group and one of the placebo group were excluded list-wise, due to missing baseline data. Additionally, data of one participant of the oxytocin group and one of the placebo group were identified as extreme outliers and were excluded from the analyses (*six inter-quartile ranges (Q3-Q1) below or above the first (Q1), respectively third (Q3) quartile).



Supplementary Figure 2

Treatment-induced changes in salivary oxytocin levels (on data without imputation of missing values).

Treatment-induced pre-to-post changes in salivary oxytocin levels are visualized separately for each treatment group (oxytocin, placebo) and assessment session, i.e., at the post session (T1), and the four-week (T2) and one-year (T3) follow-up sessions. Vertical bars denote +/- standard errors.



Mean pre-to-post change scores (in pg/ml) are listed separately for each treatment group (oxytocin, placebo) and assessment session (T1, T2, T3).

		Oxytocin				Placebo			Between-group difference
Salivary OT	N	Mean ± SD	*t*-value	*p*	N	Mean ± SD	*t*-value	*p*	Cohen's *d*
Post (T1)	14	281.13 ± 323.56	3.25	0.0063	14	-10.67 ± 82.95	-0.48	0.64	1.24
Four-week follow-up (T2)	18	102.32 ± 164.11	2.65	0.017	12	22.22 ± 229.23	0.34	0.74	0.40
One-year follow-up (T3)	12	8.94 ± 58.08	0.53	0.60	12	10.06 ± 237.23	0.15	0.89	0.006

T- and *p*-values correspond to single-sample *t*-tests assessing within-group pre-to-post changes separately for the oxytocin and placebo group. Cohen's *d* effect sizes of between-group differences (pre-to-post change-score$_{OT}$ –pre-to-post change-score$_{PL}$)/pooled SD) are reported (small effect: 0.2; medium effect: 0.5; large effect: 0.8).

Supplementary Table 1

Demographic and clinical characteristics of participants randomized to receive oxytocin or placebo.

	Oxytocin	Placebo	T-value	p-value
Number of participants	22	18		
Age	25.00 ± 4.86	24.00 ± 5.55	0.62	0.54
IQ				
Total IQ	102.27 ± 12.45	104.61 ± 21.59	-0.43	0.67
VIQ	105.57 ± 9.27	108.72 ± 16.83	-0.74	0.47
PIQ	104.76 ± 18.35	102.39 ± 22.90	0.36	0.72
ADOS(2)				
Total	7.18 ± 4.22	8.06 ± 4.26	-0.65	0.52
Communication	2.05 ± 1.40	2.39 ± 1.54	-0.74	0.46
Social interaction	4.82 ± 3.50	5.67 ± 3.33	-0.78	0.44
Use of psychostimulant medication	6	2		
Comorbidity	8	2		

Data are shown as mean ± standard deviation. IQ = Intelligence Quotient, VIQ = Verbal IQ, PIQ = Performance IQ, ADOS(-2) = Autism Diagnostic Observation Schedule(-2). Detailed information on medication use and comorbidities is provided in **(Bernaerts et al., 2020)**.

Supplementary Table 2

Treatment-induced changes in behavior.

As reported in more detail in Bernaerts et al. (2020), we previously explored behavioral improvements as a result of the four-week OT treatment in the same patient sample in terms of social functioning (Social Responsiveness Scale - Adult version: SRS-A), repetitive behaviors (Repetitive Behavior Scale – Revised: RBS-R) and attachment avoidance (State Adult Attachment Measure: SAAM).

- The SRS-A (self-report) (64 items) (Constantino et al., 2003) comprises four subscales examining social communication, social awareness, social motivation and rigidity/repetitiveness, using a four-point Likert-scale.
- The RBS-R (self-report) (43 items) (Lam and Aman, 2007) examines a heterogeneous set of repetitive behaviors including stereotypic behavior, self-injurious behavior, compulsive behavior, ritualistic behavior, sameness behavior and restricted interests behavior, using a four-point Likert-scale.
- The SAAM (self-report) (Gillath et al., 2009) comprises three subscales, of which one subscale assesses attachment avoidance (e.g., “If someone tried to get close to me, I would try to keep my distance”) (7 items) using a seven-point Likert-scale.

In short, behavioral improvements were evident immediately after treatment (T1) and until four weeks (T2) and one year (T3) post-treatment in repetitive behaviors (RBS-R) and feelings of avoidant attachment (SAAM). While the oxytocin group also reported improvements in social symptoms (SRS-A), these improvements were not treatment-specific (i.e., comparable improvements were evident in the placebo group).

The table below lists for each questionnaire the mean pre-to-post change scores separately for each treatment group (oxytocin, placebo) and assessment session (T1, T2, T3).

	Oxytocin				Placebo				Between-group difference
	N	Mean ± SD	*t*-value	*p*	N	Mean ± SD	*t*-value	*p*	Cohen's *d*
Post (T1)									
SRS-A	22	-5.55 ± 11.40	-2.28	0.033	18	-1.06 ± 10.01	-0.45	0.66	-0.42
RBS-R	22	-4.77 ± 6.47	-3.46	0.002	17	-1.76 ± 4.75	-1.53	0.15	-0.63
SAAM avoidance	22	-0.40 ± 0.71	-2.63	0.016	18	0.06 ± 0.98	0.24	0.81	-0.61
Four-week follow-up (T2)									
SRS-A	22	-5.64 ± 12.57	-2.10	0.048	18	-7.67 ± 12.09	-2.69	0.015	0.22
RBS-R	22	-4.91 ± 6.33	-3.64	0.002	17	-2.35 ± 3.43	-2.83	0.012	-0.50
SAAM avoidance	22	-0.38 ± 0.70	-2.58	0.018	18	-0.06 ± 0.76	-0.35	0.73	-0.53
One-year follow-up (T3)									
SRS-A	22	-8.59 ± 20.95	-1.92	0.07	18	-6.72 ± 21.01	-1.36	0.19	-0.12
RBS-R	22	-4.91 ± 9.46	-2.43	0.02	17	-0.41 ± 4.27	-0.40	0.70	-0.98
SAAM avoidance	22	-0.52 ± 1.18	-2.07	0.05	18	0.0 ± 0.75	0.00	1.00	-0.80

SRS-A = Social Responsiveness Scale adult version, RBS-R = Repetitive Behavior Scale – Revised, SAAM = State Adult Attachment Measure, Negative scores indicate pre-to-post improvement.
T- and *p*-values correspond to single-sample *t*-tests assessing within-group pre-to-post changes separately for the oxytocin and placebo group. Cohen's *d* effect sizes of between-group differences (pre-to-post change$_{OT}$ – pre-to-post change$_{PL}$)/pooled SD) are reported where 0.2 is indicative of a small effect, 0.5 a medium effect and 0.8 a large effect.

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