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Oxytocin and state attachment responses to secure base support after stress in middle childhood

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ABSTRACT

We tried to replicate the finding that receiving care increases children's oxytocin and secure state attachment levels, and tested whether secure trait attachment moderates the oxytocin and state attachment response to care. 109 children (9-11 years old; M = 9.59; SD = 0.63; 34.9% boys) participated in a within-subject experiment. After stress induction (Trier Social Stress Test), children first remained alone and then received maternal secure base support. Salivary oxytocin was measured eight times. Secure trait and state attachment were measured with questionnaires, and Secure Base Script knowledge was assessed. Oxytocin levels increased after receiving secure base support from mother after having been alone. Secure state attachment changed less. Trait attachment and Secure Base Script knowledge did not moderate oxytocin or state attachment responses to support. This might mean that, regardless of the attachment history, in-the-moment positive attachment experiences might have a beneficial effect on trait attachment development in middle childhood.

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According to attachment theory, with a focus on how children develop trust in the availability of parental support, care-related interactions feed into expectations about parental support (Bowlby, 1969). However, the processes explaining what exactly happens during such care-related events remain little understood, in spite of social, professional, and (mental) health implications later in life (Cassidy & Shaver, 2002). Attachment theory proposes individual differences in whether or not children develop secure attachment, corresponding to trust in parental support. Secure attachment development seems to reflect a learning process whereby each single care-related interaction affects children's expectations about their attachment figures' future support (Bosmans et al., 2020). Subsequently, it is suggested that

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children who repeatedly experience effective care during stress are more likely to develop secure attachment (De Wolff & Van Ijzendoorn, 1997; Vandevivere et al., 2018). Inversely, inconsistent or absent care during stress might lead to less secure attachment development (e.g. Ainsworth et al., 1978; Verhage et al., 2016; Verhees et al., 2021).

To date, it is unclear which processes are at play during these single learning events. Endocrinological system responses activated by care have increasingly been suggested to be relevant for attachment learning (Bosmans et al., 2020; Feldman, 2012). A putative endocrinological correlate of attachment is oxytocin (Feldman & Bakermans-Kranenburg, 2017). Oxytocin is a neurohormone produced in the hypothalamus and released from the posterior pituitary gland (Vaidyanathan & Hammock, 2017). Its most commonly known function is to induce labor and breastfeeding, but studies have also found oxytocin to be related to social affiliation (Winslow & Insel, 2002), and the establishment of social and attachment bonding (Swain et al., 2014). The recently postulated learning theory of attachment (Bosmans et al., 2020) suggests that experiences of care after stress are accompanied by a release of oxytocin. Oxytocin release is supposed to translate to positive states and feelings of being loved and cared for (Feldman, 2012). These positive states are called secure attachment states, reflecting what has been described by Sroufe and Waters (1977) as a sense of felt security (Bosmans et al., 2020). State attachment security (or insecurity) refers to the in-the-moment experience that one can (or cannot, respectively) trust in the availability of the caregiver (Bosmans et al., 2020). State attachment is seen as a more variable component of attachment, dependent upon variation in the caregiving environment. It changes positively in response to positive, care-related interactions, and negatively to conflict and absence of care during distress (Bosmans et al., 2020; Verhees et al., 2021).

