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The interaction of parenting and the serotonin transporter gene on trajectories of fearfulness in early childhood

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Abstract

Children who are more fearful and inhibited during early childhood are at greater risk for social problems (e.g., loneliness, social isolation) and clinically significant internalizing disorders during adolescence and adulthood (e.g., Rubin, Chen, McDougall, Bowker, & McKinnon, 1995; Williams et al., 2009). While the impact of fearful temperament on adjustment indices are regularly the focus of study, less well understood are biological and social processes that may affect the development of fearful temperament. The present study considered the role of the 5-HTTLPR polymorphism and parenting on change in fearful and inhibited temperamental characteristics during early childhood. The *s*/s genotype was expected to be associated with elevated and sustained levels of fearful temperament. Moreover, supportive parenting was expected to be associated with less fearful temperament while more harsh parenting would be associated with more fearful temperamental characteristics, especially for children with the *s*/s 5-HTTLPR genotype. Study hypotheses were tested using 165 families (i.e., biological mothers and fathers, 3-5 year old children) who participated in the Family Transitions Project (FTP: R. D. Conger & K. J. Conger, 2002). Children were genotyped using cheek swabs. Parents reported on children's temperamental characteristics at ages 3, 4, and 5. Independent observations of mothers and fathers completing a puzzle with their 3 and 4 year old children were used to measure parenting. Results were partially supportive of predictions. Parenting interacted with the 5-HTTLPR genotype to predict trajectories of shyness and soothability dimensions of fearful temperament, but the pattern of findings varied for mothers and fathers. Results are discussed in terms of differential susceptibility and the conceptualization of risk and resilience.

Key Words: 5-HTTLPR; Mothers and fathers parenting; Genotype by environment interaction; Fearful temperament; Soothability; Preschool

The interaction of parenting and the serotonin transporter gene on trajectories of fearful temperament in early childhood

Fearful temperamental characteristics, like wariness, inhibition, and withdrawal from novelty or social interactions, may increase children's risk for developing internalizing disorders during later developmental periods. Temperamentally fearful and inhibited children, particularly children rated high on behavioral inhibition, are generally found to be shyer and more withdrawn during childhood (Kagan, Reznick, & Snidman, 1988), report feeling lonelier and more socially rejected during middle childhood (Boivin, Hymel, & Bukowski, 1995), and seem to be at increased risk for internalizing disorders during adolescence (Rubin, Chen, McDougall, Bowker, & McKinnon, 1995; Williams et al., 2009). While elevated levels of fearful temperament during early childhood may increase children's risk for developing internalizing problems during later developmental periods, most young children evidencing high levels of fearful temperament do not go on to experience internalizing problems during adolescence or adulthood (Kagan & Snidman, 1999). Instead, only children exhibiting extreme levels of fearful temperament, including heightened levels of behavioral inhibition, which remain elevated throughout childhood, seem to be most likely to experience later internalizing disorders (Hirschfield et al., 1992). Thus, young children with extreme scores on fearful temperament may be at greatest risk for developing internalizing problems.

Individual differences in serotonin availability may partially explain variations in both the level and stability of fearful temperament. Serotonin is created when tryptophan combines with tryptophan hydroxylase forming 5-hydroxltryptamine (5-HT). After 5-HT is released into the synaptic cleft, it binds to the adjacent neuron and then either degrades outside of the cell or is transported back into the cell by serotonin transporter proteins (SERT) to be reused. Re-using 5-

HT is energy efficient because of the time and energy involved in producing 5-HT. When 5-HT is not efficiently transported back into the cell, less 5-HT is available for emotion regulation.

Insufficient extracellular availability of 5-HT may increase risk for anxiety and depression among adolescents and adults (e.g., Baldwin & Rudge, 1995; Davidson et al., 2002). That is, pharmaceutical studies targeting the serotonergic system have demonstrated that increasing the availability of extracellular 5-HT improves anxiety and depression symptoms. For example, serotonin re-uptake inhibitors (SSRIs) are a class of pharmaceuticals that are commonly prescribed to treat anxiety and depression. SSRIs block the re-uptake of 5-HT, allowing 5-HT to accumulate at the synapse, permitting the neurotransmitter to continue to bind to the adjacent neuron, improving emotion regulation (Nutt et al., 1999). SSRIs have been successful in treating both anxiety and depression in children and adolescents (e.g., Camenisch & Hilt, 2013).

