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This study examined maternal sensitivity to distress, overtly negative maternal behavior, maternal trauma and loss, and their interactions with infant genes related to dopamine, serotonin and oxytocin functioning as predictors of infant attachment disorganization while accounting for race and sociodemographic covariates. The sample included 182 mother-infant dyads (approximately 50% European American and 50% African American). Prenatally, women completed the Adult Attachment Interview. At the 6 month and 1 year visits mothers and infants participated in a series of videotaped interactive tasks designed to elicit infant distress and to assess maternal behavior. Additionally, during the 1 year visit, mothers and infants participated in the Strange Situation Procedure to assess infant-mother attachment security. During the 2 year lab visit, DNA was collected via saliva samples from children. Results indicated that overtly negative maternal behavior and sociodemographic risk were positively associated with infant attachment disorganization. Infant genotype did not moderate these relationships. These findings may have applied implications in regards to informing intervention and prevention programs.

ENVIRONMENT AND GENETIC PREDICTORS OF INFANT ATTACHMENT DISORGANIZATION

by

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CHAPTER I

REVIEW OF THE LITERATURE

Overview of Attachment Theory

John Bowlby's Theory

John Bowlby's (1980) pioneering work on attachment opened the door to understanding infant-caregiver relationships. As infants are lacking the skills necessary to be self-sufficient, they must rely on their caregivers for protection, comfort, and care. This reliance leads to the development of their first relationships. Bowlby theorized that an infant's first relationship is incredibly important as it lays the groundwork for the development of future relationships.

Bowlby (1969) described attachment as an evolutionary behavioral system that promotes infant survival and protection through the use of behaviors meant to maintain contact between infants and the caregivers who protect them, with an emphasis on mothers. When an infant is distressed, the goal of the attachment system is to gain proximity to and comfort from the mother, as this most often occurs when the mother is not in the immediate company of the infant. To achieve the goal of maintaining proximity, infants use "attachment behaviors" which either bring the infant closer to the mother, or signal to the mother to return to the infant. When infants are immobile, their behaviors are mostly signaling behaviors such as smiling at their mothers, crying for them, or calling to them. But once infants have achieved mobility, they can then take a more

active approach in maintaining proximity by approaching their mothers, following them, or clinging to them (Ainsworth & Bell, 1970). Maternal responses to these bids play a critical role in the quality and nature of the developing attachment relationship. Whether infants expect that their mothers will be available to them rarely, occasionally, frequently, or often informs their working models, or infants' conscious and unconscious mental representations of themselves and the world around them. Infants working models are constructed out of their interactions with their caregivers, their own actions, and the feedback they receive about those actions. If infants experience consistently responsive caregiving, they are likely to develop secure internal working models, characterized by feeling worthy of care and trust in others to meet their needs. This positive sense of self and trust in others to keep them safe facilitates exploratory behaviors. Exploring is especially important when infants become toddlers and gain the ability to walk, because exploring promotes learning (Bowlby, 1980). However, if infants experience inconsistent caregiving, or if their bids are responded to harshly or not at all, they are more likely to develop insecure working models, characterized by a distrust in their mothers abilities to meet their needs during times of distress. Internal working models are important as they influence how infants and developing children expect others to behave towards them, which in turn affects their future relationships, their social and emotional behavior, and their psychological well-being over time.

There are many ways in which mothers can respond to infant bids. One way in which mothers can respond is by being present. According to Bowlby (1973), being present implies being readily accessible, while being absent implies being inaccessible.

Additionally, mothers can respond in appropriate or inappropriate ways to their infants' bids. In order for a mother to be truly available to her infant, she must be both highly accessible and responsive. However, mothers differ on their levels of accessibility and responsiveness, and these caregiving differences explain individual differences in the development of infants' working models.

Mary Ainsworth's Empirical Contribution

In 1978, Bowlby's collaborator Mary Ainsworth devised a procedure, known as the Strange Situation Procedure, to observe the quality of infant-mother attachment relationships. The Strange Situation was created to evaluate parent-infant attachment during a short laboratory visit. The Strange Situation Procedure began with the twelve-month-old infant and mother in a strange room filled with toys. It then proceeded through a 20-minute series of eight, three-minute episodes that included 2 stressful separations of the mother from the infant in order to activate the attachment system. As Bowlby asserted that understanding how an infant responds to his mother after a separation of loss illustrates the bond between an infant and his mother, the three-minute episodes of most interest in the Strange Situation were those in which the mother left the infant as well as those in which she returned (1969). The behaviors of interest during the separation episodes were the extent to which the infants were distressed about their mothers leaving, while the extent to which they sought and maintained proximity to her upon her return was of interest during the reunion episodes. Prior to studying these behaviors in the lab, Ainsworth and her colleagues conducted in-home observations to observe parenting quality during the first year.

After carefully observing and rating infant behavior during the lab procedure, Ainsworth and colleagues (1978) noted that most infants could be classified into one of three distinct groups based on their behavior during the separation and reunion episodes. While Bowlby only talked of secure and insecure attachment, Ainsworth and colleagues discovered that the insecure infants in their study could be divided further into two groups. Thus, there was the secure group, and two insecure groups: avoidant and resistant. Infants in the secure group differed in whether they were distressed during the separation episode, but they characteristically tried to gain proximity to their mothers and maintain their contact during the reunion. In the avoidant group, the infants characteristically showed a lack of crying during the separation episodes, and they showed little interest when their mothers returned. Finally, the infants in the resistant group tended to maintain proximity and contact with their mothers during the reunion, similar to the secure group, but they tended to become angry and resistant towards their mothers by physically hitting or pushing their mothers away.

Consistent with Bowlby's contention that parenting influences attachment behaviors and internal working models, Ainsworth noted clear differences in the quality of mothering these three groups of infants received in the prior year. (Ainsworth, Blehar, Waters, & Wall, 1978). Infants with secure attachments had mothers who consistently responded to them in a sensitive manner in the past. Sensitivity is defined as a caregiver's ability to accurately perceive and interpret her infant's signals, as well as her ability to respond both promptly and appropriately to them (Ainsworth et al., 1978). This high level of sensitivity may have contributed to this group of infants' secure internal working

models. Alternatively, avoidant infants had mothers who responded insensitively and with rejection towards them, which may have contributed to the infants not expressing distress, as they did not expect their mothers to respond sensitively to them. Finally, resistant infants had mothers who used inconsistent caregiving practices in which they were sometimes sensitive and responsive, and sometimes not. This inconsistent caregiving possibly contributed to these infants' uncertainty of whether they would be responded to when distressed.

The Discovery of Disorganized Attachment

While the majority of infants studied by Ainsworth and colleagues, and in numerous other studies in the interim (De Wolff & van IJzendoorn, 1997), were classified into one of these three categories, some engaged in behaviors that did not fit as well, and were labeled "unclassifiable." Years later, Main and Solomon (1990) revisited this group's data as well as data offered from other studies in which there were unclassifiable infants. Main and Solomon found that in contrast to the majority of classified infants, the majority of unclassifiable infants shared a characteristic: they exhibited strange behaviors that were not clearly organized towards reaching a goal, whether of gaining proximity to their mothers or avoiding it. While the infants in the secure and two insecure groups had differences in how they behaved during the separation and reunion episodes of the Strange Situation, they all shared the ability to organize their own behaviors in light of their mother's behaviors and apparent preferences. Therefore, Main and Solomon labeled this new, fourth group "disorganized" due to the lack of any particular response pattern among infants in it. In order to more

fully describe the behaviors of disorganized infants during the strange situation, Main and Solomon (1990) compiled a non-exhaustive list of seven themes of behaviors: (1) Sequential display of contradictory behavior patterns, such as calling for mother during separation, but actively avoiding mother upon her return; (2) Simultaneous display of contradictory behavior patterns, such as approaching mother with back facing her; (3) Undirected, misdirected, incomplete and interrupted movements and expressions, such as seeming to approach mother, but then trying to follow the experimenter out of the room; (4) Stereotypies, asymmetrical movements, mistimed movements, and anomalous postures, such as extended rocking, ear pulling, or hair twisting; (5) Freezing, stilling, and slowed movements and expressions, such as interrupting distressed behavior with freezing for 10 seconds with a dazed expression; (6) Direct indices of apprehension regarding the parent, such as flinging hands over face with fearful expression when mother enters room; and (7) Direct indices of disorganization, disorientation, and confusion, such as raising hands to mouth with a confused expression when mother enters room. (p. 135).