Repeated exposure to single care-related learning events has the potential to alter children's expectations about the caregivers' capacity and availability as a support figure (Bosmans et al., 2020). This affects individual differences in attachment at trait-like level, which one could see as the more stable component of attachment (Bosmans et al., 2020). More positive support-related experiences result in a general or trait-like belief that one can trust the caregiver as a support figure (i.e. secure trait attachment). Moreover, a cognitive script reflecting an expected chain of events during care-related interactions will develop. This so-called Secure Base Script (SBS) starts with exposure to distress, followed by seeking proximity to the attachment figure. This elicits caregiver support and the resolution of distress (T. E. Waters & Roisman, 2019; H. S. Waters & Waters, 2006). These experiences get stored in the brain as an information processing heuristic, with individuals with more supportive caregiver experiences developing more knowledge about the SBS (T. E. Waters & Roisman, 2019). Several longitudinal studies demonstrate that exposure to supportive caregiving experiences is essential for SBS development (e.g. T. E. Waters & Roisman, 2019; Waters, Ruiz & Roisman, 2017). Children with more SBS knowledge are more likely to expect supportive care from their attachment figures in the future (Van IJzendoorn & Bakermans-Kranenburg, 2019; H. S. Waters & Waters, 2006). Although trait secure attachment (i.e. trust) and SBS knowledge can therefore be considered as more trait-like features of the attachment construct, they are still open for updating (T. E. Waters et al., 2022), suggesting some nuance in the differentiation between state and trait attachment (Bosmans et al., 2020).

The current study's first aim was to test a critical implication of the learning theory of attachment, namely that oxytocin levels and, relatedly, secure state attachment levels would increase after care. Preliminary support for such a care-related oxytocin effect was found in two studies. First, Seltzer et al. (2010) exposed children between 7 and 12 years old to the Trier Social Stress Test for Children (TSST-C; Kirschbaum et al., 1993) to induce stress. Afterwards, children were assigned to one of three conditions: 1) a reunion with their mother who comforted them verbally and physically, 2) comfort by their mother provided over the phone, or 3) watching a neutral movie clip alone as a control condition. Urinary oxytocin was measured before the TSST-C and after exposure to one of the conditions. Oxytocin levels increased after comfort (conditions 1 and 2), but not after watching a neutral movie clip alone. Second, Brockington et al. (2021) randomly assigned stressed hospitalized children to a support condition (during which they were told stories, assumed to have a comforting effect) and a distraction condition (during which children were asked to solve amusing riddles). Again, results suggested that being exposed to a more comforting caretaker during stress increased oxytocin levels. However, these studies' between-subject designs provided only partial evidence for the causal effect of receiving care on oxytocin, as their findings could have reflected group differences rather than the direct effect of the manipulation. To increase confidence in the causal effect of support on children's oxytocin levels, a within-subject design is necessary. Since contemporary theory and research suggest that oxytocin responds to attachment-related situations, our first research aim was to replicate the studies of Seltzer et al. (2010) and of Brockington et al. (2021) with a within-subject design, to minimize effects related to interperson variability. Furthermore, within-subject designs are more powerful than between-subject designs (Van IJzendoorn & Bakermans-Kranenburg, 2016). Thus, we compared children's oxytocin responses to being alone after stress to their oxytocin responses after receiving maternal secure base support. We hypothesized that oxytocin levels after being alone following stress would be lower than oxytocin levels after receiving secure base support from mother.

At the level of secure state attachment, research suggests that we might detect subtle changes in state attachment using a within-subject design (Bosmans et al., 2014; Verhees et al., 2022). Vandevivere et al. (2018) used a mixed between-within-subject design to manipulate secure state attachment. In their study, children between 9 and 13 years old watched a negative mood induction video after which they were assigned to one of three conditions: 40 children received secure base support from their mother, 40 children were only in the physical presence of their mother, and 40 children stayed alone in a room. Between-group results indicated that children who received secure base support showed significantly higher levels of secure state attachment compared to children who were only in the physical presence of their mother or alone. After that, all children received secure base support from their mother and differences in secure state attachment disappeared. Therefore, we aimed to replicate the findings from Vandevivere et al. (2018) in a strictly within-subject design. In line with the latter study, we hypothesized that children would show higher levels of secure state attachment after receiving secure base support from mother compared to after being alone following stress.