One mechanism by which SSRIs decrease anxiety and improve mood is by moderating activity in regions of the brain that are involved in emotion generation and regulation. Specifically, the amygdala, which is densely enervated by 5-HT neurons, is modulated by SSRI administration (Azmitia & Gannon, 1986). However, the impact of SSRIs on the amygdala is further moderated by within individual history of psychopathology. For example, in a review of studies examining the relationship of short-term administration of SSRIs and emotion recognition, individuals with a history of depression had an attentional bias toward fearful faces as compared to a group of individuals with no depression history; SSRIs decreased fear recognition for individuals with a history of depression, but did not affect fear bias in the comparison group (Godlewska, Norbury, Selvaraj, Cowen, & Harmer, 2012). An optimal level of 5-HT likely exists. For individuals who have less available 5-HT, SSRIs increase 5-HT availability to normalize function, like decreasing attentional biases to fear. Continuing to

increase 5-HT may have diminished returns, such that more 5-HT has a diminished effect on attention bias to fear.

Theoretically, the serotonin transporter gene (5-HTTLPR) may directly affect the behavioral expression of temperamental inhibition by altering the amount of available 5-HT. The 5-HTTLPR polymorphism codes for SERT and the length of the polymorphism corresponds to the amount of SERT transcribed. The 5-HTTLPR polymorphism consists of 2 alleles, short (*s*) and long (*l*) resulting in 3 genotypic configurations (*s, s*), (*s, l*), and (*l, l*). Individuals with the (*l, l*) genotype have the most SERT transcribed while those with the (*s, s*) genotype have the fewest SERT transcribed. Since replenishing 5-HT takes time, failing to re-uptake 5-HT may significantly decrease 5-HT availability. Individuals with the short variant produce the least amount of SERT, placing them at risk for low 5-HT availability particularly under conditions of elevated psychological stress.

5-HT seems to be released in response to exposure to psychological stressors (Kawahara, Yoshidab, Yokoob, Nishia,& Tanakab, 1993). More frequent and more intense periods of psychological stress may render individuals with less SERT more susceptible for decreased 5-HT availability. Individuals with the *s/s* genotype have been found to be at increased risk for depression following stressful life events. In contrast, when individuals with the *l/l* genotype experience stressful life events, they are not at increased risk for depression, suggesting that the *s/s* genotype only confers risk in conditions of stress, and that the *l/l* genotype is protective against depression in conditions of stress (Caspi et al., 2003). Furthermore, a bias towards recalling negative life events likely affects depression risk, and individuals with the *s/s* genotype may be more strongly impacted by stress. Fox and colleagues (2009) found a distinct pattern in attentional biases among children with the *l/l* genotype as compared to the *s/s* and *s/l* genotype. Children with the *l/l* genotype showed an attention bias

toward positive affective pictures and selective avoidance of negative affective pictures, the opposite pattern of responding was reported for children with the *s/s* and *s/l* genotype. Children with the *s/s* and *s/l* genotype may be a higher risk for developing depression and anxiety because they likely perceive their environment as more stressful compared to children with the *l/l* genotype.

During the early childhood period, parents largely control and moderate children's social environment. Individual differences in exposure to harsh parenting and the perception of the environment as stressful may interact with the 5-HTTLPR polymorphism, resulting in variations in the stability of fearful temperamental characteristics. In contrast, supportive parenting may be perceived as less stressful, and in combination, supportive parenting can help moderate children's perception of environmental events as a stressor. Without perceiving an event as a stressor, the activity of the 5-HTTLPR polymorphism may be diminished, resulting in reduced stability of fearful temperamental characteristics.

The current study examined the direct and interactive effects of parenting quality, specifically harsh and supportive parenting, and the 5-HTTLPR polymorphism on change in fearful temperamental characteristics during the early childhood period. As will be described, high levels of harsh parenting and low levels of supportive parenting were expected to predict elevated levels and increased stability of fearful temperamental characteristics, but only when children have the short variant of the 5-HTTLPR polymorphism. The following sections will first define temperament and the developmental significance of fearful temperament on the emergence of internalizing problems over time. Next, research suggesting that parenting quality interacts with the 5-HTTLPR polymorphism to affect child adjustment will be discussed. Finally, the proposed hypotheses will be outlined.

Defining fearful temperament during the early childhood developmental period

Common to definitions of temperament is the idea that temperament has a constitutional basis, affects the experience of emotions both positively and negatively, and includes discrete observable behaviors (e.g., approach/withdraw; Buss & Plomin, 1975; Godsmith & Campos, 1990; Rothbart, Ahadi, & Evans, 2000). Despite general commonalities in what constitutes temperament, differences in how specific temperamental characteristics are operationalized exist, particularly with regard to fearful temperament. For instance, behavioral inhibition represents more extreme forms of fearful temperament and includes children's tendency to approach or withdraw from novelty (Chess & Thomas, 1984; Kagan, Renick, & Gibbons, 1989). Behavioral inhibition tends to be measured observationally by rating children's withdrawn, avoidant, and fearful behaviors. In contrast, Rothbart and colleagues (1991) define fearful temperament on multiple dimensions, such as non-social (i.e., fear) and social (i.e., shyness). Rothbart and colleagues (1991) developed parent report questionnaires that measure both general fearfulness expressed across a variety of settings.

