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RESEARCH ARTICLE

Exploring the role of *OXTR* gene methylation in attachment development: A longitudinal study

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Abstract

The current study explored longitudinally whether oxytocin receptor gene methylation (*OXTRm*) changes moderated the association between parental sensitivity changes and children's attachment changes over three waves. Six hundred six Flemish children (10–12 years, 42.8%–44.8% boys) completed attachment measures and provided salivary *OXTRm* data on seven CpG sites. Their parents reported their sensitive parenting. Results suggest that *OXTRm* changes hardly link to attachment (in)security changes after the age of 10. Some support was found for interaction effects between parental sensitivity changes and *OXTRm* changes on attachment changes over time. Effects suggest that for children with increased *OXTRm* in the promotor region and decreased methylation in the inhibitor region over time, increased parental sensitivity was associated with increased secure attachment and decreased insecure attachment over time.

KEYWORDS
attachment, hormones, parental care

1 | INTRODUCTION

Secure attachment or children's trust in the availability of caregiver support (Ainsworth, 1973; Bowlby, 1969) is an important outcome of child development as it influences later (mental) health and social and academic competence (Cassidy & Shaver, 2016). Children's ability to form attachments to parents is embedded in their genetic makeup as the result of evolutionary selection to promote their survival (Bowlby,

1969). Whether or not children develop trust in parental care (i.e., become securely or insecurely attached) depends on parents' sensitive responses to children's distress (De Wolff & Van IJzendoorn, 1997). However, research suggests that the effect of (in)sensitive parenting on children's attachment development is not equal for all children (Vandevivere et al., 2018), raising the question of which factors explain individual differences in the association between change in sensitive parenting and change in attachment across development. According to recent theories, endocrinological systems, among others the oxytocin system, are implicated in attachment development (Bosmans et al., 2020; Feldman, 2012). This has led to the current study's hypothesis that epigenetic changes in the oxytocin receptor (*OXTR*) gene moderate the extent to which changes in sensitive parenting are linked to individual differences in attachment development over time. Moreover, we will explore whether such effects can be observed beyond the age of 5. The period until 5 years old is typically characterized by more plasticity and considered as more sensitive to change (Pérez et al., 2019). However, we are interested in whether even after that age, still changes can occur in *OXTR* gene methylation (*OXTRm*) and attachment.

Attachment theory (Bowlby, 1969) proposes that children need parental proximity and support for survival. When children experience consistent sensitive care during distress, they are more likely to expect support from parents and develop a secure attachment (van IJzendoorn & Bakermans-Kranenburg, 2019). These expectations are mentally stored in a cognitive script-like manner, called a Secure Base Script (SBS; H. S. Waters & Waters, 2006). This SBS comprises the expectation that distress will be followed by a chain of events, starting with proximity seeking to an attachment figure in response to distress. This then activates caregiver support, which resolves the distress and helps getting back on track. More securely attached individuals develop more SBS knowledge (Waters et al., 2019). The level of internalization and knowledge about the SBS represents attachment quality. The SBS guides future behavior and expectations regarding seeking and receiving supportive care from an attachment figure. In contrast, children who experience inconsistent or a lack of sensitive care during distress develop not only less SBS knowledge, but they are also more likely to develop anxious or avoidant attachment styles (e.g., Ein-Dor et al., 2011; Verhees et al., 2021). An anxiously attached child fears losing connection with the attachment figure resulting in a preoccupied search for support. An avoidantly attached child expects no care from the attachment figure and shows withdrawal (Mikulincer et al., 2003). The association between (in)sensitive parenting and (in)secure attachment development has been robustly found in meta-analyses (De Wolff & van IJzendoorn, 1997; van der Voort et al., 2014). However, these effects were on average less strong and more variable over studies than traditional attachment theory predicted, leading to the question of which factors moderate the association between sensitive parenting and attachment.

In order to try to understand the variation in effect sizes over studies, potential moderators of the sensitive parenting-attachment changes association were formulated in the learning theory of attachment (Bosmans et al., 2020). According to recent theories, an optimal functioning oxytocin system is important for attachment development, as it maximises the rewarding effect of receiving care from the attachment figure (Bosmans et al., 2020; Feldman, 2012).

Children learn to expect comfort and the care-related experiences become consolidated in SBS knowledge, hereby contributing to secure attachment development. Inversely, when the attachment figure gets associated with rejection during distress, children are less likely to experience comfort and relief, which can accumulate toward anxious or avoidant attachment expectations. Hence, hormones like oxytocin could play a central role in attachment development. Oxytocin is a neuropeptide that is produced in the hypothalamus and is released from the posterior pituitary gland (Vaidyanathan & Hammock, 2017). Once *OXTRs* process oxytocin, it affects social affiliation (Winslow & Insel, 2002) and children's attachment development (Swain et al., 2014). However, individual differences in oxytocin responses to care exist. For example, Heim et al. (2009) found that adults who experienced childhood trauma or neglect showed reduced oxytocin levels in their cerebrospinal fluid. In addition, Feldman and Bakermans-Kranenburg (2017) reviewed studies showing that differences in oxytocin levels are associated with differences in how parents and children behave toward each other during attachment interactions. These individual differences in oxytocin responses could explain why the association between changes in parental sensitivity and changes in attachment over time differ across children. Specifically, if the oxytocin response during care is blunted, children may experience parental attempts to provide support as less effective, leading to a reduced ability to learn to trust in parental care.

One possible source of variation in the oxytocin system is epigenetics (Ellis et al., 2021). Epigenetics refers to the processes that can alter gene expression without changing the underlying genetic sequence itself (Lester et al., 2016). These processes are natural and necessary for the proper functioning of the organism, but if epigenetic processes become aberrant, they can have severe health or behavioral consequences (Bakermans-Kranenburg & van IJzendoorn, 2014). There are different epigenetic mechanisms. In the current study, we focus on DNA methylation, which is the most studied mechanism in epigenetics research (e.g., Darling Rasmussen & Storebø, 2021). DNA methylation comprises the addition of a methyl group (CH_3) on cytosine nucleotides, widely found within so-called CpG islands. Often, the studied CpG islands are located close to the promotor region of a gene, which identifies where mRNA can start to read out the gene (Moore et al., 2013). In the promotor region, hypermethylation of the cytosine nucleotides presumably corresponds to less expression of the mRNA and thus the gene, while hypomethylation is assumed to lead to more expression of the mRNA and the gene (Jones, 2012). However, although this is generally the case, the relationship between DNA methylation and gene expression is highly complex and varies by genomic location (Phillips & Goodman, 2008).

Methylation patterns are established perinatally but can also change throughout one's lifespan (Skinner, 2011). DNA methylation can occur because of a wide range of environmental influences like certain foods, substances (Dolinoy, Huang, & Jirtle, 2006), and pollution (Rider & Carlsten, 2019) but also aging (Klutstein et al., 2016) and social factors like bullying (Mulder et al., 2020) or parenting (Ellis et al., 2021;

Mulder et al., 2017). Additionally, genetics (methylation quantitative trait loci, mQTLs) play an important role in programming DNA methylation patterns, with over half of DNA methylation sites associated with known common single nucleotide polymorphisms (Min et al., 2021). For the *OXTR* gene, several mQTLs are known (rs2648415, rs68129856, rs73019973, rs7647170, rs9874577; GoDMC database).

