Epigenetic Regulation of the Oxytocin System: Adaptation to Overcontrolling Parenting, and Links to Relationships and Internalizing Symptoms

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Abstract

DNA methylation of the oxytocin receptor gene (*OXTR*m) reduces the gene's expression and has been associated with a variety of environmental precursors and outcomes for social development and mental health, both favorable and detrimental. This multi-method, longitudinal study, using a diverse community sample of 184 adolescents followed from age 13 to 28, examined the links between *OXTR*m and the experience of overcontrolling parenting in adolescence and conflict with romantic partners and internalizing symptoms in adulthood. Female, but not male, adolescents who experienced overcontrolling parenting at age 13 in the form of psychological control had lower levels of *OXTR*m at age 28. Reduced *OXTR*m was associated with greater relationship conflict at age 27, as reported by their romantic partner, and reduced sensitivity to the conflict, lending support to the theory that greater oxytocin reduces the salience of negative stimuli, such as angry and fearful faces. Finally, lower levels of *OXTR*m were associated with fewer internalizing symptoms at ages 24-25, which is also consistent with oxytocin reducing the salience of negative stimuli and lends support to the positive association between *OXTR*m and internalizing symptoms, particularly depression and anxiety, in the literature. Findings are consistent with the "tend-and-befriend" hypothesis which states that social stress leads to increased oxytocin, which spurs individuals to seek social support and reduce stress. Overall, these results suggest that lower *OXTR*m, and therefore a greater ability to take in oxytocin, is not necessarily the "favorable" state in all cases, as it is linked to diverse developmental, social, and mental health correlates.

Epigenetic Regulation of the Oxytocin System: Adaptation to Overcontrolling Parenting, and Links to Relationships and Internalizing Symptoms

The neuropeptide oxytocin plays an important role in the social and emotional functioning of humans and other social animals. Evidence suggests that oxytocin is involved in the recognition and classification of emotions, regulation of affect, maternal behavior, and attachment, among other functions vital to human social life (Lee et al., 2009). Research also supports the role of oxytocin as an affiliative hormone that promotes social approach, by promoting trust and increasing orientation of gaze towards the eye region of human faces (Kosfeld et al., 2005; Heinrichs & Domes, 2008). These social perception and approach processes may then impact affiliative behavior; additional research demonstrates an increase in attachment security and fathers' engagement with their child in response to the intranasal administration of oxytocin (Buchheim et al., 2009; Naber et al., 2010).

In order for oxytocin to exert its effects, it must bind to the oxytocin receptor. The receptor's synthesis is directed by the oxytocin receptor gene (*OXTR*), which is expressed both centrally, in the brain, and in peripheral tissues. Plasma oxytocin levels measure how much oxytocin is available in the system, but it does not account for the body's ability to bind and make use of that oxytocin, which is why it's essential to consider the oxytocin receptor, as well. One way oxytocin receptors, and the downstream consequences of oxytocin utilization, are regulated is through DNA methylation of *OXTR* (*OXTR*m). This epigenetic ("above the gene/DNA") modification involves the addition of methyl groups to the DNA at cytosine residues typically in the context of CpG sites—locations in the DNA sequence where a cytosine dinucleotide is followed by a guanine dinucleotide. Research across both human and animal studies has shown that *OXTR*m decreases the expression of *OXTR*, likely leading to fewer

oxytocin receptors and a diminished ability to bind oxytocin and experience its effects (Gregory et al., 2009; Perkeybile et al., 2019; Danoff, Wroblewski, et al., 2021).

DNA methylation may be more closely related to the gene's expression, and therefore to potential downstream behaviors, in certain parts of *OXTR*. For instance, the promoter region is where transcription factors bind, initiating the process of synthesizing the receptors—DNA methylation in this region, specifically, may make it more difficult for these factors to bind and therefore reduce the gene's expression. Danoff, Wroblewski, and colleagues (2021) examined the association of different *Oxtr* regions with gene expression in prairie voles, which are well-suited animal models because 1) they are highly social rodents that exhibit similar social behaviors to humans, and 2) the CpG sites -901, -924, and -934 in the MT2 region (see Kusui et al., 2001) of the gene are highly conserved in both prairie voles and humans. They found that in prairie voles, DNA methylation in the MT2 region near the *Oxtr* promoter is associated with both early parental care and with actual gene expression, with greater methylation predicting reduced expression. Additionally, DNA methylation at these CpG sites in the MT2 region were associated with *OXTR* expression in human cortex tissue, in the same direction. These findings point to specific sites in *OXTR*, that reliably predict actual gene expression, at which to investigate how DNA methylation is associated with early experience, as well as how it might impact downstream behavior.

Several lines of research suggest that *OXTR*m is likely to be influenced by environmental factors. Specifically, there is evidence that inadequate early care may have long-lasting consequences leading to increased *OXTR*m. Research on prairie voles has found increases in de novo *OXTR*m across sites -901, -924, -934_1 and -934_2 in response to low levels of early parental care, and these changes were found in DNA samples from both brain and blood

(Perkeybile et al., 2019). In humans, one study of early experience that followed young adults from the age of six found that greater early life adversity—the experience of abuse, neglect, or poor socioeconomic conditions—was associated with greater *OXTR*m at age 27 at several CpG sites in the promoter region and intron 1, though only in females (Gouin et al., 2017). These studies suggest that negative early experiences may be precursors to elevated *OXTR*m.

However, it is possible that adversity in childhood and adolescence may instead correspond to *reduced OXTR*m. Contradictory findings have been emerging in this field, suggesting that *OXTR*m is associated with various outcomes and precursors, both adaptive and maladaptive. Psychopathology and attachment, as correlates or potential outcomes of *OXTR*m, are two areas in which these contradictions emerge. For instance, some studies have found that depression and anxiety are associated with greater *OXTR*m (Bell et al., 2015; Chagnon et al., 2015; Gouin et al., 2017), while other studies utilizing various different samples—including clinically depressed patients, U.S. war veterans, and women with post-partum depression—have exhibited the opposite relationship, where depression diagnosis or greater symptom severity is associated with *less OXTR*m (Reiner et al., 2015; Warrener et al., 2021; Kimmel et al., 2016). Similarly, Ziegler and colleagues (2015) found that *OXTR*m levels were *lower* in those with diagnoses of social anxiety disorder compared to healthy controls. Contradictory findings are also found in the literature on *OXTR*m and autism spectrum disorders (see Gregory et al., 2009 and Elagoz Yuksel et al., 2016). In the attachment literature, consistent with the theory that oxytocin promotes social affiliation and that *OXTR*m correspondingly is likely to be associated with less affiliative behavior, greater *OXTR*m in the promoter region has been associated with greater attachment anxiety (Ebner et al., 2019) and greater attachment avoidance in young adults (Ein-Dor et al., 2018). However, contrary to this finding of a positive relationship between

*OXTR*m and attachment avoidance in young adults, Ebner and colleagues (2019) found a *negative* relationship, also in young (and, notably, not in elderly) adults. The disparities in these findings emphasize two points. First, it is important to consider the specific CpG sites at which methylation is being examined, as different sites in different regions may have different impacts on actual oxytocin receptor gene expression and downstream products and behaviors (Danoff, Wroblewski et al., 2021). Second, and along with the first point, researchers cannot assume that higher levels of *OXTR*m are linked to adversity or poor outcomes of all varieties. There is a clear need to examine more closely how *OXTR*m relates to various developmental, psychological, and interpersonal factors, and why.