The current study's second, more exploratory aim was to investigate whether individual differences in oxytocin responses to care and secure state attachment changes depended on children's level of secure trait attachment and SBS

knowledge. The theory proposes that secure attachment development is characterized by upward spiral dynamics (Fredrickson, 2013). The upward spiral model suggests that repeated positive psychological (e.g. supportive attachment experiences) and biological (e.g. oxytocin release) experiences accumulate over time. This, in turn, would increase similar positive emotions during subsequent experiences. Following from this, oxytocin and secure state attachment responses to care might be enhanced by more positive past care-related experiences (Kok & Fredrickson, 2010). This hypothesis has never been directly tested. Preliminary support for an upward spiral attachment history effect on oxytocin has been found by Pierrehumbert et al. (2011) who showed that higher levels of secure trait attachment were associated with stronger oxytocin responses to stress.

Likewise, it seemed plausible that children with higher levels of secure trait attachment or SBS knowledge would show a greater increase in oxytocin during maternal secure base support after stress compared to children with lower levels of secure trait attachment or SBS knowledge. At the level of state attachment, Verhees et al. (2021) found in a diary study that more secure trait attachment was linked to more stability in state attachment after receiving support for stress (Verhees et al., 2021). Also, Cuyvers et al. (2022) found that secure trait attachment was linked to enhanced secure state attachment increases in response to a secure attachment prime. However, none of these studies directly tested secure state attachment levels immediately after receiving care. Moreover, both studies included several moderator analyses and only a few of these emerged as significant. This suggests that the moderating effect of trait attachment on state attachment changes might be harder to find. Hence, the current study examined the moderating effect of secure trait attachment on secure state attachment responses to care. Based on the previous studies, we hypothesized that secure state attachment would increase more following maternal secure base support after stress in children with higher levels of secure trait attachment or SBS knowledge than in children with lower levels of secure trait attachment or SBS knowledge.

In sum, the current study had two research aims. The first aim was to replicate Seltzer's et al. (2010), Brockington et al. (2021), and Vandevivere et al. (2018) observations that care after exposure to stress has an immediate, positive effect on children's oxytocin and secure state attachment levels. Adding to the literature, we tested these effects using a within-subject design. The second, more exploratory aim was to test whether trait attachment security or SBS knowledge moderated the change in oxytocin and secure state attachment following secure base support from mother after stress. We conducted the current study in middle childhood because in this developmental period, biological and social factors undergo important transitions (Del Giudice, 2015). Although other periods such as infancy or toddlerhood are also characterized by important developmental changes (Feldman, 2015), to date, no well-validated tools exist to measure SBS knowledge during those early life stages. In addition, the TSST-C is not appropriate to use with children of younger ages. Therefore, we opted to conduct the current study in middle childhood.

Methods

Participants

The current study was preregistered at Open Science Framework (https://osf.io/f8yw7/), approved by the medical ethics committee of UZ Leuven (S63043) and funded by the FWO (G075718N). Deviations from the preregistration can be found on OSF as well (https://osf.io/3fbvn/?view_only=d2652f207d7b4ae7b77b13717d52a4b3). We recruited 200 children from 4th and 5th grades of elementary schools in Flanders, Belgium. However, due to the COVID-19 pandemic, the experiment had to be stopped after data collection for 109 participants. To participate, children were required to have an age between 8 and 12 years old and to be fluent in Dutch speaking, and their mothers were also required to speak Dutch fluently. The final sample consisted of 38 boys, 57 girls, and 14 participants for whom data on sex was missing. They were between 9 and 11 years old ($M = 9.59$; $SD = .63$). Sixteen participants did not provide data on all questionnaires, but other information was present, so they were not excluded from the analyses. In the final sample, 87 children were Belgian, and seven had other nationalities. Of these children, 66 lived in their original families, 15 lived in newly composed families after divorce of their biological parents, 11 lived in a one-parent family and for 17 children this information was missing. Mother's highest educational level was master's degree for 22 participants, bachelor's degree for 41 participants, secondary school degree for 27 participants, and elementary school degree for 1 participant. For 18 mothers, data on educational level were missing. Data were collected at school, where both mother and child were present. Both signed an informed consent form and children received a Dreamland voucher of 30 euros as a reward for their participation.