Although theoretically, mediation of *OXTRm* in the association between parenting and child outcomes might seem a logical conclusion, many studies failed to find this effect (Mulder et al., 2017). However, it might be that these epigenetic changes could be important moderators in how parenting influences attachment development. Caspi et al. (2002) proposed that DNA methylation levels affect children's susceptibility to parenting effects, and, therefore, the strength of the association between parenting and different developmental outcomes, like attachment, might be dependent on children's (epi)genetic constellation. In a similar vein, the differential susceptibility theory states that children can differ in their susceptibility to environmental influences, such as parenting (Klein Velderman et al., 2006) with epigenetics being one potential marker for individual differences in susceptibility (van IJzendoorn & Bakermans-Kranenburg, 2015). Given the hypothesized central role of the oxytocin system in attachment development, the current study will explore longitudinally whether changes in children's *OXTRm* moderate the association between changes in parental sensitivity and changes in attachment over time.

Research on whether and how epigenetics plays a role in attachment development has only recently been touched upon (Craig et al., 2021; Darling Rasmussen & Storebø, 2021). The few existing studies seem to suggest that *OXTRm* could play a role in attachment development (Craig et al., 2021). One study showed higher levels of *OXTR* gene intron 1 methylation in young adults with a more avoidant attachment style, only when anxiety was low (Ein-Dor et al., 2018). Research from Ebner et al. (2019) indicated higher methylation levels in the -924 CpG of the *OXTR* gene (MT2 region) for younger participants (20–31 years old) with less attachment anxiety, but not for older participants (63–80 years old; Ebner et al., 2019). In addition, Lecompte et al. (2021) found that hypomethylation of the *OXTR* gene was related to more attachment security. In general, those studies found that the most epigenetically active region within the *OXTR* gene is the MT2 region, which comprises several dozens of CpG sites. Therefore, in the current study, we focus on this MT2 region.

However, the previous studies had at least two limitations. First, they did not take into account change over time. Intervention studies have demonstrated that changes in parental sensitivity can contribute to changes in child attachment (Bosmans et al., 2022). Also, Cecil et al. (2014) showed that environmental changes like parenting and *OXTRm* changes were associated across childhood. Thus, to better understand changes in attachment, it is important to investigate changes in parenting behavior because the changes might be interconnected. A second limitation of those studies is that they do not investigate whether the interaction between changes in parenting and DNA methylation explains changes in attachment. Nevertheless, prior research has suggested that such interaction effects do exist. For example, Bosmans et al. (2018) found that methylation of the *NR3C1* gene moderated the link between maternal support and change in anxious attachment. More distressed children who received less maternal support reported an increase in anxious attachment over time when their level of *NR3C1* methylation was high. Hence, we were interested in exploring whether changes in *OXTRm* levels similarly moderate the association between change in sensitive parenting and change in attachment. This moderation effect was tested in a middle childhood sample and for different components of the complex attachment construct.

Middle childhood is an important developmental period in which biological factors, as well as social factors, undergo important transitions (Del Giudice, 2015). Children's social worlds expand, and although parents remain their main attachment figures, peers become more important than before (Brumariu & Kerns, 2022). Also, metacognition, memory, emotion regulation, self-awareness, and understanding of others mature in middle childhood (Raikes & Thompson, 2005). This maturation can influence learning and attachment. Therefore, middle childhood is a valuable period to study attachment development.

The development of attachment occurs at different levels of processing (Bosmans & Kerns, 2015). Research suggests that attachment is a complex construct and individual differences in attachment reflect both more strategic levels of processing (e.g., children reporting on how much secure, anxious, and avoidant attachment they experience in their relationship with parents) and more automatic levels of processing (e.g., whether children tell stories about distressing events that contain SBS knowledge). It has been argued that it is important to do research accounting for both levels of processing to better understand at which level of processing certain developmental mechanisms operate (Gastelle & Kerns, 2021).

Additionally, parenting research shows that the investment of male versus female parents in their offspring might differ (parental investment theory; Mogilski, 2021). Thus, parental sensitivity toward children can digress between mothers and fathers, also depending on the context (Branger et al., 2019). Therefore, the current study explores whether the effect of changes in maternal sensitivity on attachment development is moderated by the change in *OXTRm*.

2 | METHODS

2.1 | Participants

Six hundred six children were recruited from 21 schools in Flanders, Belgium. To increase power to find small to medium effects (calculated a priori with G*Power; Faul et al., 2009), we needed to increase the sample size and recruited extra children outside of those schools through social media. There were three waves of data collection with 1 year between the first and second and between the second and third wave. In Wave 1, 606 children of the fifth grade (M_{age} = 10.78, SD_{age} = 0.48) took part in the study (44.7% boys); in Wave 2, 440 children of the sixth grade from the same sample (M_{age} = 11.77, SD_{age} = 0.51, 42.8% boys); and in Wave 3, 332 children of the seventh grade from the same sample (M_{age} = 12.65, SD_{age} = 0.72, 44.8% boys) took part in the study (retention rate = 77%).

Most participants were of Belgian nationality (88.7%). Most parents had a college education degree (mothers = 47.5%; fathers = 36.4%) or a university degree (mothers = 36.2%; fathers = 39.6%), 15.8% of mothers and 23.3% of fathers had a secondary school degree, and 0.5% of mothers and 0.7% of fathers had a primary school degree or no degree. A total of 78.8% of the participants lived together with both their biological parents, 12.0% lived with one parent, 9.0% lived in a blended family, and 0.2% lived in a different situation.

2.2 | Measures

2.2.1 | Measures of attachment

People in My Life

To measure self-reported secure attachment or trust in the availability of parental support, children filled out the trust subscale of the People in My Life questionnaire (Ridenour et al., 2006) in every wave. This subscale has 10 statements about attachment to their mother that children (e.g., "*My mother accepts me the way I am"*) rated from 1 (*not at all true*) to 4 (*completely true*). Cronbach's αs were.84 in Wave 1,.87 in Wave 2, and.91 in Wave 3.

Experiences in Close Relationships—Revised

We also assessed self-reported attachment anxiety and avoidance with the Experiences in Close Relationships—Revised questionnaire, adapted for middle childhood (ECR-RC; Brenning et al., 2011) in each wave. The anxious attachment (e.g., "*I worry that my mom doesn't really love me*") and avoidant attachment (e.g., "*I prefer not to show my mom how I feel deep down")* subscales each consist of six items. Children rated all questions from 1 (*strongly disagree*) to 7 (*strongly agree*). Cronbach's αs for the anxious attachment subscale were.85 in Wave 1,.86 in Wave 2, and.92 in Wave 3; Cronbach's αs for the avoidant attachment subscale were.73 in Wave 1,.97 in Wave 2, and.85 in Wave 3.

