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

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Abstract

Background and objectives. Attachment developmental processes remain little understood. The current study tried to replicate the finding that receiving care increases children's oxytocin and secure state attachment levels and tested whether prior attachment history moderates the oxytocin and state attachment response to care.

*Method*s. 109 children between 9 and 11 years old (*M* = 9.59; *SD* = 0.63; 34.9% boys) participated in a within-subject experiment in which stress was induced. After stress induction, 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, SBS knowledge was measured with a narrative task.

Results. Oxytocin levels increased after receiving secure base support from mother compared to being alone following stress. Secure state attachment levels did not change. Also, secure trait attachment and SBS knowledge did not moderate oxytocin or state attachment response to receiving support from mother.

Introduction

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, 2016). 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 children who repeatedly experience effective care during stress are more likely to develop secure attachment (De Wolff & van IJzendoorn, 1997; van der Voort et al., 2014; Vandevivere et al., 2018). Inversely, inconsistent or absent care during stress might lead to less secure attachment development (e.g. Verhees et al., 2021).

To date, it remains unclear which processes are at play during these single learning events. Endocrinological system responses activated by care are increasingly suggested to be relevant for attachment learning (Feldman, 2012; Bosmans et al., 2020). A putative endocrinological correlate of attachment is oxytocin (Buchheim et al., 2009; Feldman & Bakermans-Kranenburg, 2017). Oxytocin is a neurohormone produced in the hypothalamus and released from the posterior pituitary gland (Vaidyanathan & Hammock, 2016). Its most commonly known function is to induce labor and breastfeeding, but studies also found oxytocin to be related to social affiliation (Winslow & Insel, 2002), and the establishment of social and attachment bonding (Swain et al., 2014). A recently postulated learning theory of attachment (Bosmans et al., 2020) suggests that experiences of care after stress are accompanied by 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 state attachment, reflecting what has been described by Sroufe and Waters (1977) as a sense of felt security (Bosmans et al., 2020). State attachment refers to in-the-moment experience that one can trust in the

availability of the caregiver (Bosmans et al., 2020). State attachment is seen as a more variable component of attachment, in contrast to trait attachment, which reflects the more stable component of attachment (Bosmans et al., 2020; Verhees et al., 2021). When positive states are repeatedly experienced in the proximity of the caregiver, theory suggests that the rewarding feelings related to oxytocin increase after care can get associated with the caregiver. This has the potential to alter children's expectations about the caregivers' capacity and availability as a support figure (Bosmans et al., 2020), resulting in a general or trait-like belief that one can trust in the caregiver as support figure (i.e., secure trait attachment). Similarly, a cognitive script reflecting expected chains of events during care-related interactions might develop. This so-called Secure Base Script (SBS) starts with exposure to distress, followed by seeking proximity to the attachment figure. This stimulates caregiver support and the resolution of distress (Waters & Waters, 2006). Children with more SBS knowledge are more likely to expect care in the future from their attachment figures (Waters & Waters, 2006; Van IJzendoorn & Bakermans-Kranenburg, 2019).

The current study's first aim was to test a critical implication of this learning theory of attachment that oxytocin levels, and relatedly, secure state attachment levels should increase after care. Preliminary support for such a care-related oxytocin effect was found in two studies. First, Seltzer, Ziegler and Pollak (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 vocally 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 the conditions. Oxytocin levels increased after comfort (conditions 1 and 2), but not after watching a neutral movie clip alone. Second, Brockington et al. (2020) randomly assigned stressed hospitalized children to a support condition (during which they were with a caregiver who 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 comforting caretaker during stress increases oxytocin levels.

However, these studies' between-subject design 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, Ziegler, & Pollak (2010) and of Brockington et al. (2022) with a within-subject design. To do so, we compared children's oxytocin responses to being alone after stress versus their oxytocin responses after receiving secure base support from their mother. 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, the current study's second aim was to replicate the findings from Vandeviviere 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 aim was to investigate in the secure base support phase whether individual differences between children in their oxytocin responses to care and secure state

attachment changes depend on their level of secure trait attachment or SBS knowledge. Theory proposes that secure attachment development is characterized by upward spiral dynamics (Frederickson, 2013). The upward spiral model suggests that repeated positive psychological (e.g. secure state attachment experiences) and biological (e.g., oxytocin release) experiences accumulate over time. This, in turn, increases 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 (Burns et al., 2008; Cohn et al., 2009; Oveis et al., 2009; 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 linked 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 secure base support from mother after stress compared to children with lower levels of secure trait attachment or SBS knowledge. Verhees et al. (2021) found some support that more secure trait attachment was linked to more stability in state attachment after receiving support to stress in a diary study (Verhees et al., 2021). Also, Cuyvers et al. (2022) found some support 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 a substantial number of 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 looked at the moderating effect of secure trait attachment on secure state attachment responses to care. Based on previous studies, we hypothesized that secure state attachment would increase more during secure base support from mother 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. Research aim 1 was to replicate Seltzer, Ziegler, and Pollak's (2010), Brockington et al. (2020), and Vandevivere et al.'s (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. Research aim 2 was to test whether trait attachment security and SBS knowledge moderated the change in oxytocin and secure state attachment during 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).

Methods

Participants

The current study was preregistered at Open Science Framework (OSF; DOI 10.17605/OSF.IO/F8YW7) and approved by the medical ethical committee [redacted] Deviations from the preregistration can be found on OSF as well (https://osf.io/3fbvn/). We recruited 200 children from 4th and 5th grade of elementary schools in [redacted]. 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 fluently 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, 5 were Dutch, 1 participant was Belgian-American and 1 had the Dutch-Lithuanian nationality. Of these children, 66 lived in 'elementary' families, 15 lived in newly composed families after divorce of their biological parents, 11 lived in a one-parent family and 17 were missing. Mother's educational level was a bachelor's degree for 41 participants, for 27 participants secondary school degree, for 22 master's degree, for 1 elementary school degree. For 18 mothers data on educational level was missing. Data was collected at school, where both mother and child were present. Both