Materials

Stress induction

In order to induce stress, participants took part in an adapted version of the Trier Social Stress Task for children (TSST-C; Buske-Kirschbaum et al., 1993). In this test, children completed two tasks in front of a jury: a counting backwards task in which they counted back in steps of 5 during 5 min starting at a randomly chosen number (1027), and a presentation about themselves and their good and bad qualities in which they had to present themselves as a very popular student for 5 min. Participants were given 5 min to prepare their presentation. The jury member remained neutral without giving social feedback (e.g. nodding, smiling, etc.) and only gave children feedback when they did something wrong during the counting backwards task (i.e. *That was incorrect, can you please start over?*). If children finished their presentation before 5 min had passed, the jury asked some extra questions (e.g. *Can you tell me if it is important for you to be popular and why?*). Children were told this task was part of a school contest and was therefore videotaped such that their presentation could be shown to children from other schools who would then vote for the best contestant. In reality, this was a disguise used to create social-evaluative stress, because it is believed that social cues elicit strong biological responses (e.g. Jobst et al., 2015). As a manipulation check, we observed the videotaped materials for

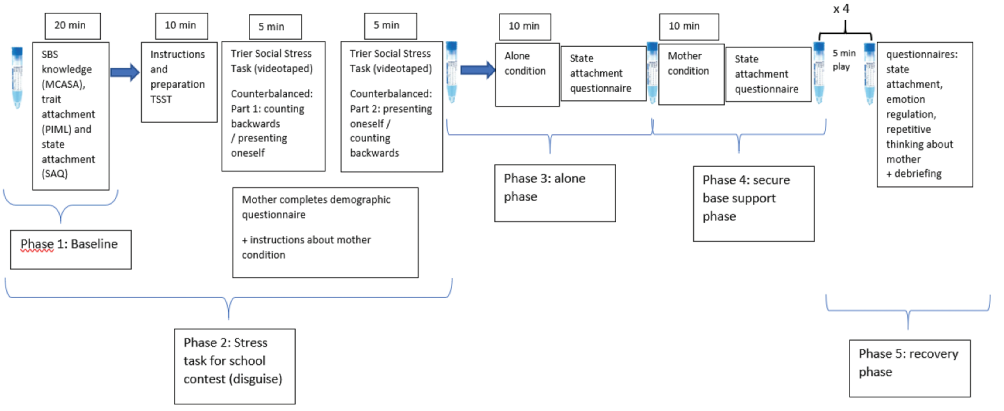

Figure 1. Study design: assessments and time-line. Note: *Salivettes represent measurements of oxytocin.*

63 children. Eighty-four percent of the children showed verbal or non-verbal signs of stress, and upon the mother's return, all children told their mothers about what happened in their absence. We may conclude that the procedure was experienced as stressful, in line with meta-analytic results (Seddon et al., 2020).

Oxytocin

During the experiment, the children were asked eight times to chew for 2 min on a salivette (Sarstedt ®) in order to collect saliva from which levels of oxytocin could be determined (see Figure 1). With Oxytocin Enzyme Immunoassay Kits (Arbor Assays®), children's salivary oxytocin levels were determined in pg/mL. All data collection moments were planned in the morning between 8:00 AM and 12:00 PM in order to keep circadian variation in oxytocin levels as similar as practically possible across children.

Some oxytocin levels had biologically impossible raw values for some assessments, probably due to a measurement error (>10,000 pg/mL) (see Table 1). No child provided only impossible levels. Therefore, we decided to exclude impossible values from the analyses without excluding participants. We can assume normality based on the central limit theorem and a reasonable sample size. However, due to the exclusion of impossible values, oxytocin levels were Log10 transformed in order to approximate normal residual distributions.

Table 1. Descriptives of oxytocin levels (pg/mL) and exclusion of impossible values.