Many of the infants who displayed these behaviors came from samples characterized as high-risk due to low-incomes, maternal depression or other psychiatric disorders, maternal substance abuse, and parental maltreatment (Lyons-Ruth & Jacobvitz, 2008). Thus, Main and Hesse (1990) argued that disorganized behaviors may be the result of having mothers who engage in frightening behavior and who are themselves frightened while caregiving. For these infants, stressful situations become complicated because their mothers are both a source of fright as well as a potential source of safety.

Because these infants cannot easily approach their frightening or frightened mothers, it appears that they develop working models in which they are unsure of how, or whether they want to approach them. This "fright without solution" is the essence of disorganized attachment (Van Ijzendoorn, Schuengel, & Bakermans-Kranenburg, 1999, p. 226).

The behaviors previously listed are also used in the coding scheme developed by Main and Solomon to classify infant disorganization during the Strange Situation Procedure during the episodes in which the mother was present. In most studies, the Strange Situation Procedure is coded in two sweeps. In the first sweep, infants are classified into one of the original three groups. Then in the second sweep, infants are rated for their disorganized behavior on a 9-point Likert scale, ranging from 1 "No signs of disorganization/disorientation. Any behaviors that initially seemed to be indices of disorganization or disorientation have been explained in other terms" to 9 "Definite qualification for D attachment status: in addition, the indices of disorganization and disorientation are strong, frequent, or extreme. Either several very strong indicators are present, or one very strong indicator and several intense exhibitions of one or several other indices " (p. 152-153). In their meta-analysis on disorganized attachment, Van IJzendoorn and colleagues (1999) found that in middle class, nonclinical samples (N = 1,882), 14% of infants were classified as disorganized. The number rose to 24% in low SES samples (N = 493), possibly due to additional life stressors in lower SES homes that may lead to more chaotic parenting environments and negative parenting behaviors (Van IJzendoorn et al., 1999). In regards to secondary classifications, they found that across 25 samples of disorganized infants (N = 1,219), 46% of the cases were given the secondary

classification of resistant, 34% were given the classification of avoidant, and only 14% were given the classification of secure.

In the 24 years since Main and Solomon first brought attention to attachment disorganization, many studies have been conducted and the general conclusion is that infant attachment disorganization is associated with negative outcomes throughout infancy and childhood and into adolescence. Children who were observed to be disorganized in infancy have been found to exhibit externalizing behaviors such as being more aggressive, disruptive, and controlling during preschool and middle childhood (Lyons-Ruth, 1996; van IJzendoorn et al., 1999; Fearon et al., 2010) as well as to have an increased likelihood for academic problems and to show poorer school performance (Moss, Rousseau, Parent, St.-Laurent, & Saintonge, 1998; Moss & St.-Laurent, 2001). Additionally, children who were classified as disorganized during infancy are more likely to exhibit role-inverting behaviors towards caregivers, such as ordering their caregivers around or being overly solicitous (Hesse & Main, 2000). Disorganized classifications are thought to be relatively stable throughout childhood and into adulthood, with unresolved/disorganized classifications in adulthood being associated with an increased frequency of criminal behaviors such as violence, as well as psychological issues (Hesse & Main, 2000). Generally, infants with disorganized attachment classifications are at the greatest risk relative to other attachment classifications for psychopathology throughout their lives (Hesse & Main, 2000). Thus, understanding the causal mechanisms that lead to these classifications may guide the development of new prevention and intervention strategies.

Predictors of Attachment Disorganization

Given the link between attachment disorganization and subsequent problems, efforts have been made to identify factors that predict attachment disorganization. As elaborated below, much of this work has focused on examining links between infant disorganization and maternal behavior and/or maternal characteristics and contexts that increase the likelihood of insensitive or pathological maternal behavior. More recently, investigators have begun to also consider that child characteristics, particularly genetic risk, may interact with maternal behaviors to increase the likelihood of infants becoming disorganized. Two perspectives currently address why the links between caregiving and attachment disorganization vary based on children's genetic risk. The dual-risk model proposes that individuals with certain genetic or biological vulnerabilities should be more vulnerable to negative environments, while individuals without them should be resilient (Sameroff, 1983). According to this, infants with certain vulnerabilities, such as having specific risk alleles, should be the most adversely affected by negative maternal behaviors, making them the most likely to become disorganized. This is due to the additive effects of negative factors. However, if they receive sensitive parenting, they should not differ in their risk of disorganization from the infants without risk alleles, who are not affected by either positive or negative caregiving and do not have an increased risk of disorganization.

In contrast, according to the differential susceptibility hypothesis, individuals with certain vulnerabilities (genetic, endophenotypic, and phenotypic factors) should not only be the most likely to be adversely affected by negative environments, but they should

also be the most likely to thrive in positive environments, including those lacking adversity (Belsky & Pluess, 2009). This hypothesis is based on evolutionary logic, in regards to reproduction. As it was not possible for parents to know which of their parenting behaviors would best promote the survival of their offspring, and hence their genes, it makes sense that natural selection would have led to offspring having varying levels of developmental plasticity, or sensitivity to environmental effects (Belsky & Pluess, 2009). According to this, infants with certain vulnerabilities, such as having specific risk or plasticity alleles, should be most adversely affected by negative maternal behaviors, while also benefiting the most from sensitive parenting. In other words, infants with specific risk alleles who also have mothers who exhibit negative maternal behaviors, such as those listed below, should be the most likely to have disorganized attachments. However, if they have mothers who are sensitive, they should be the least likely to become disorganized. Below, I begin by discussing possible main effects of maternal behavior and maternal characteristics, and then return to their likely interaction with one another in relation to attachment disorganization.

Maternal Sensitivity and Anomalous Behaviors

When examining predictors of attachment security, maternal sensitivity has consistently been found to play a role. Infants with sensitive mothers are more likely to be securely attached, while infants with insensitive mothers are more likely to be insecurely attached (De Wolff & van IJzendoorn, 1997). Therefore, it seems reasonable to expect that sensitivity may be associated with attachment disorganization. However, while the association with disorganization has been found in low SES samples, others

have reported null associations between maternal sensitivity and disorganized attachment in middle-income samples (Van IJzendoorn et al., 1999). The inconsistent findings may be due to two methodological factors. First, in most studies of attachment, maternal sensitivity has been observed during brief, non-stressful play interactions. However, maternal sensitivity during situations that elicit infant distress may be most central to the development of the attachment relationship because the attachment system is activated in situations in which infants are distressed or scared (Leerkes, 2011; McElwain & Booth LaForce, 2006). Thus maternal sensitivity during distressing tasks may be a more robust predictor of attachment outcomes including disorganization. Second, mothers can receive low scores on sensitivity for a variety of reasons, such as non-responsiveness, but only some styles of insensitive behavior, likely more extreme and overt negative behaviors, may be associated with attachment disorganization. It is possible that the association between some types of insensitive maternal behavior and attachment disorganization is being masked due to these specific behaviors being combined with other insensitive behaviors. For example, Main and Hesse (1992) theorized that some mothers engage in fearful or frightening behaviors such as entering trance-like states, sliding their hands around their infants' throats, and sexually or physically abusing their infants. Researchers have since found links between these types of chaotic and extreme parenting and infant disorganization.

Two coding schemes in particular have been used to capture the behaviors associated with these types of parenting: frightened or frightening parental behavior scales (FR) developed by Main and Hesse (1992) and the Atypical Maternal Behavior

Instrument for Assessment and Classification (AMBIANCE) developed by Lyons-Ruth, Bronfman and Parsons (1999). The FR measurement includes 6 scales addressing threatening (in non-play contexts, deep growls directed at infant), frightened (alarmed retreats or fearful affect in response to no environmental changes), dissociative (suddenly freezing with unmoving eyes), timid or deferential (submissive to infant aggressions such as hair pulling or slapping), spousal and romantic (deeply kissing infant), and disorganized parenting behaviors (mistimed movements or anomalous posture). The AMBIANCE (1999) measure has five dimensions, including: negative-intrusive behaviors (pulling infant by wrist, or teasing infant), role confusion (trying to elicit reassurance from infant), withdrawal (moving away or holding infant away from body with stiff arms), affective communicative errors (inviting infant to approach and then backing away from infant or not offering comfort to distressed infant), and disorientation (exhibiting frightened expression towards infant).