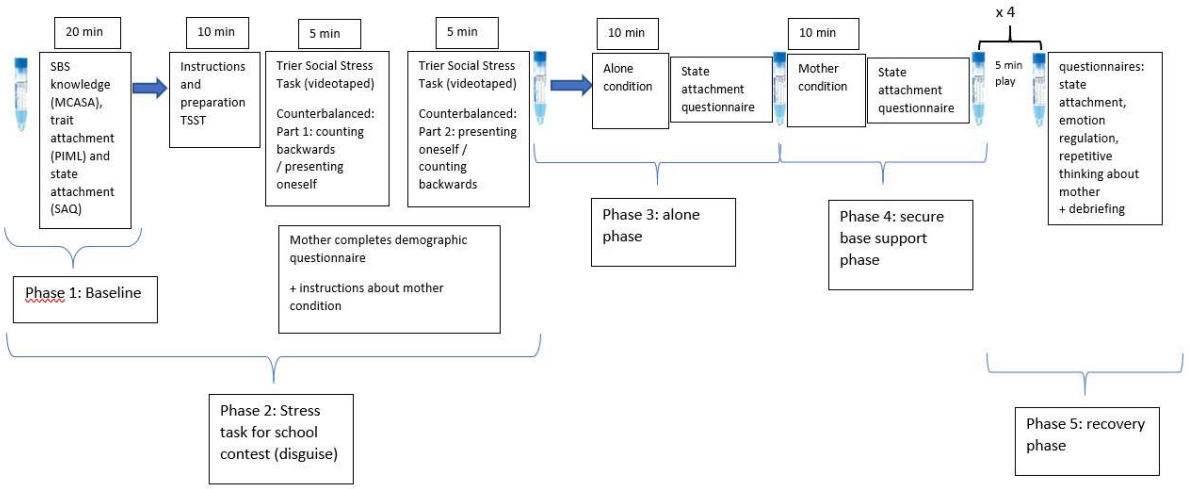
signed an informed consent form and children received a Dreamland voucher of 30 euros as a reward for their participation.

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., 1997). 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 starting at a randomly chosen number, 1027, for 5 minutes; 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 minutes. Participants were given 5 minutes to prepare their presentation. The jury member remained neutral without giving social feedback (e.g. nodding, smiling, …) and only gave children feedback when they did something wrong during the counting backwards task (e.g. *That was incorrect, can you please start over?*). If children finished their presentation before 5 minutes 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 oxytocin responds mostly to social cues (e.g. Jobst et al., 2014).

Oxytocin. During the experiment, children were asked eight times to chew for two minutes 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. These data could then be used to compare oxytocin responses to different situations within and between children. 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.

Figure 1. Study design: assessments and time-line.



Note: With every salivette presented in the figure oxytocin was measured.

Some oxytocin levels had biologically impossible raw values for some assessments, probably due to measurement error (> 10 000 pg/mL). No child provided only impossible levels. Therefore, we decided to exclude impossible values from the analyses without excluding participants. Oxytocin assessment 1: 7.7% of values were out of scale (*M* = 285.16; *SD* = 579.01; *n* = 80); Oxytocin assessment 2: 12.2% (*M* = 185.80; *SD* = 337.16; *n* = 74); Oxytocin assessment 3: 13.2% (*M* = 106.29; *SD* = 88.40; *n* = 75); Oxytocin assessment 4: 4.4% (*M* = 293.26; *SD* = 548.48; *n* = 85); Oxytocin assessment 5: 3.3% (*M* = 189.26; *SD* = 422.72; *n* = 86); Oxytocin assessment 6: 8.8% (*M* = 269.06; *SD* = 785.04; *n* = 80); Oxytocin assessment 7: 13.2% (*M* = 165.10; *SD* = 354.68; *n* = 77); and Oxytocin assessment 8: 7.7% (*M* = 124.35; *SD* = 156.81; *n* = 82). Oxytocin levels were Log10 transformed in order to approximate normal distributions.

Secure state attachment. Secure state attachment, a more variable component of attachment, was measured four times during the experiment (see Figure 1) with the State Attachment Questionnaire (SAQ). This questionnaire exists of 10 items gauging for at-the-moment secure attachment expectations about mother. All items were derived from previous diary studies by Bosmans, Van de Walle 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, a mean secure state attachment score was calculated per

measurement point. Chronbach'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 secure state attachment data were skewed, we conducted sensitivity analyses with log10 transformed values, but results remained similar. Since analyses with untransformed data are more comparable to Vandevivere et al.'s (2018) study, we report these in the current study.

Secure Base Script Knowledge. To measure the extent to which children have internalized a secure base script (SBS), the Middle Childhood Attachment Script Assessment (MCASA; Waters et al., 2015) was administered. This assessment is a storytelling task in which children were 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 implicating 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 and $ICC_{scary\ dog\ in\ the\ yard}$ 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 = .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. 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 = .30, n = 95). Cronbach's α was .77.

Control variables

Health and physical activity. Since oxytocin levels might be influenced by various health correlates or activities preceding the experiment (e.g. Mitra et al., 2010; Yüksel et al., 2019), we controlled for this with a newly composed scale, the Health Background Questionnaire (HBQ), consisting of six questions. 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 (12.8% no, 87.2% yes; n = 94), whether they performed intensive physical activity preceding the experiment (79.8% no, 20.2% yes; n = 94), whether they brushed their teeth before the experiment (35.5% no, 64.5% yes; n = 93), whether they were on medication (88.3% no, 11.7% yes; n = 94), whether they were in therapy because of a psychiatric disorder (90.3% no, 9.7% yes; n = 93), and whether they had a diagnosed endocrine disorder (100% no, 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). 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 all 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 minutes before it is detectable

in saliva (Miller et al., 2016). However, since we do not have a saliva sample exactly 15 minutes after the ending of the TSST-C, we regard the sample 10 minutes after the ending of the TSST-C as corresponding to the stress level during the TSST-C. Therefore, we included cortisol levels 10 minutes after the ending of the TSST-C as a control variable in all further analyses to account for the level of stress children had.

Other Materials

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., 2002), the Highly Sensitive Child Scale (HSC; Pluess et al., 2018; Weyn et al., 2019), Parenting questionnaires (APAR & BPAR; Parental Behavior Scale Short, Van Leeuwen et al., 2018; 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 et al., 2005) 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 two hours. First, we explained the experiment to mother and child after which they signed the informed consent form. During this 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 five

minutes 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 five minutes. 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 minutes. Meanwhile, the mother was instructed about the secure base support she had to provide to her child in the upcoming phase. After 10 minutes 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*'. After 10 minutes, 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 minutes, four more saliva samples were collected. After 20 minutes, the free play ended and participants completed the SAQ again, as well as the PTMQ about repetitive thinking about mother and 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. At the end, children received a reward of 30 euros worth of store credit from a toy store.

Statistical analyses

The current study was preregistered at OSF (DOI 10.17605/OSF.IO/F8YW7)[1]. Data were analyzed with R 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 in R (Pinheiro & Bates, 2000). The advantage of using multilevel mixed models is that both within-subject and between-subject variations 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 use general-purpose optimization based on Nelder–Mead's implementation Optim (Nash, 2019).