Oxytocin measure	Impossible values (excluded)	n after exclusion	Mean	SD
1	7.7%	80	285.16	579.01
2	12.2%	74	185.80	337.16
3	13.2%	75	106.29	88.40
4	4.4%	85	293.26	548.48
5	3.3%	86	189.26	422.72
6	8.8%	80	269.06	785.04
7	13.2%	77	165.10	354.68
8	7.7%	82	124.35	156.81

Secure state attachment

Secure state attachment, the more variable component of attachment, was measured four times during the experiment (see Figure 1) with the State Attachment Questionnaire (SAQ). This questionnaire comprises 10 items gauging for at-the-moment secure attachment expectations about mother. All items were derived from previous diary studies by Bosmans et al. (2014). Children rated all items on a visual analogue scale ranging from 0 (not at all) to 110 (very much). Items started with "*At this moment, I feel that . . . "* and were followed by an attachment-related statement such as "*I would ask my mother for help if I had a problem*." Three items were reverse coded. Then, the mean secure state attachment score was calculated per measurement point. Cronbach's *α* for the first measurement point = .67, for the second measurement point = .77, for the third measurement point = .74, and for the fourth measurement point = .75. Because the distribution of secure state attachment data was skewed, we conducted sensitivity analyses with Log10 transformed values, but the results remained similar. Since analyses with untransformed data are more comparable to Vandevivere et al. (2018) study, we report these in the current study.

Secure base script knowledge

To measure the extent to which children have developed a secure base script (SBS), the Middle Childhood Attachment Script Assessment (MCASA; T. E. Waters et al., 2015) was administered. The validity of the measure has been established in several longitudinal studies linking prior caregiving experiences to Attachment Script Assessment scores (T. E. Waters & Roisman, 2019). This assessment is a storytelling task in which children are instructed to tell five stories based on a story title and word prompts. The first two stories are practice stories about attachment unrelated themes ("Snowy day" and "Biking to the park"). The other three stories include word prompts implying a storyline following the secure base script ("Scary dog in the yard," "At the beach," and "Soccer game"). Such a storyline consists of the experience of stress by the child, followed by 1) proximity seeking to mother/monitoring by mother, who then 2) provides effective care, after which 3) the child feels relieved and back on track. A score ranging from 1 to 7 (*1 = anti-script elements; 3 = no meaningful attachment elements; 7 = all three secure base script elements extensively present*) was given by two trained coders for each attachment-related story, based on the extent to which the three secure base script elements were present. The two independent coders rated 10 stories simultaneously for the "At the beach" and "Soccer game" stories and achieved an Intraclass Correlation Coefficient (ICC) of at least .80 ($ICC_{at\ the\ beach} = .80$; $ICC_{soccer\ game} = .90$). For the "Scary dog in the yard" story, the ICC was not sufficiently high after double coding of 10 stories, so 10 extra stories were coded after which the $ICC_{scary\ dog\ in\ the\ yard}$ was .93 for these 10 extra stories. Agreement scores were assigned to the first set of 10 stories from "Scary dog in the yard." After that, all stories were rated by one coder. The final secure base script knowledge variable was calculated as the mean score over the three attachment-related stories for each child ($M = 3.5$; $SD = 0.53$; $n = 107$). Cronbach's *α* was .70.

Secure trait attachment

In order to measure the level of secure trait attachment, children completed the trust subscale of the People In My Life questionnaire (PIML, Ridenour et al., 2006) at the beginning of the experiment. The validity of the measure has been suggested, among others, in studies showing links between trust and observed attachment behavior

(Bosmans et al., 2015). This subscale consists of 10 statements about attachment to their mother (e.g. "*My mother accepts me the way I am")* that children rated from 1 (*not at all true*) to 4 (*completely true*) ($M = 3.71$; $SD = 0.30$, $n = 95$). Cronbach's α was .77.