One theory responding to this question of "why" is that methylation of the oxytocin receptor gene may act in evolutionarily adaptive or compensatory ways, adjusting the body's biological mechanisms to account for and react to the organism's experiences. Research suggests that early parental care "tunes" the oxytocin system through *OXTR*m, such that offspring who receive low care have greater *OXTR*m, reducing their body's ability to use oxytocin, conserving resources, and preparing them for both their current and future environments where they may expect to continue experiencing low levels of parental care (Perkeybile et al., 2019; Krol, Moulder, et al., 2019). This downregulation of the oxytocin system is one way that humans may adapt to adverse experiences, but it is also plausible that compensatory mechanisms could play a role, instead *up*regulating the oxytocin system in response to difficult experiences. Ziegler and colleagues (2015) posited just such a mechanism as the role of oxytocin in social anxiety disorder; they found that *OXTR*m levels were *lower* in those with social anxiety disorder as compared to healthy controls, and that greater *OXTR*m was actually correlated with lower symptom severity. These findings support previous work which had found that greater social

anxiety symptom severity and relationship dissatisfaction were both associated with *higher* plasma oxytocin levels among those with social anxiety disorder (Hoge et al., 2008), so Ziegler and colleagues concluded that reduced *OXTR*m levels in those with social anxiety disorder may be compensating for social difficulties, increasing *OXTR* expression and the quantity of oxytocin receptors in order to better utilize the oxytocin that is available and attempt to remedy the social difficulties through the neuropeptide's social affiliative properties.

Compensatory mechanisms may be particularly important in adolescence, when young people begin to transition from a state of primarily relying on caretakers toward ultimately functioning as autonomous adults. The "tend and befriend" theory posits one such potential mechanism, that oxytocin may upregulate—in this case, through decreased *OXTR*m and, therefore, more oxytocin receptors—in response to gaps in positive relationships. This theory, as proposed by Taylor and colleagues (2000), suggests an alternative to the typical "fight or flight" response to stress: That in lieu of the "fight or flight" response, some individuals may focus instead on nurturing children and seeking companionship by creating social networks of support. It is possible that processes involved in the development of autonomy in adolescence may interact with the oxytocin system in a way that is consistent with the tend-and-befriend theory; specifically, parents' undermining of their adolescent's development of autonomy—a stressful relationship experience—may be associated with an upregulation of the adolescent's oxytocin system through reduced *OXTR*m. This theoretical model is depicted in Figure 1. There are various ways to inhibit an adolescent's development of autonomy. One way is parental psychological control, which is the use of manipulation, for instance through guilt or other harsh methods, to control their child (Loeb et al., 2020). This type of overcontrolling parenting, which by nature inhibits developmentally appropriate growth in autonomy, has repeatedly been

associated with numerous poor outcomes for adolescents, including reduced autonomy observed with peers and later romantic partners, lower peer acceptance, reduced academic attainment by adulthood (age 32), and a blunted heart rate response to stress at age 29 (Oudekerk et al., 2015; Loeb et al., 2020; Loeb et al., 2021). Parental psychological control is clearly an adverse experience for adolescents, and it is possible that compensatory mechanisms may develop to help adolescents adjust to consistently controlling parenting. Namely, parental psychological control experienced in adolescence may, through decreased *OXTR*m or the prevention of de novo methylation, upregulate the body's ability to use oxytocin in an environment where they are stressed and are likely not getting the nurturing relationships that they need. It is also possible that psychologically controlling parents are also more involved parents, and research in prairie voles suggests that parents more involved in their offspring's care have offspring with lower *OXTR*m levels (Perkeybile et al., 2019), although with prairie voles, this involvement appeared primarily nurturing, not controlling.

Theory and supporting research suggest that stressful conditions, such as the experience of overcontrolling parenting in adolescence or other stressful relationships, are likely linked to adaptive responses characterized by elevated oxytocin uptake, and it's possible this compensatory mechanism may differ by gender. Taylor and colleagues (2000) specifically proposed that tend-and-befriend responses to stress are more dominant in women and that oxytocin plays a key role in this biobehavioral mechanism; this gender difference in stress responses and the potential role of oxytocin has been supported by research (e.g., Taylor et al., 2006; Cardoso et al., 2016). The tend-and-befriend theory has been expanded to elaborate on oxytocin as a mechanism: In the presence of social distress or gaps in positive social relationships, oxytocin is released to promote affiliative responses in an effort to close any

relationship gaps and remedy this social distress (Taylor, 2006). Indeed, one study found higher plasma oxytocin levels in women who had been experiencing problems in their relationships, including unrewarding marriages or partnerships and/or decreased social support from friends and family (Taylor et al., 2006).

The tend-and-befriend theory suggests that a similar compensatory effect will be found with regard to social anxiety and its relationship with the endogenous oxytocin system. Social anxiety, which involves significant fear of social situations and/or performance situations and can greatly interfere with individuals' social lives, may be thought of as social distress, and those with social anxiety disorder have both higher plasma oxytocin and lower *OXTR*m levels (Hoge et al., 2008 and Ziegler et al., 2015, respectively). Consistent with increased oxytocin then promoting affiliation, another study found that oxytocin administered intranasally increased perceived support while recalling emotional memories when an experimenter (i.e., potential social support) was available, but only in women, supporting the role of oxytocin in promoting social support seeking in distressed women (Cardoso et al., 2016). Based on this evidence, it is plausible that in response to chronic social distress, the body's ability to utilize oxytocin will be upregulated through the oxytocin receptor by reducing *OXTR*m, and this increased oxytocin uptake would spur people to seek out friends and relationships to fill social gaps and reduce social distress.