Results

Preliminary analyses

First, we inspected the data for missing values. In total, all oxytocin data were missing from 18 children due to technical reasons (e.g., not enough saliva to determine oxytocin), leaving in total 639 valid oxytocin samples. Apart from the biologically impossible oxytocin values, all other outliers from all variables were kept in the analyses, since they might include meaningful information.

Table 1 presents the correlation matrix of all main variables in the analyses. 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. Further, oxytocin levels at different points in time were correlated, suggesting that they were reliable.

Table 1. Correlation matrix with main variables

	1	2	3	4	5	6	7	8	9	10	11	12

[1] Deviations from the preregistered plan are described at https://osf.io/3fbvn/

1) Secure trait attachment	1											
2) Secure Base Script knowledge	,105	1										
3) Secure state attachment 1	**,389****	,124	1									
4) Secure state attachment 2	**,400****	,127	**,786****	1								
5) Secure state attachment 3	**,416****	,125	**,716****	**,814****	1							
6) Secure state attachment 4	**,391****	,051	**,635****	**,746****	**,840****	1						
7) Oxytocin level 1 (log10)	,145	,107	,065	,078	,045	,157	1					
8) Oxytocin level 2 (log10)	,011	,012	-,039	,082	,041	,186	**,482****	1				
9) Oxytocin level 3 (log10)	,197	-,129	,101	,155	**,237***	,163	**,462****	**,668****	1			
10) Oxytocin level 4 (log10)	,056	,093	,027	-,015	-,065	-,017	**,394****	**,472****	**,612****	1		
11) Oxytocin level 5 (log10)	,083	,057	-,052	-,028	,023	,105	,099	,196	**,471****	**,443****	1	
12) Oxytocin level 6 (log10)	,081	,201	,063	,056	,057	,093	,151	,040	**,281***	,180	**,449****	1
13) Oxytocin level 7 (log10)	,040	,180	-,132	,026	,006	,075	,195	**,257***	**,257***	,191	,111	**,311****
14) Oxytocin level 8 (log10)	,088	,016	-,143	-,038	-,031	,000	,129	,139	**,335****	,209	,109	**,385****

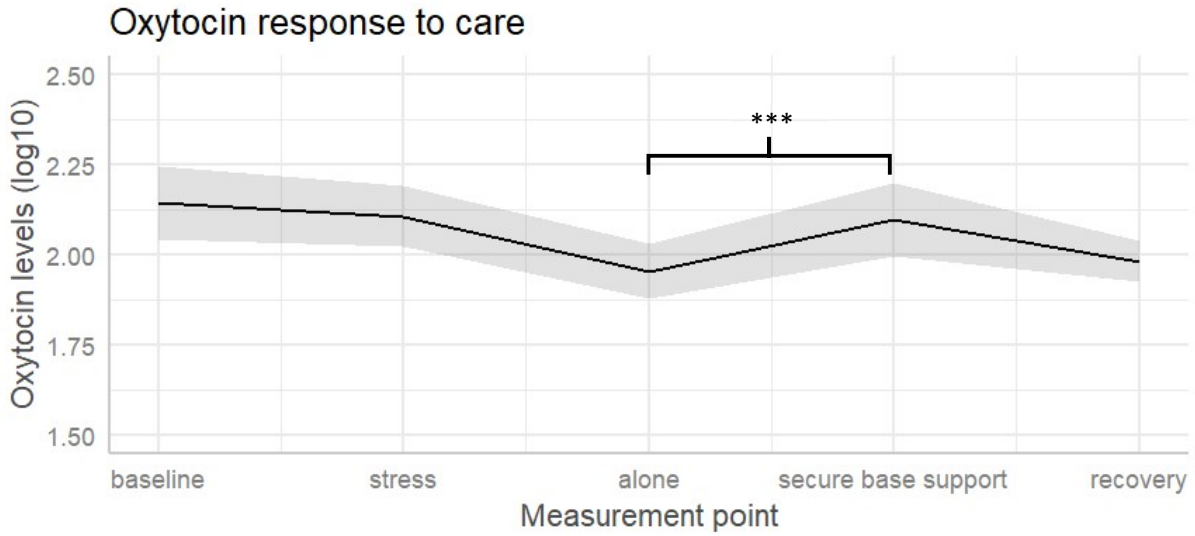
*Note. * p < .05, ** p < .01*

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 the Supplementary file (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., Kramer et al., 2004; Caldwell, 2018). In addition, brushing teeth and medication were included as control variables in analyses with oxytocin as the dependent variable. Medication use was also included as a control variable in analyses with secures state attachment as the dependent variable.

Oxytocin response to care

With our first research question, we wanted to replicate the findings from Seltzer, Ziegler and Pollak (2010). The study from Seltzer, Ziegler and Pollak (2010) found that oxytocin levels rose in children who received support from mother compared to children who remained alone after a stress induction. We wanted to replicate these findings in a within-subject design in order to avoid variations in oxytocin due to inter-person differences. Furthermore, within-subject designs need fewer participants to gain the same power as between-subject designs with more participants and they allows drawing stronger conclusions about causality.

Figure 2. Oxytocin levels throughout the experiment



Note: The x axis shows the different measurement points during the experiment. The y axis shows the estimated marginal means of log 10 transformed levels of oxytocin at each intervention phase, with 95% confidence intervals. Analyses were controlled for sex, whether children brushed their teeth before the experiment, whether they were on medication, and cortisol levels corresponding with the stress level after the TSST-C.

* $p < .05$, ** $p < .01$, *** $p < .001$

To test our core hypothesis stating that oxytocin levels after the alone phase would be lower than oxytocin levels after the secure base support phase, we compared contrasts and found that the level of oxytocin after the secure base support phase was significantly ($p < .001$) higher ($M = 2.16$; $SD = .096$) than the oxytocin level after the alone phase ($M = 2.02$; $SD = .069$) controlling for sex, teeth brushing, use of medication, and cortisol level after the TSST-C ($X^2(1, N = 83) = 6.28$; $pr^2 = .012$). This can also be seen in Figure 2. Results remained similar in a model without control variables ($X^2(1, N = 91) = 3.03$; $pr = .081$). This suggests that receiving secure base support from mother after stress induces an increase in oxytocin, compared to being alone after stress.