Control variables

Health and physical activity

Since oxytocin levels might be influenced by various health correlates (Mitra et al., 2010) or activities preceding saliva sampling (Yüksel et al., 2019), we controlled for this with a newly composed questionnaire, the Health Background Questionnaire (HBQ). This questionnaire consisted of six separate questions for which no overall score is calculated. All questions are answered with 1 = yes or 0 = no with the option to specify if yes. Questions asked about whether children ate or drank something sweet before the experiment (87.2% yes; $n = 94$), whether they performed intensive physical activity preceding the experiment (20.2% yes; $n = 94$), whether they brushed their teeth before the experiment (64.5% yes; $n = 93$), whether they were on medication (11.7% yes; $n = 94$), whether they were in therapy because of a psychiatric disorder (9.7% yes; $n = 93$), and whether they had a diagnosed endocrine disorder (0% yes; $n = 94$). Since no children had a diagnosed endocrine disorder, we excluded this item as a possible confounding variable. The other items were investigated for their correlation with oxytocin in order to decide to include them as control variables (see Table S1 in Supplementary file 1). Only brushing teeth and medication were correlated with oxytocin levels and were therefore included as control variables in analyses with oxytocin as the dependent variable. Medication use was also correlated with secure state attachment levels and therefore also included as a control variable in analyses with secure state attachment as the dependent variable.

Cortisol

Cortisol (µg/dl) was measured in order to assess the biological level of activation or stress in children during the experimental procedure. Cortisol levels were assessed in the same saliva samples as used for oxytocin, with the Cortisol ELISA kits (Enzo Life Sciences®). The samples were thus taken during the morning to keep circadian fluctuations as similar as possible across participants and stored at −20 degrees. Cortisol is shown to have a time lag of about 15 min before it is detectable in saliva (Miller et al., 2016). However, since we do not have a saliva sample exactly 15 min after the ending of the TSST-C, we regard the sample 10 min after the ending of the TSST-C as corresponding to the stress level during the TSST-C. Therefore, we included cortisol levels 10 min after the ending of the TSST-C as a control variable in all further analyses to account for the level of stress children experienced.

Other measures

The current study was embedded in a larger project in which other measures were completed as well, like the Strengths and Difficulties Questionnaire (SDQ; Muris et al., 2003), Highly Sensitive Child Scale (HSC; Pluess et al., 2018), Parenting questionnaires (APAR & BPAR; Louvain Adolescent Perceived Parenting Scale, Delhaye et al., 2012;

Perceptions of Parents Scale; Grolnick et al., 1991), Behavioral Inhibition System-Behavioral Activation System questionnaires (BIS-BAS; Luman et al., 2012), an adapted emotion regulation scale (FEEL-KJ; Braet, 2013) and more demographic information of the child. These measures are beyond the scope of the current study.

Procedure

Invitation letters were distributed through schools in Flanders. When families indicated interest in participating in the study, the researcher contacted the mother and invited both child and mother to come to the child's school during a school morning. The total experiment lasted approximately 2 h. First, we explained the experiment to mother and child after which they signed the informed consent form. During this baseline phase, children completed baseline measures (see Figure 1). The mother was brought to another room and the child stayed with the researcher in a separate room. Then, the first saliva sample was collected. After that, the child completed the MCASA storytelling task and PIML questionnaire. Last, secure state attachment was measured.

Then, in the stress phase of the experiment, children were subjected to the adapted TSST-C task. They were instructed to present themselves as very popular in front of a jury and a video camera, such that it could be shown later to other children from a different school. Participants received 5 min to prepare their presentation. Further, they were instructed to do a counting backward task. The order of both tasks was counterbalanced over participants. As part of a school contest, they were told that children from another school would select the participant that seemed most popular. In reality, this was a cover story meant to create social stress. The jury member did not give any emotional feedback and only stimulated or corrected the participant during the tasks, according to a prescribed protocol. Both tasks lasted 5 min. Immediately after the TSST-C, saliva sample 2 was collected. Meanwhile in another room, mothers completed a demographic questionnaire, the health background questionnaire, and some other questionnaires not included in the current study.