Infants whose parents use these anomalous behaviors are more likely to be disorganized (Lyons-Ruth et al., 1999; Beebe et al., 2012). Specifically, children who have experienced anomalous parenting behaviors are nearly four times more likely to form disorganized attachments, thus making it a more robust predictor of attachment disorganization (Madigan et al., 2006). In the current study, three types of anomalous maternal behavior will be considered: negative, intrusive, and mismatched affect. Negative behaviors such as the use of a harsh tone or insults, as well as physical intrusiveness or behaviors that interfere with the infant's autonomous efforts, such as insisting the infant play with a different toy when already engaged in something else, are

insensitive behaviors that may confuse or scare infants when they are distressed. Additionally, maternal behaviors that reflect mismatched affect from infant behaviors, such as laughing at an infant's distress, are also insensitive, confusing, and even frightening. The presence and quantity of these overtly negative maternal behaviors may lead to infant disorganization, and the mothers who display such egregious types of behavior have been found to share other important characteristics.

Maternal History of Trauma and Loss

Similar to infants, mothers' internal working models in regards to attachment can also be assessed. In 1985, George, Kaplan, and Main created the Adult Attachment Interview (AAI), to measure adults' representations of their childhood experiences, and to tap into their internal working models of attachment. Adults can be classified into one of four groups: secure/autonomous, dismissing, preoccupied, and unresolved/disorganized (Hesse, 2008). Adults are coded as secure/autonomous when they are able to describe their childhood experiences, whether positive or negative, clearly and concisely without becoming emotionally distraught. Adults are coded as dismissing when they attempt to minimize any discussion of attachment-related experiences, and also fail to provide supporting evidence for their answers. Preoccupied classifications are given to adults who are able to think of memories in relation to the questions asked, but are unable to focus their responses to the questions. They also often use vague language, digress to other topics, and become emotional during the interview. Finally, adults are given unresolved/disorganized classifications when there are substantial lapses in their descriptions during discussions of potentially traumatic events

involving loss or abuse, or they exhibit disorganization or disorientation during their explanations.

Hesse and Main (2000) proposed that unresolved mothers might become frightened in response to "aspects of the environment that are unconsciously associated with a traumatic event" (p. 1113). Once in this state, these mothers may exhibit the threatening, frightened, or overtly dissociated behaviors previously mentioned, which may be the mechanism through which infants become disorganized. In their meta-analysis on the AAI and infant attachment, Van IJzendoorn (1995) found that 53% of unresolved parents had children classified as disorganized in the Strange Situation and the average effect size for the association between adult unresolved status and infant disorganization was .31. Additionally, in their meta-analysis, Madigan et al. (2006) found that anomalous parental behaviors, including fearful or frightening behaviors, mediated a small portion of the association between unresolved states of mind and disorganized attachment, with the unmediated, and therefore unexplained portion of the association referred to as the transmission gap.

The results of subsequent studies investigating the transmission of adult attachment to infant attachment have been mixed with some reporting direct effects of unresolved adult attachment on infant disorganization (Hughes, Turton, McGauley, & Fonagy, 2006) and some reporting partial mediation via maternal sensitivity or anomalous behaviors (Jacobvitz, Leon, & Hazen, 2006). It may be the case that a history of loss or trauma among unresolved mothers remains a strong predictor of attachment disorganization over and above measures of parenting because it is difficult to observe

extreme maternal behaviors in brief, non-stressful observational contexts Thus, considering maternal trauma history in addition to directly observed parenting behavior is important when attempting to identify the predictors of attachment disorganization.

Although most investigators have examined associations between mothers' unresolved loss or trauma (i.e., indicating they have not come to terms with these events) and infant attachment disorganization (Madigan et al., 2006), some investigators (van IJzendoorn et al., 1999) have reported that a history of loss in and of itself (e.g., the death of parent in childhood) is linked with infant attachment disorganization. This prompted Bernier and Meins (2008), to conclude that there is no indication that having unresolved loss or trauma is a stronger predictor of disorganization than having any experience of trauma or abuse at all. In the proposed project, a history of maternal loss or trauma, in addition to the level of resolution, will be examined as a predictor of attachment disorganization.

In addition, Bernier and Meins (2008) proposed their threshold model to explain how both child characteristics and social-environmental risk factors, including maternal behavior and maternal trauma history, play a role in the formation of disorganized attachment relationships. Their model assumes that infants have individual threshold levels, which, if breached by insensitive or atypical maternal behaviors, lead them to become disorganized. Each infant has a different threshold level based on their individual characteristics, such as their genetic makeup. Infants with lower levels are more susceptible to developing disorganized attachments due to anomalous or insensitive maternal behaviors, because it takes less to breach their thresholds. The higher an infants'

threshold level, the more it takes for them to be affected by negative maternal behaviors. This takes into account why not all infants in similar environments will become disorganized, and may explain why there are characteristically small effects when main effect models are tested. Below, I elaborate on infant genotypes as a specific child characteristic that moderates links between maternal characteristics and behavior and infant attachment disorganization.

Genetic Factors

In addition to maternal characteristics, infant characteristics may play a role in disorganization, specifically through genetic factors. To date, genes related to the dopamine, oxytocin, and serotonin systems have been identified as potentially playing roles in attachment disorganization because they are linked with functional differences in attention, motivation, affect, and social cognition that may contribute to engaging in less adaptive behavior.

Dopamine is a hormone and a neurotransmitter, or a chemical that sends signals to and from nerve cells (Snyder, 2011). The dopaminergic system is related to the prefrontal cortex, which plays a role in cognition and emotional processes (Wang, Zhong, Gu, & Yan, 2003), and is involved in the attentional, motivation, and reward mechanisms (Numan, 2010; Robbins & Everitt, 1999). In previous studies, the A1 allele of the dopamine receptor D2 gene (DRD2 rs1800497), has been linked with reduced dopamine binding (Jönsson et al., 1999) and reduced D2 expression in the striatum (Noble, Blum, Ritchie, Montgomery, & Sheridan, 1991). The 7 repeat (7R) allele of DRD4, the dopamine receptor D4 gene, has also been associated with a decrease in ligand binding.

Both of these alleles require individuals to need increased levels of dopamine in order to overcome their body's blunted responses (Asghari et al., 1994). Likewise, Catechol-O-methyltransferase, or COMT (rs4680) is a gene linked with the inactivation of dopamine activity in the prefrontal cortex (van IJzendoorn et al., 2008), and the COMT val allele (also known as G) is linked with less limbic and prefrontal activation in response to negative stimuli (Smolka et al., 2005) As the prefrontal cortex plays a role in cognition and emotion, a decrease in activation might play a role in infants responses to negative maternal behaviors, one type of negative stimuli. These risk alleles may moderate the link between maternal behavior and infant attachment disorganization, as dopamine plays a role in emotional responses, such as overtly negative maternal behavior. These negative factors may interact to increase levels of disorganization, consistent with the dual-risk model.

Serotonin is a monoamine transmitter, and the 5-HTTLPR (rs25531) polymorphism is an excitatory neurotransmitter in the central nervous system that is involved in regulating serotonin levels in the presynaptic region (Zhang, Chen, Deng, & Lu, 2014) and plays a role in the regulation of fear (Hariri et al., 2002). The short allele of the serotonin transporter polymorphism 5-HTTLPR is associated with lower expression of the 5-HTTLPR gene (Ebstein, 2006; Rutter, 2006), and has been found to be associated with increased fear and anxiety-related behaviors (Hariri et al., 2002). This may play a role in infant attachment disorganization, as it is associated with exposure to fearful or frightening behaviors (Madigan, 2006). That is, infants who are fear and

anxiety prone may be more negatively affected by anomalous maternal behavior increasing their risk for attachment disorganization.

Finally, the function of the oxytocin is not entirely certain, however there is some evidence that it may play a role in attachment disorganization. The oxytonergic system is related bonding, affiliation, and empathy (Carter, 1998; Feldman, Weller, Zagoory-Sharon, & Levine, 2007), and the A allele of the oxytocin receptor gene, OXTR rs53576, has been linked to a decrease in the functional response of the amygdala (Tost et al., 2010), which plays a role in mediating fear responses (Adolphs et al., 2005). As oxytocin plays a role in bonding, infants with the risk allele may be less likely to form positive bonds in the first place, although to my knowledge, no prior studies have reported a main effect of carrying the A allele on attachment outcomes. Given that the GG allele of OXTR is related to social cognition and prosocial behaviors, it has been argued that carriers of the GG allele may be more sensitive to their social environment and therefore more strongly affected by it (Bartz, Zaki, Bolger, & Ochsner, 2011). If this is true, maternal characteristics, sensitivity, and behavior should be more strongly associated with attachment disorganization among infants with the GG genotype than among infants with the AA or AG genotype. Such an effect would be consistent with the differential susceptibility perspective rather than the dual risk perspective.