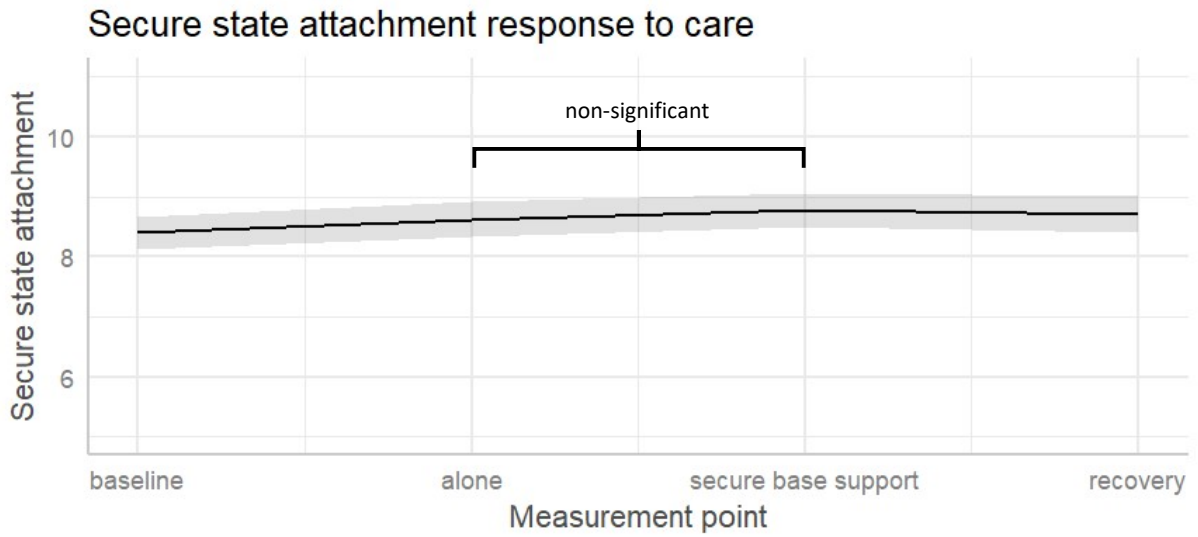
State attachment response to care

The level of secure state attachment after the secure base support phase was not significantly ($p > .05$) higher ($M = 8.89$, $SD = .174$) than the secure state attachment level after the alone phase ($M = 8.74$, $SD = .179$) controlling for sex, teeth brushing, use of medication, and cortisol level after the

[2] Pr represents the probability of obtaining results at least as extreme as the value we computed, assuming the null hypothesis holds true.

TSST-C ($X^2(1, N = 91) = 2.96$; pr = .086). Figure 3 shows secure state attachment levels over the course of the experiment. Results remained similar in a model without control variables ($X^2(1, N = 91) = 3.03$; pr = .081). This suggest that we were not able to replicate the findings from Vandevivere et al. (2018) in the current study.

Figure 3. Secure state attachment levels throughout the experiment



Note: The x axis shows the different measurement points of secure state attachment during the experiment. The y axis shows the mean secure state attachment level at each measurement point. Analyses were controlled for sex, whether the child was on medication and cortisol levels after TSST-C.

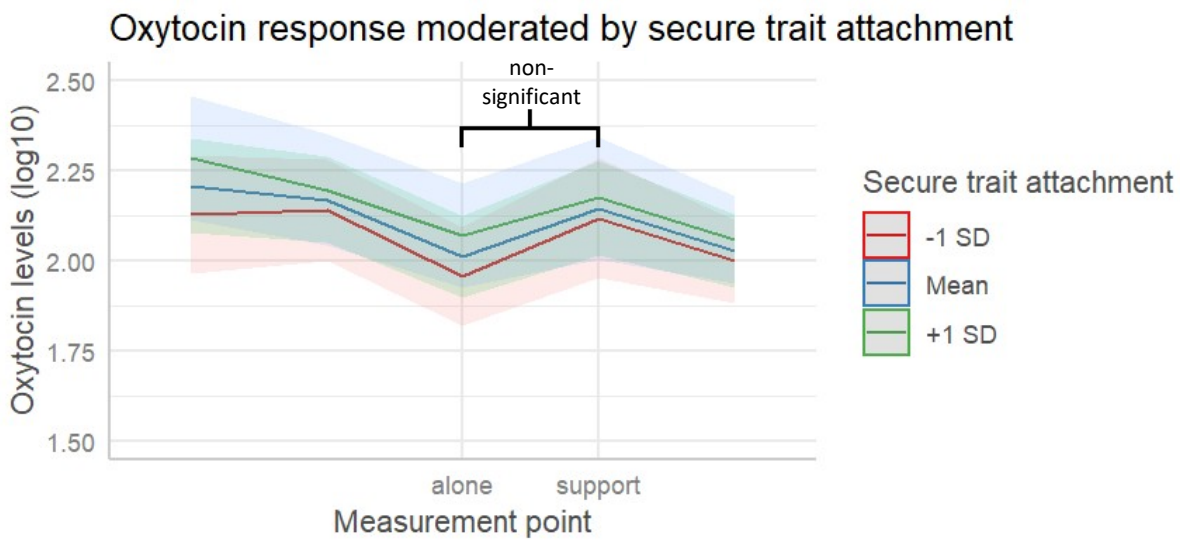
However, contrast analysis showed a significant increase from baseline to after the mother provided secure base support ($X^2(1, N = 92) = 13.79$; pr = .000; $p < .000$). Further analysis, however, showed that this increase was already significant after the alone condition ($X^2(1, N = 92) = 6.17$; pr = .013; $p < .01$), and the secure base support condition did not significantly add extra increase.

The role of trait attachment in oxytocin responses during secure base support

We investigated whether secure trait attachment (PIML) or SBS knowledge (MCASA) moderated the oxytocin response to care during the secure base support phase. Controlling for sex, teeth brushing, medication use, and cortisol level after the TSST-C), we did not find support for a moderating role of secure trait attachment measured with PIML ($X^2(1, N = 83) = .30$; pr = .587), as can