Middle Childhood Attachment Script Assessment

In addition, we measured attachment in a more implicit way using the measurement of SBS knowledge (MCASA; T. E. Waters et al., 2015). The MCASA is a storytelling task in which children are asked to tell a coherent story as if the story is happening at that moment in time, using given word prompts. There were two practice stories and three test stories. The test stories contained one about "a scary dog in the yard," "a soccer game," and "at the beach." The word prompts are selected in such a way that they suggest a SBS storyline. The stories children tell are rated on the amount of SBS elements the stories contain. Per story, children receive a score ranging from 1 to 7. A score of 1 indicates insecure elements in the script and 7 the full presence of all three SBS elements. The stories were recorded, transcribed, and coded by three independent raters. The raters all received training from the principal investigator who was trained himself in the lab of Stony Brook University. Our lab also coded SBS data already before, used in previous studies (e.g., T. E. Waters et al., 2015; Waters et al., 2019). Three raters reached good interrater reliability for a subset of 121 stories of each storyline, scored independently of each other (i.e., "scary dog in the yard," "soccer game," and "at the beach"). Intraclass correlations were assessed in a two-way mixed model with an absolute agreement for single measures for the three coders, ranging between.84 and.90 for the 'scary dog in the yard' story, between.76 and.82 for the 'soccer game' story, and between.83 and.94 for the "at the beach" story. Stories on which the raters differed more than 1 point in scores were discussed and they agreed upon a consensus score. Subsequently, the remaining stories were equally divided between the raters and coded independently. Cronbach's αs between the three stories were in Wave 1 = .60, Wave 2 = .58, Wave 3 = .62. These αs are rather low, but based on several previous studies (Bosmans et al., 2014; Verhees et al., 2021; T. E. Waters & Roisman, 2019), we deemed it acceptable to use this more implicit measure of secure attachment as an additional attachment outcome variable.

2.2.2 | Sensitive parenting

To measure the level of support by children's parents, both parents were asked to complete a self-report questionnaire on sensitive parenting (Wave 1: $N_{mothers} = 412$, $N_{fathers} = 306$; Wave 2: $N_{mothers} = 317$, $N_{fathers} = 236$; Wave 3: $N_{mothers} = 335$, $N_{fathers} = 271$). The questionnaire included 15 items, coming from the subscales of Positive Parenting (Parental Behavior Scale Short; Van Leeuwen et al., 2018), Responsivity (LAPPS; Louvain Adolescent Perceived Parenting Scale; Delhaye et al., 2012), and Autonomy Support (PPS; Perceptions of Parents Scale; Grolnick et al., 1991). An example item is "*I take into account my son's/daughter's opinion on affairs that concern me*" and all items were scored on a 5-point Likert scale ranging from 1 *"(almost) never"* to 5 *"(almost) always."* A higher score means more self-reported parental sensitivity. Internal consistency was good in Wave 1 (Cronbach's $\alpha_{mothers} = .87$, Cronbach's $\alpha_{fathers} = .88$), in Wave 2 (Cronbach's $\alpha_{mothers} = .90$, Cronbach's $\alpha_{fathers} = .89$), and in Wave 3 (Cronbach's $\alpha_{mothers} = .88$, Cronbach's $\alpha_{fathers} = .88$).

2.2.3 | OXTRm

In each wave, saliva was collected to measure DNA methylation levels on the *OXTR* gene. The *OXTR* gene contains four exons and three introns and is located on chromosome 3p25-3p26.2 (hg38, 3:8750408-8769628), with the MT2 region as the most active one. Using the Oragene DNA sample collection kit (DNA Genotek Inc.), saliva was collected, and bisulfite was converted according to the manufacturer's protocol (EZ-96 DNA Methylation Kit; Zymo Research). Research has shown that *OXTR* methylation levels in the MT2 region analyzed from the periphery (saliva, blood) samples reliably correlate with DNA methylation levels measured in the brain (Danoff et al., 2021; Gregory et al., 2009; Krol et al., 2019; Puglia et al., 2020). Therefore, and because it is less intrusive than taking blood samples, saliva sample collection is an appropriate measurement method for DNA methylation in children (Smith et al., 2015). This is also the case for the DNA region analyzed in the current study.

A pyrosequencing method was used to deduce the level of DNA methylation in the part of the *OXTR* gene (Tost & Gut, 2007). The

analysis was performed on PyroMark Q96 (Qiagen). We looked at seven CpG sites located in exon 1: *-924*, *-934*, *-959*, *-982*, *-989*, *-1001*, *-1016* at *hg19*, *chr3:8,810,729-8,810,845*, some of which were studied in previous research that showed links with attachment (MT2 region; Ebner et al., 2019; Kraaijenvanger et al., 2019).

The CpG sites *-924*, *-934*, and *-914* were analyzed via an adapted protocol from Krol et al. (2019). To analyze CpG sites *-924*, *-934*, and *-914*, we used the following Polymerase Chain Reaction (PCR) primers (*OXTR Forward: TTG AGT TTT GGA TTT AGA TAA TTA AGG ATT; OXTR Reverse: -biotin-AAT AAA ATA CCT CCC ACT CCT TAT TCC TAA*). PCR conditions (Step 1: [95°C/15 min]/1 cycle, Step 2: [94°C/30 s, 54°C/30 s, 72°C/30 s]/50 cycles, Step 3: [72∘C/10 min]/1 cycle, Step 4: 4°C hold) were used for amplification of the fragment. The following sequencing primer was used to read the DNA sequence containing CpG sites *-924*, *-934*, and *-914* (*OXTR Sequencing: AGA AGT TAT TTT ATA ATT TTT*).

To analyze CpGs *-982*, *-989*, *-1001*, and *-1016*, we used the following protocol: PCR primers (*OXTR* Forward: TTG AGT TTT GGA TTT AGA TAA TTA AGG ATT; *OXTR* Reverse: /5Biosg/GGC TGC ACC TAA TGT GAT GCT AAG C), PCR conditions (Step 1: [95°C/15 min]/1 cycle, Step 2: [94°C/30 s, 58°C/30 s, 72°C/30 s]/50 cycles, Step 3: [72°C/10 min]/1 cycle, Step 4: 4°C hold). The following sequencing primer was used to read the DNA sequence containing CpG sites *-982* to *-1016* (*OXTR* Sequencing: AGG TAT TTT ATT TTT AT). Samples were randomly placed on 16 plates.

2.3 | Procedure

The current study was part of the Methylation IN Development project. To recruit participants during the school year 2016–2017 and 2017–2018 (drop-in), information letters were distributed in schools, in public areas, and on social media. Only children in the fifth grade of elementary school who were fluently Dutch-speaking were included as participants. There were three waves of data collection with each 1 year in between. In all three waves, parents completed an informed consent prior to the start of data collection. For Wave 1 and Wave 2, participants took part in the study in their classroom when recruited through schools or came to the research center when recruited another way. In Wave 3, children moved from elementary school to secondary school. Therefore, we visited them at home. Participants who came to the research center in Wave 1 and Wave 2 could return there in Wave 3 during the summer.