In the first study of its kind to provide evidence for a genetic main effect, Lakatos et al. (2000) found support for a link between the dopamine D4 receptor gene and infant attachment disorganization. They found that the 7-repeat allele was associated with higher risk for disorganized attachment. However, future studies were unable to replicate

the findings. In fact, an examination of the association between the presence of the DRD4 7-repeat allele and disorganized attachment in a combined group of six studies with 542 infant-mother dyads showed no significant effect (Bakermans-Kranenburg and Van IJzendoorn, 2007).

In a 2011 study done by Luijk et al., dopamine, serotonin, and oxytocin were once again examined for main effects. The researchers examined the DRD2, DRD4, COMT, 5-HTTLPR, and OXTR genes within a combined cohort of 478-522 infants. The only main effect that the researchers found across the two samples was that infants with one or more COMT risk alleles were more likely to be disorganized. However, they suggested that interactions with environmental factors might be hiding some of the more important genetic effects.

In 2009, Spangler et al. examined both genetic and environmental influences on attachment disorganization in a community sample. They found an interaction between serotonin (5-HTTLPR), and maternal responsiveness. Infants with the 5-HTTLPR risk allele were more likely to be disorganized when exposed to low maternal responsiveness; this was not the case for infants without the 5-HTTLPR risk allele, which is consistent with the dual-risk model. In the interim, this interaction effect has not been replicated in larger samples (Luijk et al., 2011). Likewise, Luijk et al (2011) demonstrated only one interaction effect between genes and environment and infant attachment disorganization out of 12 tested interactions. Specifically, COMT and maternal sensitivity interacted such that sensitivity was only linked with disorganization for infants who carried the risk allele. Notably, this interaction was only apparent in one of the two samples included in their

multi-study investigation. Given simple slopes were not presented or graphed, it is unclear if the results were consistent with dual risk of differential susceptibility. Finally, Gervai et al (2007) found an interaction between DRD4 and disrupted maternal affective behavior, with only the infants carrying the short form of the allele having a higher risk for disorganization. There were no significant findings for infants with the DRD4 long (7-repeat) allele. These results were consistent with the differential susceptibility hypothesis, albeit the short allele was the plasticity factor rather than the long (7 repeat) allele as would have been expected.

As noted by Spangler (2013), at this point, indications of a genetic basis for attachment disorganization (classifications or ratings) have not been consistent. Additionally, since it takes numerous statistical tests to check for multiple gene effects, increasing the chance of finding false positives (Benjamin, Ebstein, & Belmaker, 2002), more research is needed in order to address whether there are genetic and environmental interactions. To my knowledge, no studies to date have examined these genes as a moderator of sensitivity to infant distress or the set of discrete negative maternal behaviors I am considering in relation to attachment disorganization. Based on the literature to date, I posit that infant genotypes will not predict attachment disorganization as main effects, rather they will moderate the extent to which infant attachment disorganization is predicted by maternal sensitivity to distress, maternal negative behavior, and maternal history of trauma and loss.

The Proposed Study

In sum, the goal of the proposed study is to examine whether both environmental and genetic factors predict infant attachment disorganization in a racially and socioeconomically diverse sample with a larger than typical rate of disorganization. In an effort to enhance statistical power, the continuous rating of disorganized behavior will be the key outcome variable rather than the dichotomous classification. This is consistent with recent work examining gene by environment associations in relation to attachment disorganization (Luijk, 2011). That maternal sensitivity and overtly negative maternal behaviors were observed during tasks designed to elicit infant distress is novel as is the inclusion of maternal history of loss or trauma regardless of resolution in comparison to most prior research of this type.

The Following Hypotheses Will Be Tested:

Hypothesis 1. Maternal insensitivity, a maternal history of trauma or loss, and the presence of overtly negative maternal behaviors will be positively associated with infant attachment disorganization.

Hypothesis 2. Infant's genotypes will moderate the association between maternal sensitivity, maternal history of trauma or loss, the presence of overtly negative maternal behaviors, and infant attachment disorganization. Given the prior literature is mixed about the nature of such interactions, I will explore whether they are consistent with the dual risk or the differential susceptibility perspective.

Given the characteristics of the current sample (approximately 50% each European American and African American) and concerns that gene by environment

interactions may function differently in different populations (Ellis, Boyce, Belsky, Bakermans-Kranenburg, & Van Ijzendoorn, 2011), I test maternal race as an additional moderator of all effects. I do not anticipate consistent race differences.

CHAPTER II

METHOD

Participants

The current sample will be drawn from a larger study examining how infants characteristics, like temperament, are related to their development and well-being over time, as well as what influences how mothers' parent as their infants grow. The original sample included 259 primiparous mothers (128 European American, 123 African American, and 8 who self-identified as both European American and African American). Mothers in the sample ranged from 18 to 44 (Mean=25.1). Approximately 65% had at least some college level schooling, and annual family income ranged from poverty to over $100,000, Median = $35,000. The majority (57%) of mothers were married or living with their child's father, 24% were in a relationship but not living with their child's father, and 19% were single. All infants were full term and healthy; 125 (49%) were male and129 (51%) were female.

The analytic sample for this project will include those children and mothers who participated in the Strange Situation Procedure at the 1 year time point and provided DNA at the 2 year time point. This results in an analytic sample of 182. Key reasons for attrition, missing data, or being withdrawn from the study include infant mortality (2 cases), moving from the area and an inability to return for behavioral observations (19 cases), withdrawing from the study (9 cases), declining to provide DNA (4 cases),

providing insufficient or questionable DNA (3 cases), and failure to schedule or complete data collection after multiple attempts to schedule (40 cases).

Participants in the analytic sample did not significantly differ from those not in the analytic sample on race, child gender, marital status, income level, or AAI 4-way classifications. However, participants in the analytic sample were significantly older (M = 25.64, SD = 5.26) and higher educated (M = 4.03, SD = 1.77) than those not in the analytic sample (M= 23.68, SD =5.54, t(256) = -2.7, p = 0.01); (M= 3.29, SD =1.74 , t(255) = -3.09, p = 0.02), respectively.

Procedures

Expectant mothers were recruited from childbirth classes, obstetric practices, prenatal breastfeeding classes, the Special Supplemental Nutrition Program for Women Infants and Children (WIC), and by means of flyers and presentations given by research staff members. Upon enrolling in the study, women were mailed their consent forms and a packet of questionnaires. They were instructed to return their completed questionnaires to us when they visited the laboratory for an interview 6 to 8 weeks prior to their due dates. At the visit, participants were administered the Adult Attachment Interview (AAI; George, Kaplan, & Main, 1984-1996). Upon completion of the questionnaires and the interview, participants received $50 and a small gift.

Mother were contacted by phone and visits were scheduled in our laboratory within 2 weeks of the child's 6 month birthday, 1 month of the child's 13 month birthday, and within 4 months of the child's 2.5 year birthday, respectively. Prior to each visit, mothers were mailed questionnaires to complete including updated demographic forms as

well as a measure of childhood trauma experiences at the 2 year follow-up. At the 6 month and 1 year visits mothers and infants participated in a series of videotaped interactive tasks designed to elicit infant distress and to assess maternal behavior. Additionally, during the 1 year visit, mothers and infants participated in the Strange Situation Procedure to assess infant-mother attachment security. During the 2 year lab visit, mothers and infants also participated in a series of interactive tasks not included in the current project, and then DNA was collected via saliva samples from mothers and children, and mothers were interviewed afterwards. Twelve mothers and infants who had moved from the area provided saliva samples via the mail. For completing each visit, mothers received $50, $100, and $120, respectively, and infants received a small toy.

Measures

Sociodemographic Risk

Mothers' demographic risk was measured by self-report on the demographic form, and included income-to-needs, education, and maternal age. Income-to-needs is a measure of a family's cash income divided by the official poverty line for a family that size, with scores less than 1 reflecting that a family is below the poverty line, scores of 1 reflecting that a family is at the poverty line, and scores above 1 reflecting that a family is above the poverty line. Income-to-needs, education, and mother's age were standardized and averaged, and then multiplied by -1 to reflect the fact that lower scores indicate higher risk (Cronbach's alpha = 0.82).