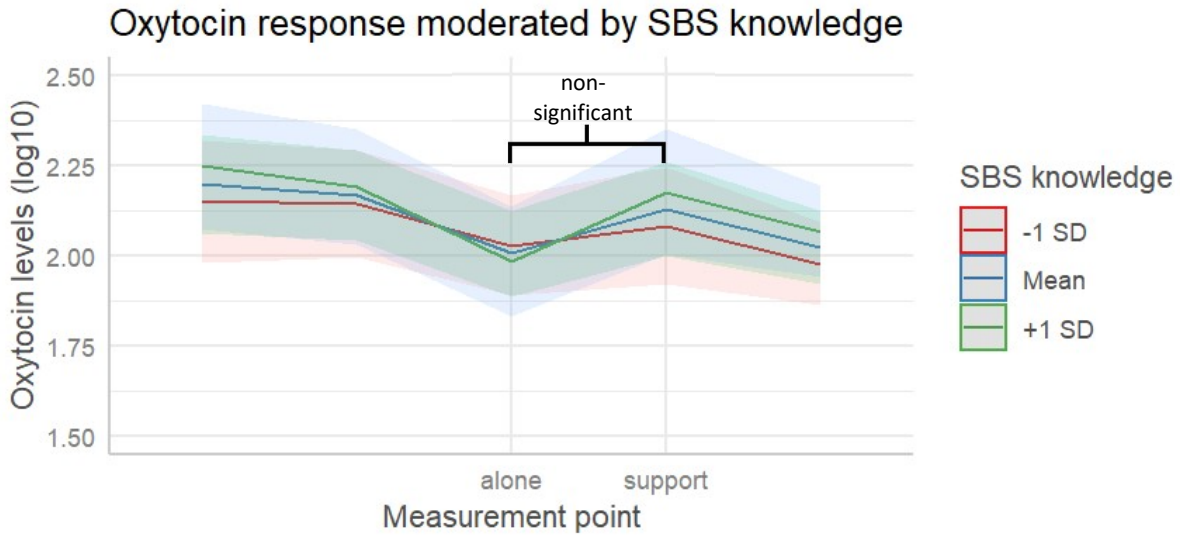
be seen in Figure 4. Results remained similar without control variables ($X^2(1, N = 83) = .27$; pr = .602). Neither did we find support for a moderating role of SBS knowledge in the oxytocin response to care during the secure base support phase, with control variables ($X^2(1, N = 82) = 1.68$; pr = .195) or without control variables ($X^2(1, N = 89) = 1.42$; pr = .233). This can be seen in Figure 5. Neither secure trait attachment, nor SBS knowledge moderated the effect of secure base support.

Figure 4. Moderation of the oxytocin response to secure base support by secure trait attachment



Note: The x axis shows the different measurement points of oxytocin levels of interest. The y axis shows the mean log 10 transformed levels of oxytocin at each intervention phase. Secure trait attachment levels were measured with the PIML questionnaire. Analyses were controlled for sex, whether children brushed their teeth before the experiment, whether they were on medication, and cortisol levels corresponding with the stress level after the TSST-C.

Figure 5. Moderation of the oxytocin response to secure base support by SBS knowledge



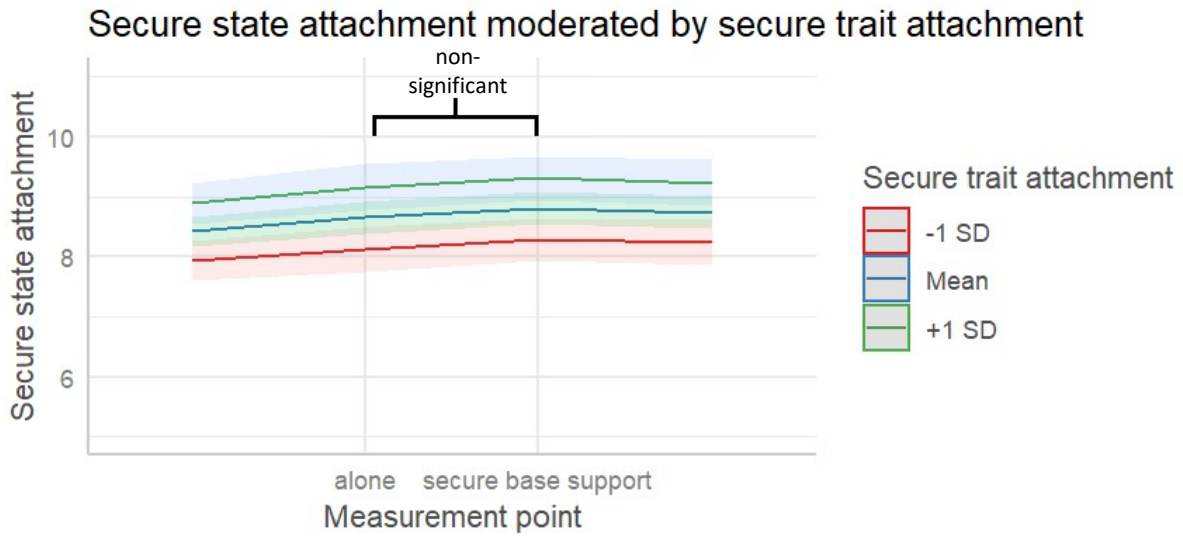
Note: The x axis shows the different measurement points of oxytocin levels of interest. The y axis shows the mean log 10

transformed levels of oxytocin at each intervention phase. SBS knowledge was measured with the MCASA storytelling task. Analyses were controlled for sex, whether children brushed their teeth before the experiment, whether they were on medication, and cortisol levels corresponding with the stress level after the TSST-C.

The role of trait attachment in secure state attachment changes during secure base support

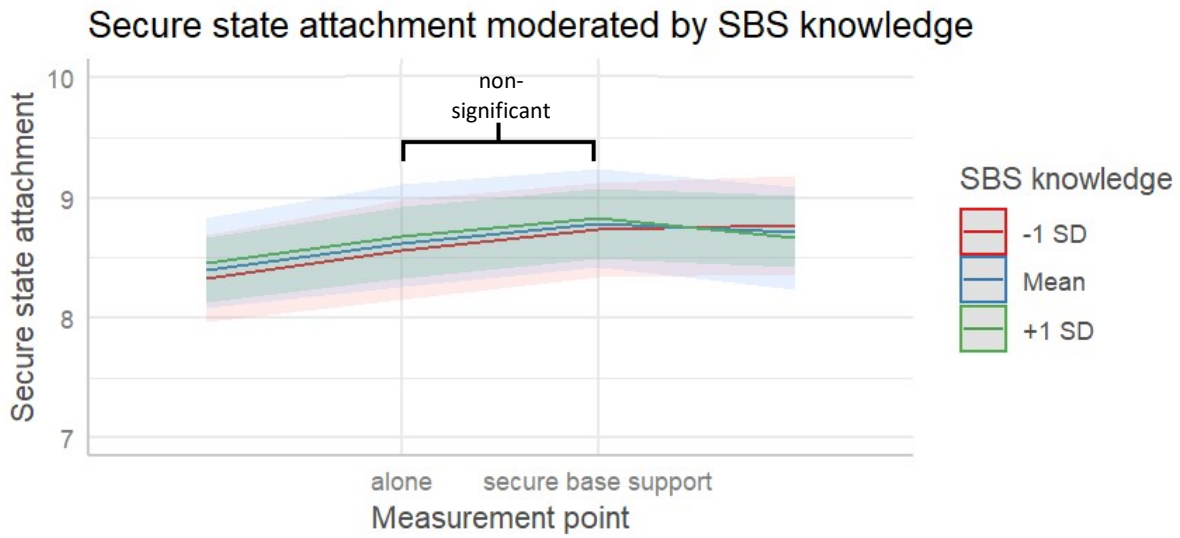
We investigated whether secure trait attachment (PIML) or SBS knowledge (MCASA) moderated secure state attachment responses to care during the secure base support phase. In a model with the control variables included, we did not find support for a moderating role of secure trait attachment measured with PIML ($X^2(1, N=92) = .01$; pr = .944). This can be seen in Figure 6. Results remained similar without control variables ($X^2(1, N=95) = .00$; pr = .948). Additionally, we investigated whether SBS knowledge moderated the secure state attachment change to care. However, the interaction effect was not significant with control variables ($X^2(1, N=91) = .01$; pr = .927), nor without control variables ($X^2(1, N = 94) = .02$; pr = .889), as shown in Figure 7. This suggests that secure trait attachment and SBS knowledge did not moderate the effect of secure base support on secure state attachment levels.