Only children whose parents gave active informed consent were invited to participate. In all three waves, the procedure was similar. First, children completed an informed assent form and were guided through the procedure. They started with completing questionnaires on attachment, among other questionnaires that are beyond the scope of the current study. Next, children followed the researcher into a separate room where the SBS task was administered individually. Finally, they were instructed to donate saliva for the DNA methylation extraction.

During the procedure at school, parents (both mother and father) were asked to complete questionnaires. For children who came to the lab or were home-visited, the parents could choose to complete the questionnaire on the spot. When children were taking part in the study at school, the parent questionnaires were distributed there to fill out at home and later brought back to school. One of the parent questionnaires was about self-reported sensitive parenting, which was used in the current study. This study was approved by the Ethical Committee of the KU Leuven.

2.4 | Data analysis

Prior to the main analyses, we examined the presence of univariate outliers using the robust median absolute deviation (MAD) and of multivariate outliers using the minimum covariance determinant (MCD) approach (Routliers R package, Leys et al., 2018). The MAD and MCD analyses detected univariate and multivariate outliers, and diagnostic tools to detect influential outliers (using the olsrr R package, Hebbali, 2020) in regression-based analyses also detected the presence of influential points. Accordingly, to avoid the potential bias because of these influential outliers and non-normality, all analyses employed were robust—that is, unbiased in the presence of outliers and non-normality. We also examined whether school affiliation (participants were nested within schools) accounted for a significant portion of the variance in attachment patterns and/or parental sensitivity. To do so, we compared the fit (by the Analysis of Variance [ANOVA] function in R) of intercept-only linear models (i.e., not nested within schools) with intercept-only mixed effect models (i.e., with participants nested within schools). A significant deviation test would support a nested design. None of the tests were significant (lowest $q\text{-value}_{FDR} = .164$), so we did not include school ID as a random effect. In addition, 25.82% of the data was missing (9.65% at age 10, 24.98% at age 11, and 37.13% at age 12). Although 81 patterns of missing data were present, Jamshidian and Jalal's non-parametric missing completely at random test indicated the data were missing at random (MAR): that is, Hawkins' test [$\chi^2_{(36)} = 829.89$, $p = 5.80^{-15}$] and Anderson–Darling rank test [$T_{median} = 66.20$, $p = 8.55^{-35}$] were significant. Given the high proportion of missing data and that it was MAR, we opted for a more conservative approach to the data and to conduct the analyses only on the complete cases.

We first assessed whether attachment (attachment security, attachment anxiety, attachment avoidance, and SBS knowledge) changed over time (ages 10 to 12) by conducting latent trajectories models using robust mixed-effects models in robustlmm R package (Koller, 2016). In these models, the predictor was time (coded 0, 1, and 2 with "year" as the unit of time), and the random effect was the participant number. The attachment patterns scores were the dependent variable. Similarly, we assessed whether parental sensitivity levels changed over the waves and whether methylation levels in the MT2 region of the *OXTR* gene changed over waves using additional robust mixed-effects models. In all sets of analyses, the unit of time was years. The models tested were linear and quadratic (more complex trajectories require more than three waves of measurements) and compared by deviance tests and Bayesian Information Criterion (BIC) values.

Next, we employed exploratory graph analysis (EGA) to assess the dimensionality of the seven selected CpG sites within the MT2

region (using the EGAnet R package, Hudson & Alexander, 2021). EGA estimates the number of dimensions using a graphical lasso and/or Triangulated Maximally Filtered Graph (TMFG) and a weighted network community detection algorithm. A bootstrap method for verifying the stability of the dimensions and items in those dimensions was also used with 1000 resampling cycles. EGA was conducted separately for each wave of measurement to corroborate the robustness of the findings. The EGAs were also followed by a series of Pearson correlations to estimate the pattern of associations between all CpGs.

Following these analyses, we estimated whether the intra-individual change in attachment over time is predicted by the change in parental sensitivity levels during that same period. To do so, we carried out three robust mixed-effects models with changes in maternal and paternal sensitivity (see Supplementary File 2) levels predicting each attachment change variable (change in attachment security, attachment anxiety, attachment avoidance, and SBS knowledge) in separate analyses. In these models, we also controlled for the contribution of biological sex (see preliminary analyses in Table S4 for the contribution of biological sex in Supplementary File 1). To control for multiple testing, we calculated false detection rates (FDRs; Benjamini & Hochberg, 1995). FDR controls the rate of type I errors. In line with molecular biology studies, the FDR is set to 10% (e.g., Kanaan et al., 2012). *p*-values (without control of FDR) and equivalent *q*-values (with control of FDR, based on the number of effects in all analyses, i.c., 16) are reported.

In the final models, we examined whether the change in methylation of the MT2 CpG sites of the *OXTR* gene moderated the association between the change in parental sensitivity levels and change in attachment over time (i.e., whether *OXTRm* interacts with the change in methylation to predict the change in attachment patterns). We first considered the option to include all analyses in one comprehensive model. However, given the complexity of the models and the number of effects, we decided to conduct separate analyses and not a multivariate approach while adjusting the significance of the effects by FDR. To do so, we conducted moderation analyses within robust mixed-effects models. To prevent multicollinearity, predictors and moderators were centered around their sample mean. Significant interactions were probed and plotted by simple slope analyses using the interactions R package (Long, 2019). In these models, we controlled for biological sex (using it as a covariate), and for batch effect (by introducing it as a random effect).

3 | RESULTS

3.1 | Preliminary analyses

All preliminary analyses are reported in detail in Supplementary File 1. The models revealed that a linear trend of change was superior to a quadratic trend across all tested models (i.e., the addition of the quadratic term did not add significantly to the explained variance; $\Delta\chi^2_{(1)}$ *p*-values > .11)), with the linear models' BIC values being lower than those of the quadratic ones. The linear models indicated that attachment security, as well as children's SBS knowledge, tended to increase over time and that attachment anxiety but not avoidance tended to decrease during that period of time. The currently observed linear trend is in keeping with past longitudinal research on these measurements of attachment (Jones et al., 2018; T. E. Waters et al., 2022). Parental sensitivity did not significantly change over time at the group level, though at the individual level, there were a substantial number of families reporting differences over time.

Our analysis identified two distinct sets of CpGs, with the first set comprising CpGs *-924*, *-934*, and *-959*, all of which exhibited high levels of methylation. These sites are situated adjacent to an inhibitory site of the *OXTR* gene. Research on these specific sites, particularly *-934*, has revealed inconsistencies: Some studies suggest a correlation between these sites and increased expression of the *OXTR* gene (e.g., Danoff et al., 2021), while others, including our findings, associate them with the inhibition of this expression or behavioral correlates (e.g., De Leon et al., 2020; Jack et al., 2012). For instance, De Leon et al. (2020) observed in a large sample of rhesus monkeys that higher methylation at *-934* correlated with increased, rather than decreased, affiliative behavior. Similarly, Jack et al. (2012) found that higher methylation at *-934* was linked to more, not less, activation of two social brain regions: the left superior temporal gyrus and the cingulate gyrus. In our study, the findings align with the latter findings, and we propose that higher methylation levels at these CpGs indicate reduced activity of the inhibitor, thereby enhancing the expression of the *OXTR* gene (Mamrut et al., 2013).