However, for other reasons, it is likely that the link from lower *OXTR*m to supportive relationships will be less than straightforward. Specifically, it is possible that oxytocin interferes with accurate or sensitive emotion perception in social relationships, which can lead to conflict—the opposite of improving the quality of supportive relationships. Research on the role of oxytocin in social perception supports this hypothesis. Puglia and colleagues (2015) found that

lower *OXTR*m levels at site -934 in adults, and therefore increased utilization of oxytocin, are associated with reduced responses to angry and fearful faces in the amygdala—a region of the brain associated with processing faces and emotions. In other words, those with lower *OXTR*m perceived negative emotional signals as less salient than those with higher *OXTR*m did. Another study found similar results in infants (five and seven months of age): Infants with lower *OXTR*m levels at site -924 displayed reduced neural activation of the right inferior frontal cortex—another area that has been implicated in emotion processing—in response to angry and fearful faces, and higher activation in response to happy faces (Krol, Puglia, et al., 2019). This confirmed and extended Puglia and colleagues' (2015) finding, indicating that infants with greater ability to utilize oxytocin (lower *OXTR*m) perceived negative emotional signals as less salient than those with higher *OXTR*m did, and they perceived these negative emotional signals as less salient than positive signals. Together, these findings suggest that oxytocin, through the mechanism of decreased *OXTR*m at two sites in the regulatory MT2 region, plays a role in attenuating the neurological fear response. Therefore, people with lower *OXTR*m levels, and theoretically better utilization of oxytocin, may find negative stimuli less salient and be less sensitive to them, perhaps including negative signals from romantic partners (see Figure 1). This could lead to interpersonal problems that decrease the quality of romantic relationships, particularly in the realm of partner conflict. Lower sensitivity to negative stimuli and reduced saliency of partners' negative signals may also lead the person to be less aware of any problems that may result from this blunted sensitivity and saliency. It is possible that those with lower *OXTR*m levels have romantic relationships characterized by greater conflict.

Beyond its effects on social perception and relationships, it is well-established that oxytocin has anxiolytic effects, decreasing stress and associated problems, such as depression

and anxiety (e.g., Carter, 1998). Numerous studies have shown links between lower oxytocin—and greater *OXTR*m—and depression and anxiety. Lower plasma oxytocin levels have been associated with greater depression (Ozsoy et al., 2009; Scantamburlo et al., 2007) and anxiety (Carson et al., 2015; Scantamburlo et al., 2007; Weisman et al., 2012). Higher *OXTR*m levels, and therefore reduced oxytocin uptake, have also been associated with anxiety and depression in women and with postnatal depression (Chagnon et al., 2015; Bell et al., 2015). While there are some discrepancies in the literature and the link between *OXTR*m and anxiety, and its various manifestations, is still somewhat unclear (see Kraaijenvanger et al., 2019 for a review), one would hypothesize from the existing findings that reduced *OXTR*m, and therefore greater oxytocin uptake, would be associated with fewer internalizing (anxiety, depression) symptoms, and this would also be consistent with the findings on social perception. Those with lower *OXTR*m levels who perceive negative stimuli as less salient may also not be as reactive to, or stressed by, lacking supportive relationships, challenging life events, or other negative aspects of their environments, leaving them less susceptible to internalizing problems (see Figure 1).

This study aimed to assess these perspectives in a diverse community sample of adolescents followed from age 13 to 28. We explored how *OXTR*m relates to experiences of autonomy-undermining parenting in adolescence, and romantic relationships and psychopathology in adulthood. To our knowledge, the current study is the first to investigate *OXTR*m as it relates to adolescent autonomy development. We decided to analyze whole blood-derived DNA methylation at CpG site -924 within the MT2 region, a conserved site located in the promoter region of *OXTR* that has been associated with emotion processing and internalizing symptoms, which are related to our outcomes of interest (Krol, Puglia, et al., 2019; Warrener et al., 2021). In addition, we explored sex effects and interactions, due to several studies that found

a significant effect of oxytocin or *OXTR*m in only one of the sexes, or even opposite trends between the sexes (Gouin et al., 2017; Lieberz et al., 2020; Nawijn et al., 2019; Weisman et al., 2013; Yuen et al., 2014). It is also likely that oxytocin plays different roles in men and women, potentially due to the role it plays in childbirth and lactation in women, with oxytocin playing more of a role in tend-and-befriend responses to stress in women (Taylor et al., 2000; Taylor et al., 2006; Lieberz et al., 2020). Our hypotheses were guided by prior research, though it is important to note the many discrepancies in *OXTR*m research findings to date in making these hypotheses. It was hypothesized that:

1) The association between perceived parental psychological control at age 13 and *OXTR*m in the MT2 region at age 28 will exhibit a pattern consistent with a compensatory mechanism, such that adolescents who perceive their parents as more controlling will have *lower* levels of *OXTR*m in adulthood.
2) Consistent with findings that greater oxytocin reduces salience of and sensitivity to negative stimuli, lower *OXTR*m levels at age 28 will predict reduced sensitivity to conflict in romantic relationships at ages 26-28, (i.e., greater conflict as reported by the participant's romantic partner, relative to the participant's own rating).
3) Consistent with much of past research, those with lower *OXTR*m levels at age 28 will also have lower levels of internalizing symptoms at ages 24-25.

Methods

This report is drawn from a larger longitudinal investigation of adolescent social development in family and peer contexts. The effective final sample of 112 participants (67 females, 45 males) was drawn from an initial sample of 184 individuals who were first assessed in the seventh and eighth grades (mean age = 13.35) and were re-assessed annually until age 28.

This sample of 112 includes only those with *OXTR*m data. The sample of 112 participants was demographically diverse and representative of the local population; baseline median family income was in the $40,000-$59,999 range, and the subset self-identified as 57% White, 29% Black or African American, 8% multiracial, 1% Asian, 1% Hispanic, 1% American Indian, and 3% other races or ethnicities. The subset of 112 participants who provided blood samples from which *OXTR*m data were successfully obtained was similar demographically to the full initial sample of 184 participants. The full sample self-identified as 58% White, 29% Black or African American, 8% multiracial, 1% Asian, 1% Hispanic, 0.5% American Indian, and 2% other races or ethnicities; their parents reported a similar median income in the $40,000-$59,999 range. Participants were originally recruited in the seventh and eighth grades from a public middle school drawing from urban and suburban populations in the Southeastern United States. The school was part of a system in which students had been together since the fifth grade. An initial mailing to all parents at the school was used to recruit students, and efforts to follow-up were made at school lunches. Adolescents who indicated they were interested in the study were contacted by telephone. Of all students eligible, 63% agreed to participate, either as a target participant or as a peer providing collateral information.