Figure 6. Moderation of secure trait attachment in state attachment response to secure base support



Note: The x axis shows the measurement points of interest of secure state attachment. The y axis shows the mean secure state attachment level at each measurement point. Secure trait attachment levels were measured with the PIML questionnaire. Analyses were controlled for sex, whether the child was on medication and cortisol levels after TSST-C.

Figure 7. Moderation of SBS knowledge in state attachment response to secure base support



Note: The x axis shows the different measurement points of interest. The y axis shows the mean secure state attachment levels at each intervention phase. SBS knowledge was measured with the MCASA storytelling task. Analyses were controlled for sex, whether the child was on medication and cortisol levels after TSST-C.

Discussion

The current study aimed to, firstly, replicate findings from two previous studies suggesting that children's oxytocin and secure state attachment levels increase after care (Seltzer et al., 2010; Brockington et al., 2020; Vandevivere et al., 2018). Adding to those studies, the current research tested these effects using a within-subject design. Additionally, the moderating effect of trait attachment (secure trait attachment and SBS knowledge) on these effects was investigated. Results replicated the oxytocin main effect, but not the secure state attachment main effect. Also, trait attachment did not moderate the effect of secure base support on oxytocin or secure state attachment responses.

In contrast with previous studies (Seltzer et al., 2010; Brockington et al., 2020; Vandevivere et al., 2018) the current study used a within-subject design. Such designs are superior to between-subject designs as they yield more statistical power and allow for drawing potentially more replicable conclusions about causality. For oxytocin, results showed that the oxytocin effect could be replicated. Specifically, oxytocin levels significantly increased when children received secure base support from

mother compared to after being alone following stress. Although the within-subject design used in the current study increased the power of the test, it might have caused other effects to disappear. Specifically, our design used no counterbalance and different phases and manipulations followed each other in the same order for all children, so we could not control for sequence effects. Such effects could have masked any effect of the secure base support manipulation. However, we deemed it less adequate to counterbalance the order of the alone versus secure base support conditions as, theoretically, prior exposure to secure base support from mother after stress might contaminate the oxytocin and state attachment responses to being alone thereafter. More specifically, if children would already have received secure base support, effects thereof cannot be undone during the alone phase. Also it seemed less appropriate to repeat the TSST-C and have it twice immediately followed by secure base support or by being alone in counterbalanced order. Repetition of the TSST-C could have resulted in habituation or overly stressed conditions hampering a good comparison between the conditions. The fact that we were able to replicate the main effect on oxytocin in response to care in spite of the possibly dampening impact of the current study's design might suggest that the oxytocin effect is rather strong. This has important theoretical implications. Oxytocin has been most commonly known for its role in breast feeding and labor induction (Winslow & Insel, 2002), but the current findings seem to contribute to the hypothesis that maternal care reliably influences oxytocin levels (Swain et al., 2014). In addition, the current findings seem to be in line with the learning theory of attachment that due to the rewarding effect of increased oxytocin in response to care, the development of secure attachment might be enhanced (Bosmans et al., 2020).

In contrast, we failed to replicate the findings of Vandevivere et al. (2018) that secure state attachment increased after maternal secure base support following stress. This suggests that Vandevivere et al.'s (2018) state attachment effect might be less robust than the oxytocin effect. This is surprising given the theoretical association between oxytocin and state attachment. Past experimental research found some support for such effects (Bernaerts et al., 2017; Bernaerts et al., 2020). However, the latter studies had a different design, testing the effect of the exogeneous

administration of oxytocin on state attachment. Concerns have been raised about the validity of the impact of exogeneous oxytocin on attachment (Leng & Leng, 2020; Quintana, 2021). Moreover, in a recent study, Daniels et al. (2022) failed to replicate the oxytocin-state attachment association in children, raising further questions on whether oxytocin and state attachment are strongly linked.

Furthermore, there were significant differences between the current study's and Vandevivere's paradigm. Vandevivere et al. (2018) compared state attachment levels between groups of children in different conditions, while we used a within-subject design. Like in our study, Vandevivere et al. (2018) did not find time effects within each condition, but an interaction between condition and time, suggesting that the secure state attachment effects might be rather weak and harder to replicate in a strict within-subject design.

Moreover, the timing of the measurement points was different in the current study compared to Vandevivere et al.'s (2018) study. In the current study, we measured state attachment after children were left alone for 10 minutes, while in the study of Vandevivere et al. (2018), state attachment was already measured after five minutes in the alone condition. It might be that after 10 minutes the immediate effect of absent care on secure state attachment was erased. For example, the additional time might have given children the opportunity to self-regulate any negative appraisals that got activated by being left alone after being stressed. Post-hoc, we wondered whether adaptive emotion regulation could have had a positive impact on children's secure state attachment levels during the alone condition. Given that we measured adaptive emotion regulation, we tested this post-hoc hypothesis and reported the analyses in Supplementary file 2. We found that effective emotion regulation did moderate the state attachment response while being alone after stress. Children with more effective emotion regulation increased more in state attachment after being alone than children with less effective emotion regulation (see Supplementary file 2). Hence, it is not unlikely that after 10 minutes alone children's adaptive emotion regulation skills may have dampened the secure state attachment decrease we expected, making it harder for the secure base support condition to have a positive effect on children's secure state attachment levels. Similarly, secure state attachment levels

were already high at baseline, which could have created a ceiling effect. More research in future studies, with shorter measurement timings is necessary to further explore the impact of care on children's state attachment levels.