Adult Attachment Interview

During the prenatal visit to the laboratory, the pregnant mothers were administered the Adult Attachment Interview (AAI; Main, Goldwyn, & Hesse, 2003-2008). The AAI is a semi-structured interview in which participants describe their early childhood relationships with their primary caregivers and reflect on the influences those experiences had on them. AAI transcripts were coded, using the standard coding system for the AAI developed by Main and Goldwyn (Main & Goldwyn, 1998/2003), by coders trained through and reliable with the lab of Dr. Mary Main. Fifty transcripts were double coded to calculate reliability, and agreement was good 82%, $k = .67$, $p < .001$. In the current sample, the majority of mothers were classified as secure/autonomous ($n = 173$), with 65 mothers classified as dismissing, and 11 as preoccupied. An additional 10 mothers were classified as unresolved, which will be too few for analyses, so I will focus exclusively on history of loss/trauma.

Coders noted whether or not the respondent experienced applicable loss or trauma (0 = no, 1 = yes) for each. Then they rated the extent to which the loss was resolved (i.e. the respondent had come to terms with it) on a 9-point scale where 1 indicated "no evidence of U/d" and 9 indicated "marked U/d." Agreement for "applicable loss" was $k = .94$, $p < .001$, 98%, while agreement for "applicable trauma" was $k = .78$, $p < .001$, 96%. A single score for unresolved loss/trauma will be computed so that 0 indicates no loss or trauma, 1 indicates completely resolved loss or trauma, 2 indicates mostly resolved loss or trauma, and 3 indicates a 3 or higher on scale scores for unresolved loss or trauma.

Observed Maternal Sensitivity and Behavior at 6 Months and 1 Year

During the 6-month laboratory visit, infants and mothers participated in 3 videotaped distress-eliciting tasks. During the *arm restraint task*, designed to elicit frustration, infants were seated in a car seat while the experimenter kneeled in front of them and held their arms still while not interacting. During the *novelty task*, designed to elicit fear, infants were seated in their car seats in front of a table with a short barrier that prevented a toy from falling into the infant's lap. A remote controlled dump truck with an action figure in it moved across the table and approached the infant twice. While sitting immobile in front of the infant, the truck vibrated and flashed its light while a voice sounded and the truck made sounds to mimic a horn and ignition. This sequence was repeated twice, and then the truck sat silent and immobile in front of the infant for 1 minute. Both of these tasks lasted for 4 minutes. Mothers were seated beside the infants and within reach of a toy basket. They were instructed for the first minute to stay in their chair and be neutral, unless they wanted to end the activity. For the remaining 3 minutes the mothers could do whatever they wanted except get the infant out of the seat or touch the toy truck during the novelty task.

The mothers and infants also participated in a *still-face task* (Tronick, Als, Adamson, Wise & Brazelton, 1978). This task lasted 6 minutes. The infants were seated in their car seats and the mothers were seated across from them so they were at eye level. For the first two minutes the mothers played with their infants as they normally would using their voice and hands. Then mothers were instructed to look away briefly and then look back at their infant with a still face for 2 minutes. Finally, the mothers looked away

briefly and then were instructed to play with their infant as they normally would for two minutes. For this task, maternal behavior and sensitivity during the reengagement portion, is the focus because that is when the infants were most likely to become distressed.

During the 1-year laboratory visit, infants and mothers and participated in 2 videotaped distress-eliciting tasks. At the onset of both tasks the infants were seated on a rug on the floor. Before the *limitations task* began, a toy phone that lit up and played music was introduced to the infants and the infants were allowed to play with it for a minute. Once the infants seemed interested, the experimenter gently took the phone from the infants and put it in a clear plastic jar that the infants couldn't open on their own. The task began when the experimenter placed the jar on the floor near the infant. The infants were prompted by the experimenter to get the phone during the entire phone task. After the task, the jar was opened and the infants were allowed to play with the phone.

During the *novel character approach*, a research assistant dressed in a green ogre costume entered the room and stood quietly near the door for 10 seconds. The research assistant then spoke from a script in a neutral voice ("Hello, I'm an ogre. Do you know what an ogre is?" etc.) and addressed the infant by name. The research assistant slowly approached within two feet of the infant and crouched down while repeating the script. Then she crossed the room and danced while humming a nursery rhyme, and next slouched in a chair pretending to sleep while snoring loudly. After pretending to wake up, the research assistant approached the infant again, crouched down, and repeated the script until the task ended. Both of these tasks lasted for 4 minutes. Mothers were seated beside the infants and within reach of a toy basket. They were instructed for the first minute to

stay in their chair in the room and remain neutral. For the remaining 3 minutes the mothers could do whatever they wanted except open the jar during the first task or talk to or touch the research assistant dressed as the ogre during the second.

Observed maternal sensitivity. Trained raters rated maternal sensitivity separately for each distressing task (6 month: arm restraint, novelty, still face reengage; and 1 year: limitations, novel character approach) using Ainsworth's global 9 point sensitivity scale from (1) *highly insensitive* to (9) *highly sensitive* (Ainsworth et al., 1974). The focus of this scale is the extent to which the mother reads and responds to her infants' cues and demonstrates an awareness of the infant's state by adjusting her own behavior. Twenty percent of the current sample was double-coded for reliability, with the kappa = .77 at the 6-month time point and .80 at the 1-year time point. An overall maternal sensitivity to distress score was calculated by averaging the sensitivity ratings across tasks and time to yield a highly reliable measure that reflects the quality of caregiver across the first year of life (Cronbach's alpha = 0.82).

Observed maternal behavior: Discrete maternal behaviors during the distress eliciting tasks at 6-month and 1-year was continuously coded from digital media files using INTERACT 9 (Mangold, Arnstorf, Germany) Event based coding was used, meaning once a code was activated, it remained active until another code was selected. Maternal behavior was coded using 12 mutually exclusive categories described below. Thirty cases were double-coded for reliability with kappa = .77 at the 6-month time point and .80 at the 1-year time point.

Maternal behavior codes were: negative (directs negative affect toward the infant); intrusive (forces own agenda on the infant); withdraw (abruptly moves away or ends interaction with infant); mismatched affect (laughs at infant's distress); distracted from infant (engages in behaviors unrelated to parenting such as checking cell phone); persistent ineffective (continues to respond to infant in same ineffective manner when alternative responses are available); monitor (watches infant/situation without intervening); task focused (engages with infant focusing on the arousing task); calming (soothes infant physically or vocally); supportive (provides soothing support for engagement with the task); non-task focused engagement (plays with or distracts the infants without using the arousing task); and routine care (engages in practices like wiping nose, straightening clothing). For the current study, negative, mismatched affect, and intrusive behaviors will be examined because these most closely parallel anomalous behaviors coded in other studies about the origins of attachment disorganization. Scores reflecting the percentage of time mothers engaged in each of these three interactive behaviors were computed for both the 6 month and 1 year time points and then averaged over time to yield measures of the extent to which infants experienced these anomalous parenting behaviors across the first year of life.

The Strange Situation Procedure

Infant-mother attachment security was assessed at 1 year using the Strange Situation Procedure (Ainsworth & Wittig, 1969; Ainsworth, Blehar, Waters, & Wall, 1978). The Strange Situation Procedure is a 25-minute procedure, which contains brief episodes of increasing stress for the infant, including two mother-infant separations and

reunions. The Strange Situation was administered according to standard procedures and E. Carlson coded videotapes of all Strange Situations. Four 7-point interactive behavior scales in the two reunion episodes (Episodes 5 and 8) of the Strange Situation Procedure were coded: proximity/contact seeking, contact maintenance, avoidance, and resistance. Coders also rated the degree of disorganization for the entire SSP on a 9-point scale (Main & Solomon, 1990). Strange Situations were scored using the standard 3 way classification of secure (n = 168), insecure-avoidant (n = 16), and insecure-resistant (n = 19). In the current sample, 17% of the sample (n = 44) were classified as disorganized, by being rated 5 or higher on the disorganization scale. Inter-rater reliability on the D rating, the primary dependent variable, was established based on 30 double coded cases, and was adequate, ICC =0.6. The disorganization scale ratings were chosen as the dependent variable, given that greater statistical power is afforded by using a continuous measure, as well as to be consistent with, recent molecular genetic work by Luijk (2011).

DNA Collection and Genotyping

Children's DNA was collected via saliva samples during the 2 year visit (or at the child's home in rare instances in which saliva samples were mailed) using the Oragene Collection Kit 500OrageneTM, DNAgenotek, Ottawa, Ontario, Canada, www.DNAgenotek.com). Children's saliva samples were collected by using a q tip-like swab (the Oragene swab format; #OG-575) to collect the saliva and twist it into a tube that when capped releases a stabilizing lsis buffer. All samples were given a bar coded label linked only to the research records maintained by the PI before sending the tubes for DNA processing. The DNA was prepared at the Molecular/Cellular Biology Core

Laboratory at the University of North Carolina at Greensboro using the methodologies described by Oragene. Then, DNA was quantified by spectrophotometry (Nanodrop Spectrophotometer) and standardized to a working concentration of 20 ng/µl. Genotyping was performed for genes in the dopaminergic system; DRD2 (rs1800497), DRD4, COMT (rs4680), the serotonergic system; 5-HTTLPR (rs25531), and the oxytonergic system; OXTR (rs53576). Two individuals scored genotypes independently, and inconsistencies were reviewed and rerun when necessary.