For the purposes of the present study, data were drawn from five time points: First in early adolescence (*M* age = 13.35, *SD* = 0.64), and then two time points one year apart in adulthood (*M* age = 24.65, *SD* = 0.96; *M* age = 25.69, *SD* = 0.99). At the next time point (covering a three-year timespan when participants were 26-28), participants' romantic partners were invited to participate in survey measures if they reported a relationship duration of at least three months (M_{duration} = 4.01 years, *SD* = 3.16 years). Ninety-nine (54%) of the original 184 participants were in eligible romantic relationships and both they and their partners agreed to

participate. Finally, target participants had blood drawn for the DNA methylation analysis (*M* age = 28.74, *SD* = 1.21, range = 26.32–32.12).

For all data collection, adolescents provided informed assent, and their parents provided informed consent. Participants began providing consent at age 18. Romantic partners provided consent. Assessments and interaction tasks took place in private offices in a university academic building. Adolescents and romantic partners were all financially compensated for their participation. Participants' data were protected by a Confidentiality Certificate issued by the U.S. Department of Health and Human Services, which further protects information from subpoena by federal, state, and local courts. Transportation and child care were provided to participants and their families, if needed.

Attrition

Attrition analyses were conducted to determine whether individuals who did versus did not provide blood samples for DNA methylation analysis differed in terms of baseline variables. Of the original sample of 184 adolescents, 112 (61%) provided blood samples from which methylation data were successfully obtained. There were no significant differences between those who did versus did not provide blood samples in terms of gender, family income, or parental psychological control.

To best address any potential biases due to attrition and missing data in longitudinal analyses, full information maximum likelihood (FIML) methods were used, with analyses including all variables that were linked to future missing data (i.e., where data were not missing completely at random). These procedures yield less biased estimates than approaches that use listwise deletion, and so the full sample of 184 adolescents was utilized for these analyses (Arbuckle, 1996).

Measures

Parental Psychological Control (Age 13)

At age 13, participants completed the 10-item Psychological Control versus Psychological Autonomy subscale of the Childhood Report of Parenting Behavior Inventory (Schaefer, 1965), which is widely used to assess psychological control separately from behavioral control (Barber, 1996). Adolescents reported the extent to which their mothers and fathers used guilt, love withdrawal, or other psychologically controlling behaviors using a 3-point Likert scale, where 1 is "not like my mother [father]" and 3 is "a lot like my mother [father]." Example items include "my mother/father figure is a person who is less friendly with me, if I do not see things her/his way" and "my mother/father figure is a person who says, if I really cared for her/him, I would not do things that cause her/him to worry." Scores across items were summed, and the scores for the two parents were averaged together, or a score for a single parent was used. Scores ranged from 10 to 26. Adolescents' scores for their mothers and fathers were highly correlated ($r = .68$, $p < .001$). Reports of mothers' use of psychological control were higher than fathers' ($M_{mothers} = 15.59$, $SD = 3.66$; $M_{fathers} = 14.43$, $SD = 3.80$; $t = 2.88$, $p = .004$). Internal consistency of both parental measures was good (Cronbach's $\alpha = .82$ for both).

Parental Expressive Affection (Age 13)

When adolescents were 13, their parent(s) completed the expressive affection subscale of the Expression of Affection scale, which has been used in prior work in families (Hetherington et al., 1992). This instrument measures how often parents hug, spend time with, and laugh, joke, or talk with their child. Parents reported how often they engaged in these activities on a 7-point scale, with higher scores corresponding to greater frequency. Acceptable internal consistency and test-retest reliability for this measure have been demonstrated in prior work (Hetherington et

al., 1992). Scores across items were summed, and the two parents' scores were averaged together, or a score from a single parent was used. Scores ranged from 12 to 61. Scores from mothers and fathers were significantly correlated (r = .28, p = .005). Mothers' reports of expressive affection were higher than fathers' ($M_{mothers}$ = 36.10, SD = 11.29; $M_{fathers}$ = 30.22, SD = 10.13; t = 4.53, p < .001). Internal consistency of both parental measures was good (Cronbach's α = .88 and .86 for mothers' and fathers' reports, respectively).

Internalizing Symptoms (Ages 24-25)

At ages 24 and 25, participants completed the 39-item Internalizing subscale of the Adult Self-Report (Achenbach & Rescorla, 2003), which includes items relating to symptoms of anxiety and depression, as well as somatic symptoms and social withdrawal. In the development of the Adult Self-Report, items were validated with mental health professional raters using DSM-oriented criteria (Achenbach et al., 2003; Diagnostic and Statistical Manual of Mental Disorders, American Psychiatric Association, 2013). Items were scored on a 3-point Likert scale where 0 = "not true," 1 = "somewhat or sometimes true," and 2 = "very true or often true," such that higher scores indicate more reported internalizing symptoms. Participants' scores were averaged across the two waves, or a score from a single wave was used, in order to create an aggregate measure of internalizing behaviors in young adulthood. Internal consistency for each of these two waves was excellent (Cronbach's α = .93 for both waves). Scores across the two waves were highly correlated (r = .74, p < .001).

Romantic Partner Conflict, Self- and Partner-Report (Ages 26-28)

Romantic conflict was assessed using participants' and their romantic partners' reports of conflict in the relationship on the 3-item conflict subscale of the Network of Relationships Inventory (Furman & Buhrmester, 1985). Participants and their romantic partners completed this

assessment once when target participants were between ages 26 and 28. The Network of Relationships Inventory is a 45-item scale that measures various qualities of close relationships, and the conflict subscale assesses how much anger, disagreement, and argumentation there is in the relationship. Items were scored on a 5-point Likert scale where 1 = "little or none" and 5 = "the most." Internal consistency was excellent for both self- and partner-reports (Cronbach's αs = .93 for both reports).

Blood Collection, DNA Isolation, and DNA Methylation Analysis (Age 28)

One hundred twelve participants consented to a blood draw between ages 26 and 32, and venipuncture was performed. For each participant, 8.5 milliliters of whole blood were drawn into a PAXgene Blood DNA Tube (PreAnalytiX, Hombrechtikon, Switzerland) and held at -20°C for short-term storage (< 3 months) and at -80°C for long-term storage. Following manufacturer instructions, the PAXgene Blood DNA kit (PreAnalytiX, Hombrechtikon, Switzerland) was used to extract DNA. Two hundred nanograms of DNA isolated from whole blood were subjected to bisulfite conversion using MECOV50 Kits (Invitrogen, Carlsbad, CA), which allows for the differentiation of methylated and unmethylated cytosines in the DNA sequence. Samples were eluted in 10 microliters.