A final difference is that Vandevivere et al., (2018) used a negative mood induction video clip to induce stress. Such a stressor is significantly less intense than the TSST-C procedure used in the current study (Gunnar et al., 2009). Previous research in middle childhood showed that differences in intensity of stress impacted a range of attachment-related behaviors (Bosmans et al., 2015). Likewise, it might have been be that the difference in stressor intensity had a differential impact on self-reported secure state attachment responses to care. Future research should investigate the role of stressor intensity in predicting oxytocin and secure state attachment changes in response to care.

Regarding our second research we did not find support for our hypothesis that children with higher levels of secure trait attachment or SBS knowledge would show a stronger oxytocin or secure state attachment increase after secure base support. One explanation might be that this moderation effect was tested at a younger age that previous studies (Girme et al., 2018 ; Tops et al., 2007; Strathearn et al., 2009; Pierrehumbert et al., 2012; Venta et al., 2017). Such an age-effect would be in keeping with research on early maladaptive schemas showing that such schemas become moderators of the association between stress and psychopathology towards late adolescence (Braet et al., 2013), and that only later schemas are sufficiently developed and crystallized to have a moderating effect on how individuals interact with their environment (Muris et al., 2006; Waters et al. , 2019). The few available studies showed that it is hard to find moderating effects of trait attachment on state attachment changes as a consequence of changing experiences in the caregiving environment (see Verhees et al., 2021, and Cuyvers et al., 2022). Several meta-analyses have shown that there is less trait attachment stability among children than in adults (Fraley, 2002; McConnell & Moss, 2011; Pinquart et al., 2014), further supporting our post-hoc interpretation of the current study's results (Frederickson et al., 2013). At the same time, the absence of a moderating effect of trait attachment could also point at substantial plasticity in children's state attachment responses. It could mean, at

least in middle childhood, that exposure to positive attachment experiences in their environment have a beneficial effect on their secure trait attachment development in spite of a history of insecure trait attachment development .

Limitations and suggestions for future research

Although we found an effect on oxytocin responses of receiving maternal secure base support after stress, findings should be interpreted cautiously since the current study also has some limitations. First, we used an experimental design in order to measure oxytocin responses to care versus no care, but it remains unclear how long it takes until oxytocin responses in the brain are detectable in saliva. We based the timing of our saliva samples on studies from Seltzer et al. (2010) and Bernhard et al. (2018). Nevertheless, more basic neuroendocrinological research is needed to reveal the time correspondence between brain oxytocin responses and salivary oxytocin responses.

Second, criticism exists about the validity of using peripheral salivary oxytocin levels as an operationalization of central levels of oxytocin (Horvat-Gordon et al., 2002; McCullough et al., 2013). Obviously, because of ethical reasons, it is impossible to collect oxytocin samples from the brain. Oxytocin can also be measured in cerebrospinal fluid, plasma or urine, but collecting saliva is much less intrusive, especially in children. Based on research findings that salivary oxytocin is interrelated with plasma oxytocin (Feldman, Gordon and Zagoory-Sharon, 2010), and with cerebrospinal fluid oxytocin levels (Martin et al., 2018), we deemed it justified to assess oxytocin levels through saliva. However, results are sometimes mixed (Martins et al., 2020) and thus more research is necessary in order to assess the validity of salivary oxytocin measures.

Third, the current study encountered some unexpected and biologically impossible oxytocin values after extraction from saliva. We do not know the cause of these findings. Oxytocin is a fragile hormone to measure for which strict data collection protocols should be followed. Since data collection was conducted by ten different experimenters, small differences in data collection may have occurred. Additionally, we used Enzyme Immunoassay Kits (Arbor Assays®) to assess oxytocin levels. However, McCullough et al. (2013) warned about such commercial assays because next to oxytocin,

other molecular species showing similar oxytocinergic reactivity could be extracted, which would lead to an overestimation of oxytocin levels. Therefore, future research might consider using different manners to extract oxytocin levels and standardize the collection of data even more.

Fourth, we did not assess the subjective level of stress after the TSST-C. Therefore, we cannot be sure whether the TSST-C induced enough subjective stress for children to desire comfort from their mother (but see Seddon et al., 2020). Nevertheless, future research may register the stress response. In the same vein, we did not check if children interpreted the secure base support from mother as supportive or not as we did not do an integrity check on how effective the support was.

Sixth, we only looked at attachment to mother, not to father. However, fathers also may play an important role in attachment development in middle childhood (Lucassen et al., 2011). In addition, we only used self-reported data of secure trait and state attachment levels which might be considered less valid than interview or observational assessment, which is reason why we included SBS knowledge as a more implicit measure of trait attachment.

Last, due to the COVID-19 pandemic, we only were able to collect data from 109 participants instead of the 200 participants we aimed for. power?

Implications

We found some evidence for the hypothesis that oxytocin increases in response to care, by replicating the results of Seltzer and colleagues (2010). Moreover, this implies that oxytocin could play a significant role in children's attachment development. More research is needed to test whether oxytocin would also have a reinforcing effect and plays a role in learning itself. A better understanding of the processes playing a role in attachment development is of great value, because attachment impacts important developmental outcomes later in life (Cassidy & Shaver, 2016). A better understanding of the role of endocrinological factors such as oxytocin responses and accompanying psychological responses such as state attachment changes could be used in the design of future psychological treatments aiming to increase trait attachment security. By identifying situations or

contexts in which oxytocin gets released or secure state attachment increases, we can adapt existing therapies by stimulating such situations and increasing beneficial mental health outcomes (Crockford et al., 2014). For example, the current study's finding that oxytocin increases following maternal secure base support could be integrated in mother-child therapy by stimulating maternal touch in order to increase oxytocin both in mother and child (Feldman, 2012).Although the current study's findings support the idea that oxytocin release can be impacted by care, our findings also suggest that in middle childhood, no strong upward spiral effects (Frederickson et al., 2013) are present yet. Oxytocin and secure state attachment responses to care do not (yet) depend on secure trait attachment and SBS knowledge levels. Thus, there seems to be still room for improvement of more secure attachment based on naturally occurring or intervention-generated (e.g., in the Middle Childhood Attachment-Based Family Therapy; Van Vlierberghe et al., in press) learning events.