The assay of the dopamine D2 receptor gene, (DRD2 rs1800497) was done using a fluorogenic 5'nuclease (Taqman®, ABI, Foster City, CA) method (Haberstick and Smolen, 2004) on an ABI Prism® 7000 Sequence Detection System using the allelic discrimination mode (Livak, 1999). Primer and probe sequences were: forward: 5'-GTGCAGCTCACTCCATCCT-3'; and reverse: 5'-GCAACACAGCCATCCTCAAAG-3'; with A1 Probe: 5'- VIC-CCTGCCT***T***GACCAGC-NFQMGB-3'; and A2 Probe: 5'-FAM-CTGCCT***C***GACCAGC-NFQMGB-3'. As other studies have grouped participants by whether or not they carry one or more of the A1 alleles, (Jönsson et al., 1999), participants were classified as either having one or two A1 alleles (risk = 1) or none (no risk = 0).

The assay of the dopamine D4 receptor gene, (DRD4; Anchordoquy et al, 2003) was a modification of an extant method (Lerman, et al., 1998). The primer sequences were forward: 5′-VIC-GCT CAT GCT GCT GCT CTA CTG GGC-3′; and reverse: 5′-CTG CGG GTC TGC GGT GGA GTC TGG-3', which yielded PCR products from 279 (2R) to 663 (10R) bp. As other studies have grouped participants by whether or not they

carry one or more of the 7R alleles (Robbins & Everitt, 1999) participants were classified as either having one or two 7R alleles (risk = 1) or none (no risk = 0).

The assay of the Catechol-O-methyltransferase (COMT rs4680) gene was performed using a fluorogenic 5'nuclease (Taqman®, Applied Biosystems, Foster City, CA) method (Haberstick and Smolen, 2004. Primer and probe sequences were: forward: 5'-TCGAGATCAACCCCGACTGT-3'; and reverse: 5'-AACGGGTCAGGCATGCA-3'; with *Val Probe*: 5'-FAM-CCTTGTCCTTCA*C*GCCAGCGA- NFQMGB-3'; and *Met Probe*: 5'-VIC-ACCTTGTCCTTCA*T*GCCAGCGAAAT- NFQMGB-3' (Mattay et al., 2003). As other studies have grouped participants by whether or not they carry one or more of the val (or G) alleles, (Smolka et al., 2005), participants were classified as either having one or two val alleles (risk = 1) or none (no risk = 0).

The assay of the serotonin transporter polymorphism gene, 5-HTTLPR rs25531 is a modification (Anchordoquy et al, 2003) of the method of Lesch *et al*, (1996) using the primer sequences from Gelernter et al. (1999). The primer sequences were Forward: 5'-NED - ATG CCA GCA CCT AAC CCC TAA TGT - 3', and Reverse: 5'- GGA CCG CAA GGT GGG CGG GA - 3' which yield PCR products of 376 (S) and 419 (L) base pairs (bp). As other studies have grouped participants by whether or not they carry one or more of the short alleles, (Ebstein, 2006; Rutter, 2006), participants were classified as either having one or two short alleles (risk = 1) or none (no risk = 0).

The assay of the oxytocin receptor gene, OXTR rs53576 was performed using a fluorogenic 5'nuclease (Taqman®, LifeTechnologies, Grand Island, NY) method using the 40x primer-probe reagents obtained from the company (assay number

C___3290335_10_M). Reactions were performed in an ABI Prism® 7000 Sequence Detection System using the allelic discrimination mode (Livak, 1999). Reactions containing 5-20 ng of DNA were performed in 15 µl reactions with TaqMan® Universal PCR Master Mix using the standard cycling conditions. As other studies have grouped participants by whether or not they carry one or more of the A alleles, (Ebstein, 2006; Rutter, 2006), participants were classified as either having one or two A alleles (risk = 1) or none (no risk = 0). Having no A allele (0) also indicates the infant has the GG genotype which would be considered the "plasticity" genotype (Sturge-Apple, Cicchetti, Davies, & Suor, 2012).

The Hardy-Weinberg Equilibrium (HWE) test was run separately by race to see if the gene frequencies in the sample are similar to gene frequencies in the general population. Frequency distributions conformed to the HWE, except for OXTR rs53576 for White participants (p = .0029).

CHAPTER III

RESULTS

Preliminary Analysis

Preliminary analyses included examining the frequencies and distributions of all study variables. Descriptive statistics were calculated for all primary variables and potential covariates and appear in tables 1 and 2. Possible covariates were identified by examining simple correlations between the outcome variable of disorganization and the predictor variables of race, maternal sensitivity, overtly negative maternal behavior, trauma and loss, and the 5 individual genes, with sociodemographic risk, child gender, and race. Sociodemographic risk was positively associated with infant disorganization and DRD2, and negatively associated with race, maternal sensitivity, and 5HTTLPR, underscoring the importance of using it as a covariate to ensure that the observed associations between the predictor variables and disorganization were not artifacts of this covariate. Race was associated with a number of primary variables such that European American women were rated as more sensitive, had lower sociodemographic risk, and infants who were less likely to have the risk genes for DRD2 and COMT, and more likely to have the risk gene for 5HTTLPR relative to African American women. Race differences in gene frequencies are not uncommon in research; controlling for race and testing race by gene interactions are common approaches to dealing with this issue (Barr & Kidd, 1993; Mills-Koonce et al., 2007; Propper et al., 2008).

Zero-order correlations among primary variables and identified covariates are displayed in Table 3. As expected, maternal sensitivity to distress and overtly negative maternal behavior were negatively correlated. Consistent with prediction, negative maternal behavior correlated positively with attachment disorganization. Finally, 5HTTLPR risk correlated positively with overtly negative maternal behavior.

Hypothesis Testing

All continuous variables to be involved in the interactions were centered (maternal sensitivity to distress, the three maternal behaviors, trauma and loss, and sociodemographic risk). Two-way interactions were created by multiplying each of the 5 risk alleles by centered maternal sensitivity to distress, overtly negative maternal behavior, trauma and loss, and race. In addition, 3 way interaction terms between each allele, each moderator, and race were calculated by multiplying each of the two-way interaction terms by race.

Five initial multiple regressions were run, one for each gene. In each, sociodemographic risk, race, maternal sensitivity to distress, overly negative maternal behavior, and trauma and loss were entered in the first step. Next, one genotype was entered in step two. Then, the two-way interactions between the gene and maternal sensitivity to distress, overtly negative maternal behavior, trauma and loss, and race were entered in step three. Finally, the three-way interactions between the gene and race, and maternal sensitivity to distress, overly negative maternal behavior, or trauma and loss were entered in step 4. None of the three-way interactions were significant for any of the 5 regressions, and so results are presented only for step 1, 2, and 3 in Table 4.

Given concerns about multicollinearity due to the number of interactions in the complete models, an issue magnified by the fact that interactions within each model were all composed of the target gene, I retested significant interactions in reduced models. The reduced models included all covariates and main effects (steps 1 and 2), but only one interaction term in step 3. The results of these reduced models are summarized in the text.

Hypothesis 1. Main Effects

Consistent with prediction, negative maternal behavior predicted higher infant attachment disorganization independent of the covariates, maternal sensitivity to distress and history of unresolved trauma and loss over and above all controls. Inconsistent with prediction, maternal sensitivity to distress, and trauma and loss were not significant independent predictors of attachment disorganization. Additionally, as anticipated, there were no main effect associations between any gene and disorganization. Although maternal race was not linked with attachment disorganization in the zero-order correlations, it was linked with attachment disorganization when other main effects were controlled such that attachment disorganization was higher among infants of European American mothers than infants of African American mothers. Although this association was not significant in the DRD2 and OXTR models, likely based on the slightly reduced analytic sample, it was a trend in each, both $p = 0.06$.

Hypothesis 2. Two-way Interactions

Four of 20 tested two-way interactions were significant in the complete models. OXTR interacted with maternal sensitivity to distress and race and COMT interacted with maternal sensitivity to distress and history of unresolved trauma and loss in relation to

disorganization. None of the interactions involving DRD2, DRD4, and 5HTTLPR were significant.