The second set of CpGs, comprising CpGs *-982*, *-989*, *-1001*, and *-1016*, exhibited low levels of methylation and were located adjacent to the promoter site of the gene. It is presumed that higher methylation levels near the promoter region lead to decreased expression of the *OXTR* gene (Mamrut et al., 2013). For instance, Andari et al. (2020) demonstrated that increased methylation at *-989* was associated with reduced brain resting-state functional connectivity between independent components representing the theory of mind, specifically the superior temporal sulcus and the posterior cingulate cortex.

However, the methylation levels within these sites were not highly correlated; thus, in line with research from Weaver et al. (2004), we decided to keep studying the effects of the changes in methylation levels of the different CpGs separately instead of clustering. Further, sex was related to less *OXTRm* in the promotor region and more methylation in the inhibitor region in girls. Finally, over time, an increase in sensitive parenting was linked to a greater increase in attachment security, and a greater decrease in attachment anxiety and avoidance, but not with a change in SBS knowledge (see Tables A1 and A2 in the Appendix).

3.2 | Does *OXTRm* moderate the association between the change in parental sensitivity and the change in attachment patterns over time?

Results are presented in Table A1 (maternal sensitivity). Results on the moderation of change in *OXTRm* in the link between change in paternal sensitivity and change in attachment over time can be found

in Table S5 (see Supplementary File 2). Our exploratory analyses revealed some interactions between changes in maternal sensitivity with changes in *OXTRm* on changes in attachment over time, but the majority of effects were non-significant. However, the significant interaction effects revealed a consistent pattern of results. If *OXTRm* (within the MT2 region) increased in the promotor region and decreased in the inhibitor region over time, a significant association between change in maternal sensitivity and change in attachment emerged. Increasing maternal sensitivity over time was then linked to increasing attachment security and decreasing insecurity. If *OXTRm* (within the MT2 region) decreased in the promotor region and increased in the inhibitor region over time, there was no association between change in maternal sensitivity and change in attachment. The specific effects are depicted below.

3.2.1 | Maternal sensitivity

The interactions between the change in maternal sensitivity and in methylation for predicting the change in attachment over time are presented in Figure 1. Simple slopes tests indicated that increased levels of maternal sensitivity were not associated with increased levels of attachment security and/or SBS knowledge for children whose pattern of methylation decreased in the promotor region and increased in the inhibitor region over time. Specifically, when methylation increased on the *OXTR* gene inhibiting *-959* CpG (i.e., +1 *SD*; an average increase of 7.64%) and methylation decreased on the *OXTR* gene promoting *-989* CpG (i.e., -1 *SD*; an average decrease of 4.67%), the level of attachment security and/or SBS knowledge was high regardless of maternal sensitivity ($b = .06$, $SE = 0.05$, $t = 1.9$, $p = .199$ for security [*-959*], $b = -.07$, $SE = 0.09$, $t = -0.79$, $p = .423$ for SBS knowledge [*-959*], and $b = -.03$, $SE = 0.09$, $t = -0.38$, $p = .705$ for SBS knowledge [*-989*]). Effects remained significant after controlling for multiple tests with FDR.

Conversely, simple slopes tests indicated that increased levels of maternal sensitivity were associated with increased levels of attachment security and/or SBS knowledge for adolescents whose pattern of methylation increased in the promotor region and decreased in the inhibitor region over time. Specifically, for children with less methylation on the *OXTR* gene inhibiting *-959* CpG (i.e., -1 *SD*; an average decrease of 7.35%) and more methylation on the *OXTR* gene promoting *-989* CpG (i.e., +1 *SD*; an average increase of 7.65%), the level of attachment security and/or SBS knowledge was dependent on maternal sensitivity: The higher the increase in maternal sensitivity, the greater the increase in attachment security ($b = .22$, $SE = 0.04$, $t = 5.07$, $p = 5.00^{-07}$ on *-959*) and SBS knowledge ($b = .29$, $SE = 0.09$, $t = 3.37$, $p = .0008$ on *-959*, and $b = .25$, $SE = 0.09$, $t = 2.74$, $p = .006$ on *-989*). As maternal sensitivity increased over time (+1 *SD*), the estimated level of attachment security and SBS knowledge increase over time was as high among adolescents who showed methylation increase in the promotor region and decrease in the inhibitor region as in adolescents who showed a decrease in methylation in the promotor region and increase in the inhibitor region of the *OXTR* gene over time.

4 | DISCUSSION

The current study explored whether the association between change in sensitive parenting and change in attachment is moderated by the change in *OXTRm* in middle childhood. Across different CpGs, some significant interaction effects emerged, but most effects were non-significant. However, all significant interaction effects reflected a consistent pattern. When *OXTRm* increased in the promotor region and decreased in the inhibitor region over time, increasing parental sensitivity was linked to increasing attachment security and decreasing attachment insecurity. When *OXTRm* decreased in the promotor region and increased in the inhibitor region over time, there was no association between change in sensitive parenting and change in attachment (in)security. More specifically, when *OXTRm* decreased in the promotor region and increased in the inhibitor region over time, attachment security remained high.

The current study's exploratory findings add to a novel, but growing literature regarding links between *OXTRm* and attachment development. Most of these studies looked at cross-sectional associations between *OXTRm* and attachment (see Ebner et al., 2019; Ein-Dor et al., 2018; Lecompte et al., 2021). The current study's main effects of changes in *OXTRm* on changes in attachment suggest that these effects might not be strong or robust. This calls for caution against strong claims about the direct impact of *OXTRm* changes on attachment changes within the age period of between 10 and 12 years old. As could be expected from previous research, children's age in the current study might be past the most sensitive period of epigenetic changes and attachment development (Pérez et al., 2019). However, it is important to highlight that our study accounted for the change in all the variables, in contrast to previous studies. Significant changes in *OXTRm* and attachment levels were found in the current study, but the changes were small. This might have rendered it harder to find effects, compared to when we would have looked into cross-sectional levels of *OXTRm* and attachment. Also, existing *OXTRm* —attachment research was only done in adults. We tested these associations in middle childhood, a time period in which change occurs at multiple domains of maturation (Del Giudice, 2015). Hence, it might have been harder to find change-effects at this age. Nevertheless, the literature does suggest that one needs to stay prudent when assessing the relevance of *OXTRm* for attachment development (Maud et al., 2018), and the current study's results further support this concern.