Following bisulfite conversion, procedures were conducted in triplicate. Two microliters of bisulfite-converted DNA were amplified via polymerase chain reaction (PCR) using a Pyromark PCR kit (QIAGEN, Hilden, Germany) and 0.2 μM primers [TSL101F, 5′-TTGAGTTTTGGATTTAGATAATTAAGGATT-3′ (forward); TSL101R, 5′-biotin-AATAAAATACCTCCCACTCCTTATTCCTAA-3′ (reverse)]. Each PCR plate contained methylation standards (0, 50, and 100% methylated) and negative (no DNA) controls from bisulfite conversion and PCR. Thermocycling was conducted as follows: Step 1, 95°C for 15

minutes; Step 2, 50 cycles at 94°C for 30 seconds, 56°C for 30 seconds, and 72°C for 30 seconds; Step 3, 72°C for 10 minutes; and Step 4, held at 4°C until analysis. The primers used amplify a 116-base pair region on the coding strand of *OXTR* containing CpG site -924 (Genome Reference Consortium Human Build 38, chromosome 3: 8,769,044 to 8,769,159), which was confirmed by agarose gel electrophoresis on a representative of the sample and replicates. Samples were randomized for pyrosequencing to account for plate and run variability. DNA methylation level for each sample was assessed using pyrosequencing, using sequencing primer TSL101S, 5′-AGAAGTTATTTTATAATTTTT-3′ (PyroMark Q24, QIAGEN). Epigenotypes reported are an average of the three replicate values. On average, methylation levels within replicates deviated from the mean by ±1.86%.

Results

Primary Analyses

Table 1 presents means, standard deviations, and intercorrelations of variables of interest. All analyses were conducted in R (R Core Team, 2021). Primary analyses were conducted via linear regression using full information maximum likelihood (FIML) analyses; these specific analyses were conducted using the lavaan package (Rosseel, 2012). To examine the possible moderating effect of gender on each of the relations described, interaction terms were created based on the product of centered variables.

Hypothesis 1

Greater perceived parental psychological control at age 13 will be associated with lower levels of OXTR*m at CpG site -924 at age 28.*

First, analyses examined the relationship between *OXTR*m and experiences of autonomy-undermining parenting in early adolescence. Consistent with our first hypothesis, accounting for

gender and baseline family income, greater perceived parental psychological control at age 13 predicted lower levels of *OXTR*m at site -924 at age 28 (β = -.28, p = .007); see Table 2. Additionally, gender significantly moderated this association ($\beta_{interaction}$ = -.26, p = .009), such that there was a significant negative association between parental psychological control and *OXTR*m for women (β = -.47, p < .001), but no significant association for men (β = .19, p = .272); see Figure 2. These results suggest that girls, but not boys, who experienced greater parental psychological control in early adolescence tended to have lower levels of *OXTR*m in adulthood.

Hypothesis 2

Lower OXTR*m levels at age 28 will predict reduced sensitivity to conflict in romantic relationships at ages 26-28, i.e., greater conflict as reported by the participant's romantic partner, relative to the participant's own rating.*

Analyses next examined whether *OXTR*m relates to perception of conflict in adult romantic relationships. Consistent with our second hypothesis, accounting for gender and baseline family income, lower levels of *OXTR*m at age 28 were significantly associated with greater partner-reported conflict in romantic relationships between ages 26-28, even after controlling for the target participants' own reports of conflict (β = -.25, p = .011); see Table 3. Gender did not significantly moderate this association ($\beta_{interaction}$ = .10, p = .342). These results suggest that those with lower levels of *OXTR*m have greater conflict—more arguments and anger—in their romantic relationships as perceived by their romantic partners.

Hypothesis 3

Lower levels of OXTR*m at age 28 will be associated with lower levels of internalizing symptoms at ages 24-25.*

The last planned analysis examined whether *OXTR*m relates to adult internalizing psychopathology. Consistent with our third hypothesis, accounting for gender and baseline family income, lower levels of *OXTR*m at age 28 were significantly associated with lower reported internalizing symptoms across ages 24-25 ($\beta = .24$, $p = .007$); see Table 4. Gender did not significantly moderate this association ($\beta_{interaction} = .16$, $p = .090$). These results suggest that those with lower levels of *OXTR*m experience less depression, anxiety, and social withdrawal in early adulthood.

Post-Hoc Analyses

Upon further inspection of the plotted data (see Figure 2), there appeared to be a possible curvilinear relationship between parental psychological control and *OXTR*m at site -924. To test this, we ran another regression model using FIML. We squared the mean-centered psychological control variable and added it to the regression model, along with the original psychological control variable, gender, and baseline family income. The quadratic psychological control term significantly predicted *OXTR*m ($\beta = .23$, $p = .038$). To investigate whether gender moderated this curvilinear relationship, we added an interaction term of the mean-centered gender and squared psychological control variables. Gender did not significantly moderate the association between squared psychological control and *OXTR*m ($\beta_{interaction} = -.11$, $p = .364$). Because prior evidence and the results from the current study indicate that an effect may exist in females but not in males, we nonetheless explored the simple effects to see if the curvilinear effect was also only found in females. Though the interaction was not significant, this indeed was the case: The curvilinear relationship was significant in females ($\beta = .32$, $p = .007$), but not in males ($\beta = .28$, $p = .184$). Plotting these relationships (Figure 3) illustrates that participants who reported parental psychological control levels around and slightly above the mean (15.21) had the lowest levels of

*OXTR*m. For lower levels of psychological control, *OXTR*m decreases as psychological control increases, and for the very highest levels of psychological control, this relationship appears to reverse direction slightly, though this may be driven by some extreme higher *OXTR*m levels in this range.

Additionally, prior literature, particularly in prairie vole models, supports the possibility that parental involvement may play an important role in regulating offspring's *OXTR*m levels (Perkeybile et al., 2019). To approximate this in our human participants, we explored expressive affection as a proxy for parental involvement and its relationships with psychological control and *OXTR*m. The self-report measure of expressive affection includes parenting behaviors such as spending time with their child, playing games with their child, and talking with their child about something that is worrying them. This expressive affection variable, which was an average of mothers' and fathers' reports for each adolescent, was not significantly correlated with psychological control (r = -.03, p = .677), nor with *OXTR*m at site -924 (r = .08, p = .426). Adding expressive affection to the regression analyses predicting *OXTR*m from psychological control does not change any of the results. These results suggest that our proxy for parental involvement in adolescence does not relate to later adult *OXTR*m levels.