In the reduced regression models that included all main effects and only one identified two-way interaction, the two interactions involving OXTR were not significant: $\beta = .12$, $p = .21$ for sensitivity to distress by OXTR, and $\beta = -.18$, $p = .18$ for OXTR by race. Likewise, the two interactions involving COMT were not significant: $\beta = -.17$, $p = .34$ for sensitivity to distress by COMT, and $\beta = .35$, $p = .10$ for trauma and loss. This suggests that the interactions were a function of suppressor effects in the full model and are not particularly robust. Thus, the hypothesis that infant genetic risk moderates the links between infant attachment disorganization and maternal sensitivity to distress, overtly negative maternal behavior, and loss and trauma was not supported.

As shown in table 5, across the three blocks in each of the 5 regressions, only the change in R^2 for the first block was significant ($R^2 = .076$-$.079$, all $p < .05$). This means that as a set the contextual variables (race, SES, maternal sensitivity to distress, overtly negative maternal behavior, and trauma) predicted significant variability in disorganization. However, including the genes and the gene by environment interactions did not predict significant variability in infant disorganization. An R^2 of .076 translates to an f^2 of .08, indicating a small to moderate effect size for the association between environmental factors and infant attachment disorganization (Cohen, 1988).

CHAPTER IV

DISCUSSION

The goal of this study was to examine predictors of infant attachment disorganization, which has been linked to negative outcomes into adulthood. Maternal sensitivity to distress, overtly negative maternal behaviors, maternal trauma and loss, and their interactions with infant genes related to dopamine, serotonin and oxytocin functioning were identified as possible predictors. The results of past research on gene by environment interactions in relation to attachment disorganization has been somewhat inconsistent. The null results in this study add to accumulating evidence that single infant genes linked with emotional maladjustment do not pose a risk for attachment disorganization. Moreover, that overtly negative maternal behavior was linked to higher attachment disorganization is highly consistent with prior research as outlined below.

The first goal of the study was to determine if maternal insensitivity to distress, overtly negative maternal behaviors, or maternal history of trauma or loss predicted higher infant attachment disorganization. A unique feature of this study was the focus on maternal sensitivity and behavior during distress eliciting tasks based on the rationale that such tasks may increase observed variability in insensitive and anomalous maternal behavior and enhance predictive validity to attachment disorganization. This was not the case for sensitivity to distress, which was unrelated to attachment disorganization in the present study. Although sensitivity has been

found to be negatively linked to attachment disorganization in prior research, the effect sizes have been small, indicating that sensitivity is not a strong predictor (Moran, Forbes, Evans, Tarabulsy, & Madigan, 2008). Perhaps sample characteristics, particularly the presence of other risks, influences the nature of the association between sensitivity and disorganization. Consistent with this view, while maternal insensitivity has been found to be linked with disorganization in low SES samples, these findings have not often been found in middle-income samples (Van IJzendoorn et al., 1999). The lack of a simple association between sensitivity to distress and disorganization may be a function of the economic diversity within the sample. Additionally, it may not be insensitivity in general that leads to attachment disorganization, but specific negative or fearful behaviors.

Consistent with this view and prediction, infants whose mothers engaged in more overtly negative maternal behavior were rated higher on disorganization relative to infants whose mothers who engaged in fewer of these types of behaviors. As these behaviors can be confusing or scary to infants when they are distressed, they may make infants fearful of their mothers to whom they should be able to rely on for comfort, a key feature of disorganization's "fright without solution" dilemma (Hesse & Main, 2000). This finding is consistent with prior research using the AMBIANCE coding system to assess maternal behavior (Lyons-Ruth et al., 1999), even though the current study did not use that coding system, and instead examined the presence of any one of three discreet overtly negative maternal behaviors. However, the effect size was small, thus demonstrating that negative behaviors during distress eliciting tasks was not a stronger predictor of disorganization than has been the case in studies that observed maternal

behaviors in more neutral settings. The effect size may have been small due to the fact that while the current sample is diverse, it was not an at-risk sample, which led to limited variability of these behaviors. Nevertheless, these findings suggest that even limited exposure to these types of negative maternal behaviors has the potential to disrupt the development of an organized attachment between mother and infant.

Contrary to prediction, a relationship was not found between a history of maternal loss or trauma, in addition to the level of resolution, and disorganization. Relationships have been found between mothers' unresolved AAI classifications in regards to trauma and loss, and infant disorganization. However, as this sample had a very low incidence of unresolved mothers, a history of trauma or loss was tested instead, as Bernier and Meins (2008) postulated that being unresolved with respect to loss and trauma was not a stronger predictor than just having experienced loss or trauma. While this sample had a large number of mothers who had experienced loss or trauma, relatively few scored high on being unresolved. Thus, contrary to Bernier and Meins (2008) hypothesis, it seems whether the trauma or loss is resolved is the key feature that plays a role in the transmission of attachment disorganization.

The second goal of the study was to examine whether infants' genotypes would moderate the associations between maternal insensitivity to distress, the presence of overtly negative maternal behavior, maternal history of trauma or loss, and infant attachment disorganization. Although 4 out of the 20 tested interactions were significant in the full regression models that included all of the 2-way interactions (2 involving COMT and 2 involving OXTR), they were not significant when I retested them in the

reduced models. This suggests that these interactions are not particularly robust, and therefore it seems reasonable to conclude that single infant genes related to the dopamine, oxytocin, and serotonin systems do not confer a risk for disorganization by themselves, nor do they moderate the extent to which negative environments may confer risk. This is consistent with the conclusion drawn by Luijk et al. (2011) who tested interactions between these genes and maternal sensitivity in relation to disorganization in two large samples. The null findings of the current study in regards to single genetic alleles lends greater confidence to the view that the few studies that have found single gene by environment interactions may be chance findings (Lakatos et al., 2000; Spangler et al., 2009). Additionally, I have further expanded upon these conclusions by demonstrating that there were also no interactions with overtly negative maternal behavior or trauma and loss, two variables that have not, to my knowledge, been examined before in conjunction with genetic risk.

The present results also suggest that demographic variables are important in relation to disorganization. As the current sample included approximately half European American and African American participants, race was considered, primarily to determine if it moderated other associations with disorganization, which it did not. This lends confidence that the observed association between negative maternal behavior and disorganization and the lack of genetic effects, both main and moderating, in relation to disorganization is comparable across these two racial groups. However, race was linked to disorganization as a main effect when the other covariates were included such that infants of European American mothers were more likely to be disorganized than infants

of African American mothers. This occurred when the variability in SES was controlled, leading to the possibility that it is the result of a suppressor effect, in which SES accounted for the residuals left in the model. Such race differences in disorganization have not been reported in the prior literature, thus replication is warranted prior to drawing strong conclusions from this unexpected result.

Also of note, sociodemographic risk was associated with disorganization such that infants were at greater risk of disorganization if their mothers were younger, poorly educated, and had a low income. This is consistent with studies that have found a higher rate of disorganization in at-risk samples, including low SES samples (Van IJzendoorn et al., 1999) One explanation for this is that low SES families have more stressors, leading to more chaotic environments and a greater incidence of negative parenting behaviors. For instance, lower income-to-needs ratios have been found to be associated with both a greater fear for safety and family conflicts, which lead to increased rates of harsh parenting (Barajas-Gonzalez & Brooks-Gunn, 2014). Additionally, that effects of SES were apparent over and above measures of maternal sensitivity to distress and behavior in the current study suggest that other contextual aspects associated with SES may lead to disorganization. Future research should examine environment-by-environment interactions, such as whether SES and maternal sensitivity or maternal behaviors interact to predict attachment disorganization. This would be consistent with the dual-risk hypothesis, in which the presence of two risk factors increases an individual's risk of negative outcomes moreso than the presence of a single risk factor.

The current study contributes to our knowledge of what factors predict infant attachment disorganization, as well as provides additional evidence for the limited role of gene by environment interactions, in regards to single alleles, in the development of attachment disorganization. The strengths of this study include the diverse sample in regards to SES and race, the relatively high rate of disorganization, and the use of the Strange Situation Procedure and Adult Attachment Interview, both gold standard measures. Of unique interest is the careful coding of maternal behavior over two time points, and using both a traditional global measure of sensitivity and micro coding of specific negative maternal behaviors in distressing contexts, which are highly likely to activate the attachment system. Although the use of overtly negative maternal behavior is rare, especially in a lab context, I still was able to find effects.