Slightly more support was found for the idea that *OXTRm* could moderate the association between change in sensitive parenting and attachment development. For the significant effects that emerged, the interpretation of the significant interactions was consistent and points at a potential moderating role of changes in *OXTRm* in the link between changes in sensitive parenting and attachment development. When *OXTRm* increased in the promotor region and decreased in the inhibitor region over time, increasing parental sensitivity was linked to increasing attachment security and decreasing attachment insecurity. When *OXTRm* decreased in the promotor region and increased in the inhibitor region over time, there was no association between change in sensitive parenting and change in attachment (in)security. This observation

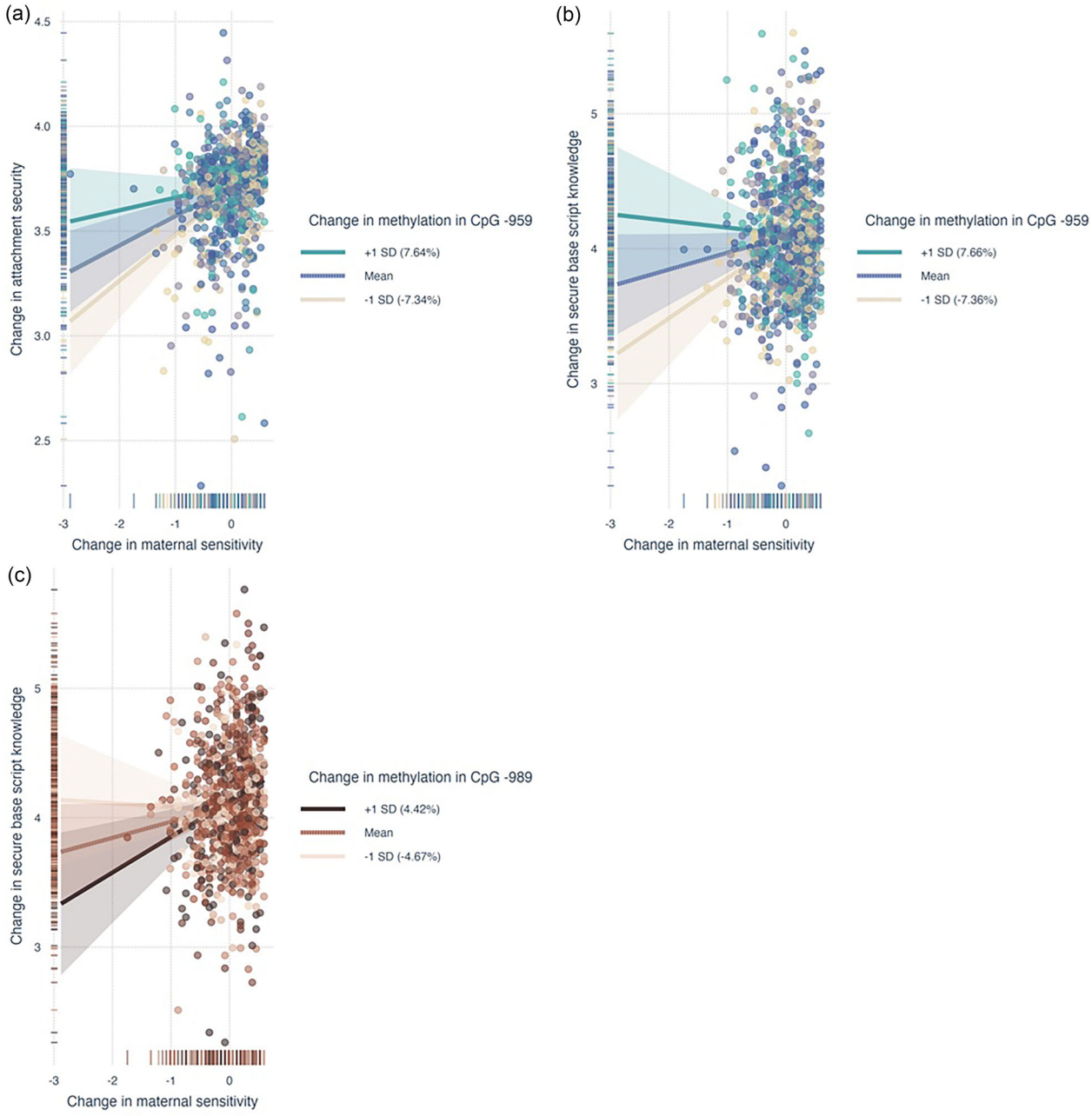

FIGURE 1 Simple slopes for the effect of the change in maternal sensitivity on the change in attachment patterns (a = security, b, c = SBS knowledge) as a function of change in methylation on *-959* (inhibitor) and *-989* (promoter) CpGs.

is new and adds to the *OXTRm*—attachment research literature, and converges with past research showing a similar effect for *NR3C1* gene methylation in the association between sensitive parenting and change in anxious attachment (Bosmans et al., 2018).

However, we need to remain cautious, especially related to the meaning of the significant findings with different CpG sites. Concern has been raised about the difficulty of replication of *OXTRm* -related effects (Min et al., 2021; Rijlaarsdam et al., 2016). Although the latter studies are epigenome-wide association studies (EWAS), which are not completely generalizable to candidate gene studies, the selection of a few CpGs out of so many remains arbitrary. Mulder et al. (2017) found great instability in the methylation of CpGs, and test–retest research is lacking. Further, the methylation levels might be influenced not only by environmental exposures but also genetically predisposed, by both direct and more distal genes (Min et al., 2021). It might thus be that methylation levels are not just the impacting factor on psychological outcomes like attachment development but also are the result of psychological outcomes (Min et al., 2021). This shows how vulnerable methylation research is and how cautious we should be about drawing conclusions about the current findings. Finally, the previously discussed inconsistencies in the direction of the methylation effects related to CpGs *-924*, *-934*, and *-959* in different studies

suggest that more research is needed to test whether the currently found inhibitory effects hold for explaining individual differences in attachment. It is safe to say that at this point in the child developmental research on epigenetics, we are merely scratching the surface, exploring whether epigenetics has applied significance for child development in general and attachment in specific (van IJzendoorn & Bakermans-Kranenburg, 2024). Nevertheless, studies like the current one are essential to generate knowledge and help guide future studies toward a stronger empirical knowledge base. The current study's interaction effects are theoretically meaningful and could reflect a differential susceptibility effect. It could be that with increasing *OXTRm* in the promotor region and decreasing *OXTRm* in the inhibitor region, children's susceptibility to parenting effects increases, for example, leading to a stronger association between changes in sensitive parenting and changes in attachment (in)security (Boyce & Ellis, 2005; Klein Velderman et al., 2006). Thus, it could be that especially in those children whose methylation of the *OXTR* gene expression increased in the promotor region and decreased in the inhibitor region over time, more sensitive parenting can still have a compensatory effect while less sensitive parenting could further deteriorate children's ongoing insecure attachment development. More research will be needed to investigate whether the current results can be replicated and to investigate the meaning of these results. Nevertheless, the current findings could prove relevant to better understand the relation between changes in sensitive parenting and attachment development.