Discussion

This long-term, multi-method study identified new links between *OXTR*m, adolescent experiences of overcontrolling parenting, and romantic relationship quality, and clarified prior findings regarding *OXTR*m and its associations with depression and anxiety symptoms. Having more experience with psychologically controlling parental behaviors in early adolescence predicted lower levels of *OXTR*m in adulthood, and those with lower *OXTR*m levels also had

greater conflict in their romantic relationships, according to their partners, and fewer internalizing symptoms in adulthood.

The negative association, for women only, between parental psychological control and *OXTR*m indicates that a potential compensatory mechanism may be at work, such that the oxytocin system is upregulated, via decreased methylation, in women who reported higher levels of parental psychological control. This upregulation is consistent with the tend-and-befriend hypothesis, as outlined in Figure 1, which posits that more oxytocin can spur people to seek support when experiencing stress or gaps in positive relationships. Our findings suggest the possibility that the body's ability to take in oxytocin is increased in response to a social-developmental environment that likely feels quite negative for the adolescent. The curvilinear nature of this association is also important to note, and it appears that this compensatory mechanism may only work up to a certain extent, as *OXTR*m levels off, and even slightly increases, once parental psychological control reaches and exceeds the average in our sample. The fact that this association was only found for women is also consistent with the gendered nature of the tend-and-befriend hypothesis. The women in our sample may be responding to the stress of autonomy-undermining parenting in early adolescence by increasing their ability to use and reap the affiliative rewards of oxytocin.

In testing the affiliative behavior part of the tend-and-befriend hypothesis, that then those who upregulate oxytocin through decreased *OXTR*m do actually reach out and form more, and better, relationships, we examined the association between *OXTR*m and an important aspect of adult romantic relationships, relationship conflict. Our results do not support this conclusion, as lower *OXTR*m is associated with more conflict as rated by the romantic partner, not less—an enhanced ability to use oxytocin was not associated with forming better, or at least less conflict-

ridden, relationships. Although reduced *OXTR*m may be an attempt to compensate for the stressful experience of parental psychological control, it did not fully compensate through improved relationships, as the hypothesis suggests it would. However, this finding is consistent with prior research on *OXTR*m and social perception, that lower *OXTR*m is associated with reduced salience of angry and fearful faces (Puglia et al., 2015; Krol, Puglia et al., 2019); reduced *OXTR*m in our study was associated with more relationship conflict, of which the participant was relatively unaware, which could result from reduced salience of and attunement to negative signals from a romantic partner in the relationship. Without data in some way measuring attention to negative versus positive stimuli, however, we cannot conclude with certainty that this is the mechanism at work. Future research will need to explore *OXTR*m and social perception, along with its possible consequences, further.

In support of this potential explanation of reduced attunement to negative stimuli as a result of enhanced ability to use oxytocin, lower levels of *OXTR*m were also associated with fewer internalizing symptoms. Past research has found that enhanced attunement to negativity, in the form of attentional biases, is associated with depression and anxiety (Gotlib et al., 2004; Disner et al., 2017; Klein et al., 2018; Schechner et al., 2012), which is consistent with our finding that greater *OXTR*m—and therefore reduced oxytocin intake and potentially enhanced sensitivity to negative stimuli—is associated with greater internalizing symptoms (depression, anxiety, and social withdrawal), and vice versa. This finding also adds to the mixed literature on *OXTR*m and internalizing psychopathology, which has found both positive and negative relationships between *OXTR*m and depression (e.g., Warrener et al., 2021 vs. Bell et al., 2015). While we have outlined an explanation for our positive association between *OXTR*m and internalizing symptoms, it is clear from the inconsistent findings in the literature that more work

is needed to understand and clarify this association, outlining conditions under which there may be positive and negative associations between *OXTR*m and depression and anxiety.

It is important to note, in discussing the findings across the literature, that our results are specific to DNA methylation at CpG site -924 on *OXTR*. Many sites across different regions of *OXTR* have been investigated (see Kraaijenvanger et al., 2019 and Maud et al., 2018 for reviews); site -924 is located in the MT2 region of the promoter, and DNA methylation in this region regulates transcription of the gene and therefore the gene's expression (Kusui et al., 2001; Danoff, Wroblewski, et al., 2021), so we can, with reasonable certainty, conclude that our measure of *OXTR*m is assessing a regulatory mechanism with effects on *OXTR* expression and downstream oxytocin receptor production. While support for the specific role of CpG site -924 is still growing, and though our results are only correlational, it appears from the current study and others that this site may play a role in social perception, psychopathology, and aspects of child and adolescent development (Gregory et al., 2009; Krol, Moulder et al., 2019; Krol, Puglia et al., 2019; Warrener et al., 2021). Future work will be needed to continue clarifying the specific role of site -924, as well as other sites in the MT2 region such as -934, and how developmental experiences may tune the oxytocin system via DNA methylation at these different sites.

In addition to the limitations already noted, the current study had only one measure of *OXTR*m, in adulthood (age 28). Therefore, we cannot assess change in *OXTR*m, and we cannot determine when increases or decreases in methylation occurred. It's possible that *OXTR*m is quite stable in early- to mid-adulthood (Krol, Moulder, et al., 2019), and little is known about the stability of *OXTR*m in adolescence, so it is difficult to determine why, exactly, there is such a strong association between psychological control and *OXTR*m in the current study. It is possible that, as we hypothesize, further methylation is prevented or reduced in adolescence in response

to the experience of overcontrolling parenting (which was also likely occurring before our measure at age 13, though we can't know for certain), but we cannot eliminate the possibility that earlier parenting, even parenting in infancy, or some other third variable impacted *OXTR*m levels earlier in development, and that those levels persisted until our measure at age 28. Another limitation regarding our measure of *OXTR*m is that we did not control for or assess female participants' menstrual cycles or the use of hormonal birth control at the time of the blood draw, and there is preliminary evidence that *OXTR*m (derived from saliva) does fluctuate in naturally cycling women but is more stable in women on birth control (Krol et al., 2020). Additionally, we were unable to fully explore whether parental psychological control is associated with lower *OXTR*m because these parents are also more involved in their children's lives, which would be consistent with prior animal and human work (Perkeybile et al., 2019; Krol, Moulder, et al., 2019). The expressive affection scale did not directly measure parental involvement or engagement—the human equivalent of early care in prairie voles. Though expressive affection was not associated with either parental psychological control or *OXTR*m, it remains possible that parents who engage in more manipulative methods and seek to control their teenagers are also more involved in their children's lives, and this involvement could be driving the negative association—more parental care associated with lower *OXTR*m levels. Finally, we used a limited measure of one narrow aspect of romantic relationships—conflict—and explored its associations with *OXTR*m; if our hypothesis regarding oxytocin dulling sensitivity to negative stimuli is correct, then lower *OXTR*m levels are likely to be associated with other aspects of romantic relationships, as well, which future work can explore, perhaps also with observational measures of romantic relationship quality.