Despite these strengths the current sample did not have a large enough proportion of unresolved mothers in order to examine the relationship between unresolved status and attachment disorganization. Additionally, the current study did not use 3 common measures of anomalous behavior: role confusion, withdrawal, and disorientation. However, the overtly negative maternal behavior composite still yielded comparable results, even though the effect size was small. Finally, I did not examine any gene by gene interactions or consider the role of epistasis or epigenetics. While single gene by environment interactions may not predict infant attachment disorganization, gene by gene by environment interactions may play a role through epistasis to predict disorganization. Epistasis refers to the effect that one gene has on another gene, such as one allele blocking the effect of another allele (Phillips, 2008). Likewise, epigenetics, a process by

which the environment may alter whether or not a gene is expressed, may play a role (Meaney, 2010). For example, infants with the DRD4 7 repeat allele may vary in the expression of characteristics associated with that gene depending on the degree of methylation. This would affect the extent to which interactions between the DRD4 7 repeat allele and the environment would be observed in relation to outcomes such as attachment disorganization. Future research should consider these possibilities in relation to disorganization.

Although the sample size is relatively large for a single site study focused on antecedents of attachment, it is rather small for molecular genetic work (Luijk et al., 2011). Moreover, a racially diverse sample is considered a strength in the developmental literature, but from a molecular genetic perspective, homogenous samples are often preferred to increase power and to reduce the chance of false-positives (Tian, Gregersen, & Seldin, 2008). Thus, the nature of the sample may be a limitation in regard to testing molecular genetic effects. Future research should also look at environment and gene interactions among samples with a higher incidence of unresolved mothers, as it is possible that an interaction may exist between mothers' unresolved adult attachment status and infant genotypes in relation to disorganization.

As infant attachment disorganization is associated with negative outcomes from infancy through adulthood, efforts are being made to identify the predictors in order to inform prevention and intervention programs. The most successful programs have focused on interventions with the aim of increasing maternal sensitivity (Bakermans-Kranenburg, Van IJzendoorn, & Juffer, 2005), although as I have shown, sensitivity to

distress is not a robust predictor of disorganization. The results of this study indicate that even the presence of infrequent overtly negative maternal behaviors contributes to infant disorganization regardless of individual differences in infants' genetic risk. Parenting programs may need to address the effects that negative maternal behaviors have on infant development, in order to reduce the occurrence of such behaviors. As SES was linked to disorganization, programs should target younger, less educated mothers, and mothers with lower income-to-needs ratios. Programs could educate mothers on the way that specific behaviors affect their relationships with their infants, as well as help mothers who use these behaviors to minimize their use of them. Researchers have noted that the most effective intervention programs have targeted infants over the age of 6 months (Bakermans-Kranenburg et al., 2005). However, results of this study suggest that maternal behavior that is in place by 6 months is a predictor of disorganization, thus it is possible that intervention prior to 6 months may in fact be useful in preventing disorganization.

In conclusion, results of this study demonstrate that negative maternal behavior and socioeconomic risk uniquely predict infant attachment disorganization. In contrast, maternal sensitivity to distress, history of trauma and loss, and single genetic risk factors are unrelated to infant attachment disorganization as main effects and in conjunction with one another. A more nuanced approach to study the complexities of environment by environment, gene by gene, and gene by environment predictors of attachment disorganization is warranted.

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APPENDIX A

TABLES

Table 1. Descriptive Statistics

Continuous Variables	N	Min	Max	Mean	Std. Deviation
Sociodemographic Risk	182	-1.87	1.58	0.02	0.84
Total Maternal Sensitivity to Distress	182	1.50	7.92	5.05	1.45
Overtly Negative Maternal Behavior	182	0.00	4.91	0.79	1.00
Trauma and Loss	182	0.00	3.00	1.34	0.91

Table 2. Descriptive Statistics

Dichotomous Variables		N	n	%
Race		182		
	Non-White		86	47.3%
	White		96	52.7%
OXTR		180		
	Plasticity		99	54.4%
	No Plasticity		81	44.5%
DRD2		180		
	Risk		110	60.4%
	No Risk		70	38.5%
COMT		182		
	Risk		28	15.4%
	No Risk		154	84.6%
DRD4		181		
	Risk		114	62.6%
	No Risk		67	36.8%
5HTTLPR		181		
	Risk		72	39.6%
	No Risk		109	59.9%

Note: Risk = 1 or 2 risk alleles, No risk = 0 risk alleles

Table 3. Intercorrelations Among Key Variables

	1	2	3	4	5	6	7	8	9	10	11
1. Race[1]	--										
2. Sociodemographic Risk	-.44**	--									
3. Total Maternal Sensitivity to Distress	.48**	-.61**	--								
4. Overtly Negative Maternal Behaviors	-.12	.10	-.34**	--							
5. Trauma and Loss	-.06	-.07	.06	-.05	--						
6. OXTR[2]	.13	.06	.00	.10	-.01	--					
7. DRD2[2]	-.19*	.17*	-.10	.06	.04	-.08	--				
8. COMT[2]	-.22**	.12	-.07	-.07	.04	-.03	-.05	--			
9. DRD4[2]	-.06	.10	-.11	.09	-.02	-.07	-.08	-.15*	--		
10. 5HTTLPR[2]	.23**	-.23**	.20**	-.12	.05	.03	-.09	-.13	-.03	--	
11. Disorganization	.04	.17*	-.14	.18*	-.02	.12	-.07	.01	.01	-.08	--

Notes: [1] 0 = Non-White, 1 = White

[2] 0 = No Risk (0 risk alleles), 1 = Risk (1 or 2 risk alleles)

*$p \leq .05$, **$p \leq .01$

Table 4. Multiple Linear Regressions

		DRD2 (n = 180)			DRD4 (n = 181)			COMT (n = 182)			5HTTLPR (n = 181)			OXTR (n = 180)		
		B	*SE B*	*β*	*B*	*SE B*	*β*	*B*	*SE B*	*β*	*B*	*SE B*	*β*	*B*	*SE B*	*β*
1.	Race[1]	.59	.31	.16	.65	.31	.18*	.63	.31	.17*	.65	.31	.18*	.59	.31	.16
	SES	.43	.21	.20*	.44	.21	.20*	.43	.21	.20*	.44	.21	.20*	.43	.21	.20*
	Maternal Sensitivity	-.05	.13	-.04	-.06	.13	-.05	-.06	.13	-.05	-.06	.13	-.05	-.05	.13	-.04
	Overtly Negative Maternal Behaviors	.12	.05	.17*	.12	.05	.17*	.11	.05	.16*	.12	.05	.17*	.12	.05	.17*
	Trauma and Loss	-.00	.15	.00	-.01	.15	-.01	-.01	.15	-.00	-.00	.15	.00	-.00	.15	.00
2.	Gene[2]	-.33	.28	-.09	-.08	.28	-.02	.19	.38	.04	-.19	.28	-.05	.27	.27	.07
3.	Gene X Maternal Sensitivity	.14	.23	.06	-.07	.24	-.04	-.66	.31	-.47*	.36	.24	.23	.49	.23	.25*
	Gene X Maternal Behavior	.01	.11	.01	-.10	.11	-.10	-.20	.14	-.27	.05	.11	.05	.03	.11	.03
	Gene X Trauma and Loss	-.03	.30	-.01	.00	.31	.00	1.08	.48	.51*	.18	.30	.07	-.15	.30	-.05
	Gene X Race	-.72	.63	-.15	-.06	.67	-.01	1.01	1.00	.27	.21	.65	.06	-1.41	.62	-.34*

Notes: [1] 0 = Non-White, 1 = White

[2] 0 = No Risk (0 risk alleles), 1 = Risk (1 or 2 risk alleles)

*$p \leq .05$, **$p \leq .01$

Table 5. R Squared Change

		DRD2 (n = 180) $R^2\Delta$	DRD4 (n = 181) $R^2\Delta$	COMT (n = 182) $R^2\Delta$	5HTTLPR (n = 181) $R^2\Delta$	OXTR (n = 180) $R^2\Delta$
1.	Race[1]	.076*	.079*	.077*	.079*	.076*
	SES					
	Maternal Sensitivity					
	Overtly Negative Maternal Behaviors					
	Trauma and Loss					
2.	Gene[2]	.007	.000	.001	.002	.005
3.	Gene X Maternal Sensitivity	.007	.005	.039	.021	.035
	Gene X Maternal Behavior					
	Gene X Trauma and Loss					
	Gene X Race					
Total R^2		.090	.085	.117	.102	.117

*$p \leq .05$, **$p \leq .01$