References

Supplementary file 1

Table S1. *Correlation matrix of possible control variables and oxytocin data*

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
1) Secure state attachment 1	1																	
2) Secure state attachment 2	**,786****	1																
3) Secure state attachment 3	**,716****	**,814****	1															
4) Secure state attachment 4	**,635****	**,746****	**,840****	1														
5) Oxytocin level 1 (log10)	,065	,078	,045	,157	1													
6) Oxytocin level 2 (log10)	-,039	,082	,041	,186	**,482****	1												
7) Oxytocin level 3 (log10)	,101	,155	**,237***	,163	**,462****	**,668****	1											
8) Oxytocin level 4 (log10)	,027	-,015	-,065	-,017	**,394****	**,472****	**,612****	1										
9) Oxytocin level 5 (log10)	-,052	-,028	,023	,105	,099	,196	**,471****	**,443****	1									
10) Oxytocin level 6 (log10)	,063	,056	,057	,093	,151	,040	**,281***	,180	**,449****	1								
11) Oxytocin level 7 (log10)	-,132	,026	,006	,075	,195	**,257***	**,257***	,191	,111	**,311****	1							
12) Oxytocin level 8 (log10)	-,143	-,038	-,031	,000	,129	,139	**,335****	,209	,109	**,385****	**,584****	1						
13) Child's sex	-,162	-,107	-,021	-,045	-,155	-,113	-,068	-,102	,092	,129	,140	,190	1					
14) Sugary drink 1-2 hours before experiment	,006	-,089	-,087	-,082	-,206	,031	,065	,150	,212	-,008	,049	,212	-,083	1				
15) Great physical effort before experiment	-,001	,062	,020	,015	-,061	,121	-,129	-,061	-,081	-,162	,173	-,013	,191	,193	1			
16) Teeth brushed 2 hours before experiment	-,053	-,086	-,088	-,094	**-,262***	-,174	-,186	,009	,117	-,016	-,145	,076	,006	**,385****	**,209***	1		
17) Child on medication	**,308****	**,226***	**,209***	**,224***	-,204	-,050	,068	-,021	**,232***	,051	-,082	-,064	-,022	,040	,146	-,076	1	
18) Psychological therapy for diagnosed disorder	,083	,042	,004	,089	,106	-,122	-,009	-,090	,012	,115	-,071	-,011	,188	-,091	-,076	,017	,004	1

*Note. * p < .05, ** p < .01*

Table S2. Cortisol levels during the experimental procedure

Measurement point	Cortisol mean level	95% CI	SD	n
1	.21	[.19, .23]	.11	103
2	.17	[.14, .19]	.12	104
3	.18	[.15, .21]	.17	103
4	.15	[.13, .17]	.10	104
5	.13	[.12, .15]	.08	104
6	.12	[.11, .14]	.07	104
7	.11	[.10, .13]	.06	104
8	.11	[.10, .12]	.06	103

Note: Cortisol 3 was included as control variable in all analyses since it reflects on the level of stress due to the TSST-C

Supplementary file 2

Secure state attachment and effective emotion regulation

Table S3: Correlation matrix between effective emotion regulation and secure state attachment levels

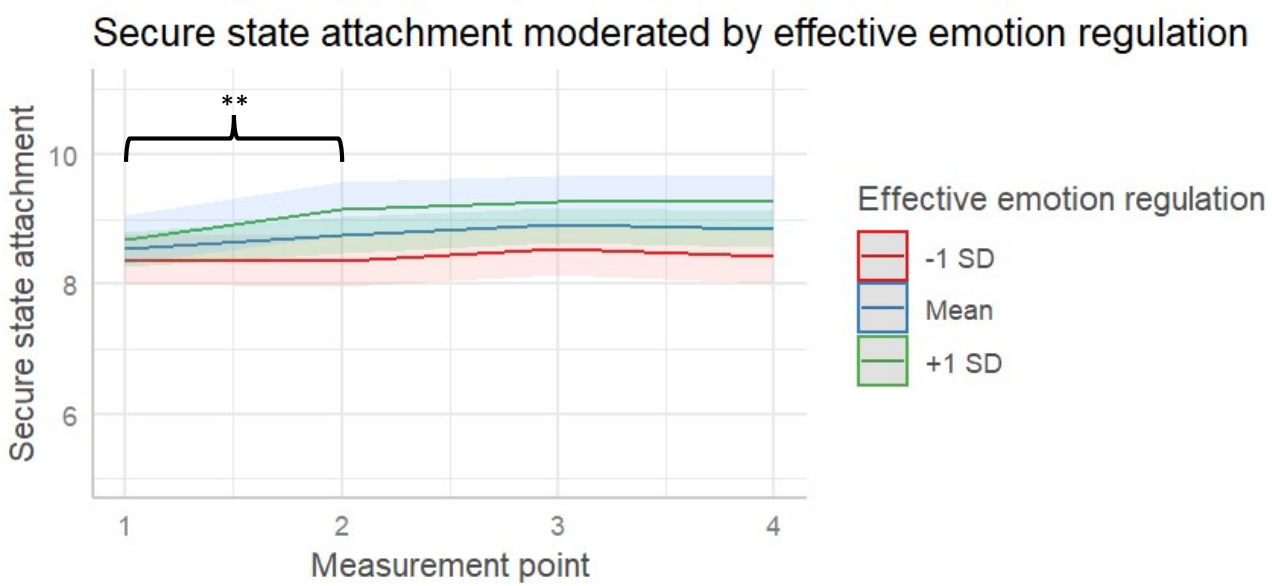
	1	2	3	4	5
1) Effective emotion regulation	1				
2) Secure state attachment 1	,082	1			
3) Secure state attachment 2	**,253***	**,786****	1		
4) Secure state attachment 3	**,242***	**,716****	**,814****	1	
5) Secure state attachment 4	**,274****	**,635****	**,746****	**,840****	1

*Note. * p < .05, ** p < .01*

As Table S3 shows, effective emotion regulation was highly correlated to levels of secure state attachment over time. In a multilevel mixed model analysis, secure state attachment significantly increased from baseline to after the alone phase, with control for sex, medication use and cortisol measure 3 (X^2(1, N = 92) = 6.17; pr = .013, p < .01). Results were similar without control variables (X^2(1, N = 95) = 6.86; pr = .008, p < .001).

Using linear regression in SPSS, results showed that effective emotion regulation during the alone phase impacted the increase in secure state attachment from baseline to after the alone phase (β = .19, SD = .13, p = .003). Thus, with more adaptive emotion regulation, a greater increase in secure state attachment

occurred from baseline towards after being alone. Effective emotion regulation during the alone phase did not impact the secure state attachment changes in response to the secure base support phase ($X^2(1, 91) = .15$; pr = .699).



Note: The x axis shows the different measurement points during the experiment: 1 = baseline phase, 2 = after alone phase, 3 = after secure base support phase, 4 = after recovery phase. The y axis shows the mean levels of secure state attachment at each intervention phase. Analyses were controlled for sex, whether they were on medication, and cortisol levels corresponding with the stress level after the TSST-C.

* $p < .05$, ** $p < .01$, *** $p < .001$