Then, during the alone phase of the experiment, the researcher left the room and the child stayed alone in the room for 10 min. Meanwhile, the mother was instructed about the secure base support she had to provide to her child in the upcoming phase. After 10 min, saliva sample 3 was collected. The child also completed the SAQ again.

Subsequently, during the secure base support phase of the experiment, the mother was brought into the room and the researcher left again. The mother provided secure base support to her child, asking about the task and saying things like "*If I hear what you had to do, I would also feel stressed*". As a manipulation check, we observed the videotaped materials of 63 mother-child dyads. All but four of these mothers (94%) showed support saying things like *"If I were you, I would also feel stressed"* and the same percentage of mothers said things like *"It doesn't matter if you did not perform well"* or *"It is over now, you can relax, it's okay."* We can conclude that mothers followed the researcher's instructions. After 10 min, the researcher entered the room again and saliva sample 4 was collected. Then, the mother left again and the child completed the SAQ again.

During the recovery phase of the experiment, children engaged in free play in the presence of the researcher. Coloring books, puzzles, single player games, etc., were provided. Every 5 min, four more saliva samples were collected. After 20 min, the free

play ended and participants completed the SAQ again, as well as the FEEL-KJ about emotion regulation during the alone phase. Finally, the mother was brought back into the room, and both mother and child were debriefed about the study and the cover story. In the end, the children received a reward of 30 euros worth of store credit from Dreamland.

Statistical analyses

The current study was preregistered at OSF (https://osf.io/nza57).[1] Data were analyzed using R version 4.2.2 and IBM SPSS Statistics Version 28. We used Pearson correlations between all main variables in preliminary analyses, with a significance level of $\alpha = .05$. Next, we conducted multilevel mixed model analyses using the nlme package version 3.1 in R (Pinheiro et al., 2017). The advantage of using multilevel mixed models is that both within-subject and between-subject variability are taken into account. In all models, we included experimental phase as a random factor such that it could predict oxytocin levels at the five different phases (1 = baseline phase, 2 = stress phase, 3 = alone phase, 4 = secure base support phase, 5 = recovery phase). To avoid convergence issues when fitting the models, we used a general-purpose optimization based on Nelder–Mead's implementation Optim (Nash, 2022).

Results

Preliminary analyses

First, we inspected the data for missing values. Eighteen children missed all oxytocin data due to technical reasons (e.g. not enough saliva to determine oxytocin), leaving in total 639 valid oxytocin samples. Apart from biologically impossible oxytocin values, all other outliers from all variables were kept in the analyses, because they might provide meaningful information. Since the current study uses mixed model analyses, missing data in response variables can be handled. However, in sensitivity analyses, missing data in covariates (sex, teeth brushing, medication use, and cortisol after TSST-C) were completed using multiple imputations. The sensitivity analyses can be found in Supplementary file 2.

Table 2 presents the correlation matrix of all main variables. No significant correlations between secure trait attachment or SBS knowledge with oxytocin levels at any point in time emerged. However, secure trait attachment was correlated with secure state attachment levels during the whole procedure, and secure state attachment levels were correlated to each other. This is in line with findings from previous research (e.g. Cuyvers et al., 2022). In general, secure state attachment levels did not correlate with oxytocin levels. Furthermore, oxytocin levels at different points in time were correlated, suggesting that they were reliable.

Correlations between oxytocin and secure state attachment levels and possible control variables (e.g. health background questionnaire or child's sex) can be found in Supplementary file 1 (Table S1). Sex did not correlate with any of the other variables and no differences in oxytocin mean levels emerged between both sexes. However, it was considered a possible confounding factor based on findings suggesting that women have higher levels of oxytocin than men (e.g. Caldwell, 2018). In addition, brushing teeth and medication use were included as control variables in analyses with oxytocin as the