Infancy has been identified as a sensitive period in which the oxytocin system is dynamic and can change in response to the social environment (Krol, Moulder, et al., 2019), but very little work has been done on how developmental experiences in adolescence, another period of rapid and important developmental change, may impact the oxytocin system. This study expands on the limited literature by providing support for the theory that adolescent females may attempt to compensate for autonomy-undermining parenting by upregulating their oxytocin system via decreased *OXTR*m. Additionally, it expands further the consequences, on romantic relationships and internalizing symptoms, of dampened attunement to negative stimuli that may result from greater uptake of oxytocin and lower *OXTR*m. This study has tested only a few parts of the model outlined in Figure 1, and has left other parts, such as additional precursors or consequences of these pathways, unexamined or as assumptions, several of which have been discussed and may be tested in future work. Although prior work has suggested the virtues of low *OXTR*m, from improved social attachment to reduced risk for various mental health concerns, these preliminary findings suggest that greater *OXTR*m, and thus less oxytocin, is not necessarily a negative state, predicted by and resulting in negative experiences. Rather, it is clear that the oxytocin system and its regulation by *OXTR*m is nuanced, and many more pieces still need to come together to clarify the role of *OXTR*m in development and human social lives.

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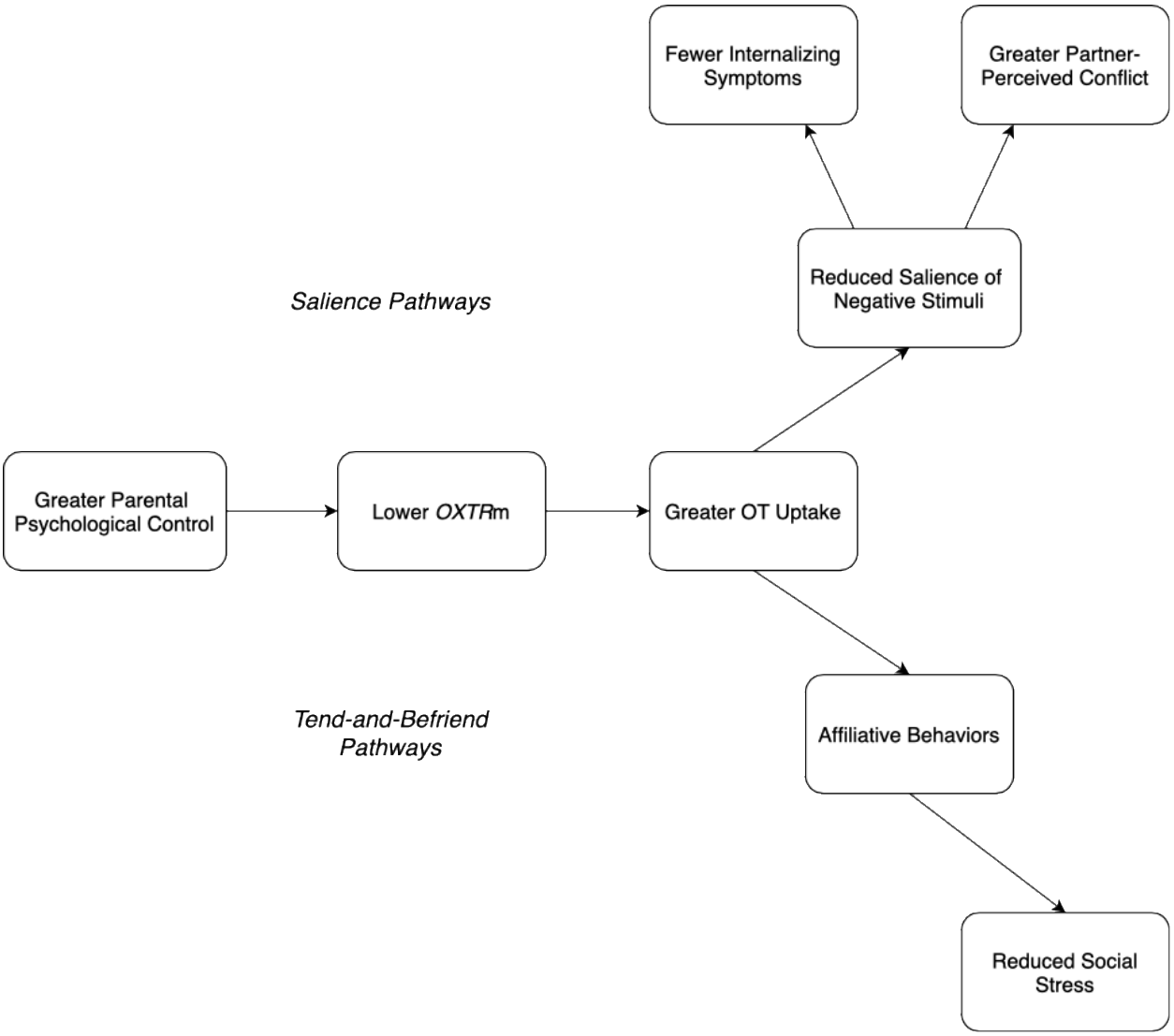
Appendix

Figure 1

Hypothesized Tend-and-Befriend and Salience Pathways from Adolescence to Early Adulthood



Note. *OXTR*m = oxytocin receptor gene methylation. OT = oxytocin.