4.1 | Limitations

We found limited significant results, and they should be interpreted with caution. First, the current study used a candidate gene approach to explore the role of *OXTRm* in the association between parental sensitivity and attachment development. In such an approach, only one candidate gene is investigated, and replication of findings is very difficult due to different sample characteristics (e.g., clinical samples vs. population samples), measurement differences, different analysis methods used (e.g., pyrosequencing vs. Infinium 450K), or the fact that the chance of false positives is higher in candidate gene studies (Dall'Aglio et al., 2020; Mulder et al., 2017). In addition, it is important to note that next to the oxytocin system, also other systems might impact parent–child attachment development, such as the reward system (e.g., LeRoy et al., 2019). The current sample was also not completely representative of the general population in Flanders since parents were more highly educated in our sample (mothers = 83.7%; fathers = 76.0%), compared to the population (52.4%; www.statbel.fgov.be). As Ioannidis et al. (2006) noted, effect sizes in candidate gene research are generally very small, and large sample sizes are needed to reach sufficient statistical power. A wide variety of (epigenetic) influences might be at play on attachment development, while by studying just one candidate gene, only a very small subregion is investigated.

Recently, hypothesis-free genome-wide association studies and EWAS were performed in an attempt to provide a solution for this problem that makes replication more difficult (Parade et al., 2021). An example is a study by Cicchetti et al. (2016) showing that children with a maltreatment history had a different whole-genome methylation pattern than controls and not just in one candidate gene. On locations where methylation was generally low in controls, methylation was generally high in children with a maltreatment history, and vice versa, spread out over different genes. Furthermore, Dall'Aglio et al. (2020) found no association between maternal sensitivity and DNA methylation (among which *OXTR*) in candidate gene studies but did find an association in EWAS with 13 DNA methylation regions linked to maternal sensitivity. However, for EWAS, even bigger sample sizes are needed to be able to identify effects after adjusting for multiple testing. In addition, the financial cost of such studies is enormous, which is why we chose to conduct a candidate gene study.

Second, different CpG sites were involved in different interaction effects. It is unclear why certain effects are found with only particular CpG sites in the same region. As a result, we cannot draw strong conclusions about the meaning of the interaction that includes certain CpG sites specifically. We can only generalize the interactions of specific CpG sites with sensitive parenting to interaction with either the promotor region or the inhibitor region of the *OXTR* gene in general with sensitive parenting.

A third limitation is that methylation levels can only be derived from peripheral tissues and not from cells in the brain directly. Therefore, criticism exists about methylation levels derived from different peripheral tissues such as saliva and its correspondence to blood and plasma levels of methylation. Braun et al. (2019) examined salivary, blood, buccal, and brain levels of methylation in patients who needed brain surgery and concluded that for some CpG sites, brain methylation levels corresponded best with blood methylation levels and for other CpG sites with salivary methylation levels. Levels of methylation differed depending on the peripheral tissues used. Puglia et al. (2020) indicated that saliva sampling was a reliable method to derive methylation levels specifically of the *OXTR* gene. One limitation of the current sample is that we did not have data on the different cell types (buccal or white blood cells) in the saliva from which methylation levels were derived, a technique often used in EWAS (Langie et al., 2017). Still, we believe that, especially in children, it is important to choose the least invasive method to measure methylation levels that are also reliable, which is demonstrated in previous research (Langie et al., 2017).

A fourth limitation is that we had no information regarding the pubertal status of participants. Szyf and Bick (2013) suggested that epigenetic changes and gene expression are sensitive to developmental changes. In addition, the parent–child relationship might change due to puberty (Marceau et al., 2015). Dadds et al. (2014) showed that from the age of 9, children start to show signs of pubertal development and show *OXTRm* changes. However, since our sample included children of at least 10 years old, we can assume that there is little difference in whether children started puberty or not. In addition, sex hormones start to increase in puberty as well and also might influence methylation levels (Kaminsky et al., 2006). Therefore, we chose to control for sex in all analyses, to compensate partly for the lack of information about pubertal status. However, future research should take into account pubertal status as a possible covariate.

A fifth limitation is that parental sensitivity was measured using self-report. Self-reported measures might have induced a social desirability bias (Van de Mortel, 2008). This in turn could explain why parental sensitivity did not change a lot over time in the current study.

Although the current study has some limitations, the design also has important advantages. To our knowledge, this is the first study that investigates changes over time in sensitive parenting, attachment (in)security, and *OXTRm*, and how they are related to each other. By looking at the changes over time, we included both within- and between-subjects information that makes the directional relations more meaningful since a change in the predictor (parental sensitivity) is associated with a change in the outcome variable (attachment (in)security). However, one still needs to be cautious in interpreting the results because we cannot be certain about other factors impacting the changes over time in all measures. Using changes over time also reduces information in that sense (Griffin et al., 1999). Although the current study was exploratory, the fact that we found some consistent patterns of effects might guide future research more to model-based approaches to try and replicate the findings of the current study (Griffin et al., 1999).

In addition, the current study examined CpG sites that have not been examined before. This led to new insights about the different effects of methylation, depending on the region studied. For example, more methylation on CpG sites in the promotor region (*-982, -989, -1001, and -1016*) is assumed to lead to less expression of the *OXTR* gene, which has been demonstrated before (Krol et al., 2019). In contrast, more methylation on CpG sites in the inhibitor region (*-924, -934*, and *-959*) is assumed to lead to more expression of the *OXTR* gene since the gene is less inhibited then. In psychological research, often DNA methylation is stated as always leading to less expression of the gene. However, in general, the levels of methylation are higher in the inhibitor region than in the promotor region, leading to opposite effects. This is an insight that has often been overlooked in previous research, although similar high methylation percentages were found in inhibitor regions versus promotor regions (e.g., Moerkerke et al., 2021; Puglia et al., 2018).

4.2 | Conclusion and implications

In sum, the current study found that changes in maternal sensitivity were associated with attachment development, depending on the changes in *OXTRm*. Only for children with increased *OXTRm* in the promotor region and decreased methylation in the inhibitor region, an association between change-sensitive parenting and attachment development was found. Decreased *OXTRm* in the promotor region and increased *OXTRm* in the inhibitor region over time functioned as a buffer against possible detrimental effects of decreased sensitive parenting on attachment development. In line with the differential susceptibility hypothesis, for clinical practice, it is thus important to note that changes in sensitive parenting will not have the same effects on attachment development in all children.

Our findings suggest that those elementary school children whose *OXTR* gene got more methylated due to being exposed to adverse stimuli earlier in life might still benefit substantially from interventions aimed at improving the parent–child relationship in general and the attachment relationship in specific. This is an optimistic message in times where clinicians are inclined to emphasize the importance of the first years of life for child development (Bakermans-Kranenburg et al., 2019). The current study suggests that in later years, important shifts in attachment can still happen when parents improve their sensitive parenting over time and that the more disadvantaged children might even be more likely to benefit from interventions that stimulate such shifts (like has been observed in differential susceptibility research; Bakermans-Kranenburg & van IJzendoorn, 2015). Nevertheless, more research is needed to replicate and understand the current findings in order to infer more meaningful implications from these results. If supported, research could explore whether interventions aimed at stimulating a secure attachment development in elementary school children (e.g., Middle Childhood Attachment-Based Family Therapy; Van Vlierberghe et al., 2023) are more effective in children displaying higher levels of *OXTRm*.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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APPENDIX

TABLE A1 Robust mixed-effects models for predicting the change in attachment patterns by the change in maternal sensitivity as a function of OXTRm while controlling for biological sex and batch effects.

	Attachment security					Secure base script				
Predictors	*b*	*Beta*	*95% CI Beta*	*p*	*q*	*B*	*Beta*	*95% CI Beta*	*p*	*q*
(Intercept)	3.77	.18	.10–.26	**<.001**		4.08	.13	.03–.23	**<.001**	
CpG -*924* (inhibitor)	0.00	.00	−.04–.05	.861	.861	−0.00	−.06	−.13–.01	.094	.139
CpG -*934* (inhibitor)	−0.00	−.00	−.05–.04	.959	.899	0.00	.06	−.01–.12	.126	.139
CpG -*959* (inhibitor)	0.00	.02	−.03–.06	.436	.654	0.00	.01	−.06–.07	.761	.406
CpG -*982* (promoter)	−0.00	−.02	−.07–.02	.243	.602	0.00	.01	−.06–.07	.903	.452
CpG -*989* (promoter)	−0.00	−.04	−.08–.00	.058	.218	0.01	.05	−.01–.12	.130	.139
CpG -*1001* (promoter)	0.00	.01	−.03–.05	.594	.783	0.01	.04	−.02–.10	.207	.151
CpG -*1016* (promoter)	0.00	.01	−.04–.05	.755	.814	−0.00	−.02	−.08–.05	.640	.366
Maternal sensitivity	0.15	.15	.10–.20	**<.001**	**<.001**	0.12	.06	−.01–.13	.058	.116
Sex (boys)	−0.04	−.10	−.22–.02	.099	.297	−0.25	−.37	−.52–−.22	**<.001**	**<.001**
CpG -*924* (inhibitor) × Sensitivity	0.00	.02	−.03–.07	.434	.654	−0.01	−.05	−.13–.02	.156	.139
CpG -*934* (inhibitor) × Sensitivity	−0.00	−.01	−.05–.04	.760	.814	0.00	.02	−.05–.09	.509	.313
CpG -*959* (inhibitor) × Sensitivity	−0.01	−.05	−.10–−.01	**.012**	**.090**	−0.02	−.10	−.17–−.03	**.003**	**.011**
CpG -*982* (promoter) × Sensitivity	0.01	.04	−.00–.08	.053	.218	0.03	.05	−.02–.11	.153	.139
CpG -*989* (promoter) × Sensitivity	0.00	.02	−.02–.06	.321	.602	0.03	.08	.01–.14	**.025**	**.067**
CpG -*1001* (promoter) × Sensitivity	0.01	.02	−.02–.07	.321	.602	−0.01	−.03	−.10–.04	.368	.245
CpG -*1016* (promoter) × Sensitivity	0.00	.01	−.03–.05	.626	.783	−0.02	−.04	−.11–.02	.201	.151
	Random effects									
σ^2	0.03					0.28				
τ_{00}	0.04_{id}					0.12_{id}				
	0.00_{batch}					0.00_{batch}				
ICC	0.56					0.30				
N	448_{id}					444_{id}				
	16_{batch}					16_{batch}				
Observations	914					889				
Marginal R^2/conditional R^2	0.072/0.595					0.073/0.353				

Note: *q* = significance of all effects was adjusted by adaptive false discover rate (FDR) of 10%.

TABLE A2 Robust mixed-effects models for predicting the change in attachment patterns by the change in paternal sensitivity as a function of *OXTRm* while controlling for biological sex and batch effects.

	Attachment anxiety					Attachment avoidance				
Predictors	*b*	*Beta*	*95% CI Beta*	*p*	*q*	*B*	*Beta*	*95% CI Beta*	*p*	*q*
(Intercept)	1.32	−.21	−.27−−.15	**<.001**		2.33	−.16	−.27−−.04	**<.001**	
CpG -*924* (inhibitor)	0.00	.00	−.04–.04	.911	.944	0.00	.02	−.05–.08	.652	.963
CpG -*934* (inhibitor)	−0.00	−.01	−.06–.03	.500	.784	0.00	.00	−.06–.07	.963	.963
CpG -*959* (inhibitor)	−0.00	−.04	−.08–.00	.052	.264	0.00	.01	−.050.07	.815	.963
CpG -*982* (promoter)	0.00	.00	−.04–.04	.829	.944	0.00	.01	−.05–.07	.737	.963
CpG -*989* (promoter)	0.00	.02	−.02–.06	.231	.615	0.00	.01	−.05–.07	.664	.963
CpG -*1001* (promoter)	0.00	.01	−.03–.05	.637	.784	−0.01	−.05	−.11–.01	.112	.448
CpG -*1016* (promoter)	−0.00	−.01	−.05–.03	.570	.784	−0.00	−.01	−.07–.05	.668	.963
Maternal sensitivity	−0.27	−.13	−.17−−.09	**<.001**	**<.001**	−0.43	−.15	−.22−−.08	**<.001**	**<.001**
Sex (boys)	0.00	.00	−.07–.08	.944	.944	0.29	.25	.08–.42	**.004**	**.030**
CpG -*924* (inhibitor) × Sensitivity	−0.00	−.01	−.06–.03	.631	.784	−0.01	−.02	−.09–.05	.547	.963
CpG -*934* (inhibitor) × Sensitivity	0.00	.02	−.02–.06	.405	.720	0.00	.01	−.05–.08	.747	.963
CpG -*959* (inhibitor) × Sensitivity	0.01	.04	−.00–.08	.066	.264	0.01	.03	−.03–.09	.347	.925
CpG -*982* (promoter) × Sensitivity	−0.03	−.04	−.08−−.00	**.040**	.264	−0.04	−.05	−.11–.02	.142	.454
CpG -*989* (promoter) × Sensitivity	0.01	.02	−.02–.06	.269	.615	0.00	.00	−.06–.07	.931	.963
CpG -*1001* (promoter) × Sensitivity	−0.01	−.03	−.07–.02	.246	.615	−0.00	−.00	−.07–.06	.913	.963
CpG -*1016* (promoter) × Sensitivity	0.01	.02	−.02–.06	.361	.720	−0.04	−.06	−.12–.01	.083	.443
	Random effects									
σ^2	0.22					0.61				
τ_{00}	0.00_{id}					0.67_{id}				
	0.00_{batch}					0.00_{batch}				
ICC	0.01					0.52				
N	445_{id}					445_{id}				
	16_{batch}					16_{batch}				
Observations	905					905				
Marginal R^2/conditional R^2	0.071/0.082					0.051/0.549				

Note. *q* = Significance of all effects was adjusted by adaptive FDR of 10%.