Table 1

Descriptive Statistics and Intercorrelations Among Variables of Interest

	M	*SD*	*N*	1.	2.	3.	4.	5.	6.	7.
1. *OXTR*m at CpG site -924 (%; age 28)	62.09	6.39	112							
2. Parental psychological control (age 13)	15.21	3.50	183	-.25**						
3. Depressive symptoms (age 13)	5.07	4.30	182	-.14	.35***					
4. Internalizing symptoms (age 24-25)	10.01	9.19	168	.26**	-.08	.16*				
5. Self-rated conflict in romantic relationship (age 26-28)	6.92	2.43	109	-.16	.20*	.08	.05			
6. Partner-rated conflict in romantic relationship (age 26-28)	7.29	2.62	99	-.32**	.36***	.33***	-.03	.46***		
7. Adolescent gender (1 = male, 2 = female)	—	—	184	.04	.06	.05	.13	-.13	.03	
8. Family income	43,600	22,400	181	.09	-.32***	-.11	.06	-.11	-.11	-.12

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

Table 2

Predicting OXTR*m at Site -924 (Mean Age 28) From Parental Psychological Control in Early Adolescence (Age 13)*

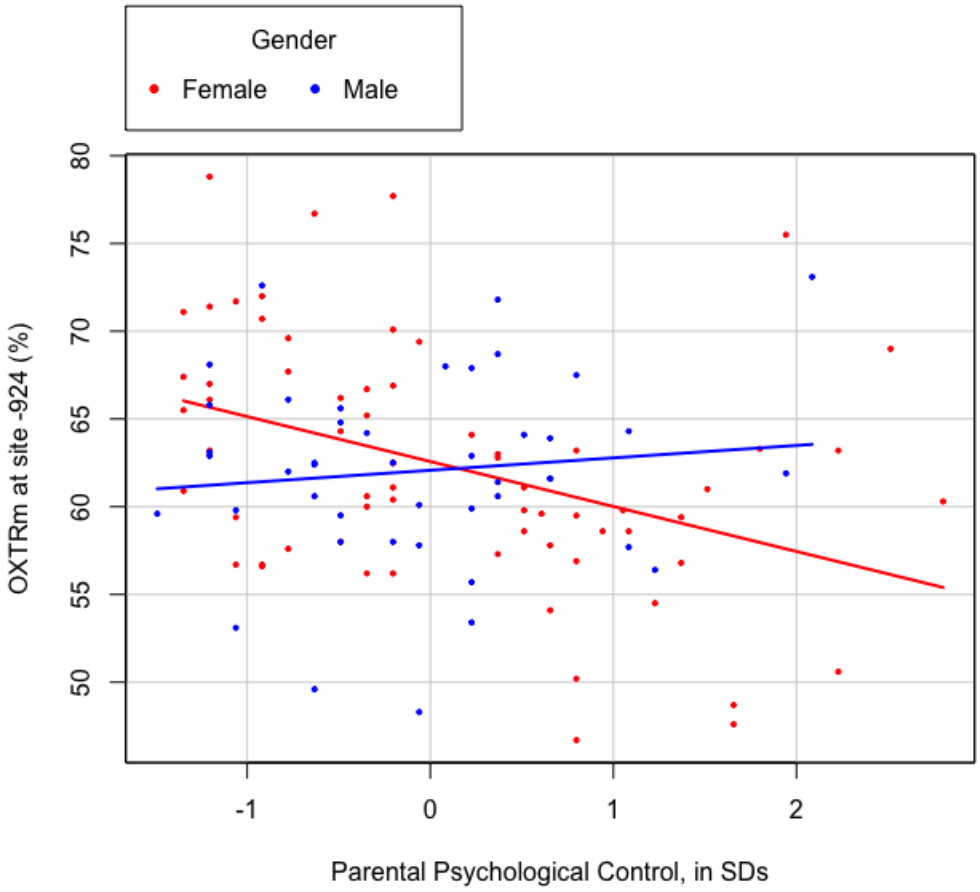
	***OXTR*m at site -924 (~age 28)**			
	β entry	β final	ΔR^2	Total R^2
Step 1				
Gender (1 = male, 2 = female)	.05	.05		
Total family income (age 13)	.10	-.05		
Statistics for step			.011	.011
Step II				
Parental psychological control (age 13)	-.28**	-.19		
			.064**	.075*
Step III				
Psychological control X gender interaction	-.26**	-.26**		
			.038**	.113**

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

Note. *OXTR*m = oxytocin receptor gene methylation

Figure 2

Scatterplot of Parental Psychological Control (Age 13) and OXTR*m (Age 28) with Plotted Gender Simple Effects*



Note. *OXTR*m = oxytocin receptor gene methylation

Table 3

Predicting Romantic Partner-Reported Conflict (Ages 26-28) from OXTR*m at Site -924 (Mean Age 28)*

	Partner-Reported Conflict (Ages 26-28)			
	β entry	β final	ΔR^2	Total R^2
Step 1				
Gender (1 = male, 2 = female)	.02	.07		
Total family income (age 13)	-.10	-.03		
Statistics for step			.010	.010
Step II				
Teen-reported conflict (ages 26-28)	.47***	.42***		
			.213***	.223***
Step III				
*OXTR*m at site -924 (~age 28)	-.25*	-.29**		
			.060*	.283***
Step IV				
*OXTR*m X gender interaction	.10	.10	.017	.300***

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

Note. *OXTR*m = oxytocin receptor gene methylation

Table 4

Predicting Internalizing Symptoms (Ages 24-25) from OXTR*m at Site -924 (Mean Age 28)*

	Internalizing Symptoms (Ages 24-25)			
	β entry	β final	ΔR^2	Total R^2
Step 1				
Gender (1 = male, 2 = female)	.14	.13		
Total family income (age 13)	.07	.06		
Statistics for step			.021	.021
Step II				
*OXTR*m at site -924 (~age 28)	.24**	.17		
			.056**	.077*
Step III				
*OXTR*m X gender interaction	.16	.16		
			.018	.095*

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

Note. *OXTR*m = oxytocin receptor gene methylation

Table 5

Predicting OXTR*m at Site -924 (Mean Age 28) From Linear and Quadratic Effects of Parental Psychological Control in Early Adolescence (Age 13)*

	***OXTR*m at site -924 (~age 28)**			
	β entry	β final	ΔR^2	Total R^2
Step 1				
Gender (1 = male, 2 = female)	.05	.01		
Total family income (age 13)	.10	-.06		
Statistics for step			.011	.011
Step II				
Parental psychological control (age 13)	-.28**	-.39**		
			.064**	.075*
Step III				
Parental psychological control squared	.23*	.28*		
			.040*	.115*
Step IV				
Quadratic term X gender interaction	-.11	-.11		
			.013	.128*

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

Note. *OXTR*m = oxytocin receptor gene methylation

Figure 3

Scatterplot of Parental Psychological Control (Age 13) and OXTR*m (Age 28) with Plotted Curvilinear Effect, in Full Sample (Top) and Women Only (Bottom)*