How temperament is operationalized has important implications for stability over time. The behavioral inhibition approach tends to categorize children as either inhibited or non-inhibited; that is, only children with extreme scores on observational ratings of both social and non-social fear are categorized as inhibited. That is, children are typically observed in a variety of social (e.g. stranger approach) and non-social (e.g. touching a novel object) novel tasks, and an aggregate of observed inhibition scores is used to identify children who score in the top 10-15% in behavioral inhibition (e.g., Hirshfeld et al., 1992; Biederman et al., 2001). Reznick and colleagues (1989) reported that this extreme group of behaviorally inhibited children tend to remain behaviorally inhibited during the early childhood period.

One challenge with categorical approaches is that the subset of the affected population is rather small. In addition, the stability of categorical approaches, like behavioral inhibition, make it difficult to study mechanisms of change in fearful temperament. Instead, continuous indicators of fearfulness allow for an assessment of within individual growth or attenuation of fearfulness as well as an examination of mechanisms that may affect rates of change in expressed fearfulness. The current study considers the extent to which fearful temperament more broadly is shaped by the quality of parenting during early childhood.

Normative vs. Non-normative Fear responses during Early Childhood

Fearful and wary behaviors first emerge during the infancy period and is normative, expected, and adaptive. Beginning around 6 to7 months of age and coinciding with increases in mobility, infants begin to develop separation anxiety, or become distressed and wary in the presence of strangers, particularly when separated from parents (Waters, Matas, & Sroufe, 1975). Separation anxiety has protective benefits in that infants are less likely to approach strangers and are more likely to maintain contact with parents during periods of exploration (Bretherton & Ainsworth, 1974). In addition, infant bids for parental attention reinforces the attachment bond by reinforcing infants' need for parents and providing opportunities for parents to respond to infants (Ainsworth, 1973). Cognitive advances during the toddler period give rise to specific fears, particularly fear of the unknown (e.g. fear of the dark, monsters; Evans, Gray, & Leckman, 1999) but wariness in the presence of strangers remains. While fear of unfamiliar people and settings typically declines by middle childhood, individuals vary in the intensity and duration of childhood fears (e.g., Brooker et al., 2013).

Early childhood fears that are intense and stable may represent trait-like temperamental characteristics that increase children's risk of developing internalizing disorders over time.

Brooker and colleagues (2013) identified trajectories of stranger fear that emerged from 6 to 30 months of age. In their study, 23.9 percent of the sample evidenced elevated levels of parent reported stranger fear at 6 months of age, with half of these children remaining chronically elevated over the 24 month period. Only those chronically elevated children also were observed to be more inhibited at 36 months suggesting that stably high levels of fear may represent a risk for more intense fearfulness (Brooker, et al., 2013). Similarly, Hirshfeld and colleages (1991) reported that elevated and sustained levels of temperamental inhibition, measured four times from 21 months to 7.5 years of age was associated with significantly higher rates of anxiety disorders at 7.5 years than children that who did not evidence chronically elevated levels of inhibition. Likewise, Prior and colleagues (2000) reported that 42 percent of adolescents rated as shy and withdrawn in 6 of 8 childhood assessments measured from 4 months of age to 13 years of age developed anxiety problems during adolescence as compared to 11 percent of children who were never rated as shy. Taken together, children who demonstrate chronically elevated levels of fearful temperamental characteristics across the early childhood period may be more likely to develop internalizing problems than children who evidence more variability in fearful temperament ratings across early childhood withdrawal or teacher-reported over anxiousness.

In conclusion, some fear and social wariness is normative during early childhood, but elevated and sustained levels of fearfulness expressed even during low-risk contexts appears to place children at risk for internalizing disorders. Since more children demonstrate instability than stability in temperamental fearfulness across early childhood (e.g., Brooker et al., 2013), understanding mechanisms associated with stability and instability in temperamental fearfulness may improve intervention efforts aimed at reducing children's risk for internalizing disorders. In addition to genetics (i.e., 5-HTTLPR polymorphism) parenting may be one mechanism that

moderates the expression and development of fearful temperamental characteristics. The following sections will explore the role of genetics and parenting in the stability of inhibited temperamental characteristics in early childhood.

Quality of parenting as a context for gene expression in temperamental fearfulness

The short allele of the 5-HTTLPR polymorphism has been associated with fear and anxiety traits among preschool aged children (Hayden et al., 2007) and shyness among elementary school aged children (Battaglia et al., 2005). Both fearful temperamental characteristics and the short 5-HTTLPR polymorphism share common neural substrates like stronger and more frequent amygdala activation to fear faces (Blackford, Avery, Cowan, Shelton, & Zald, 2011; Schwartz, Wright, Shin, Kagan, & Rauch, 2003), but the association between 5-HTTLPR polymorphism and fearful temperament is not consistent. For instance, Schmidt and colleagues (2002) reported no relationship between the 5-HTTLPR polymorphism and extreme forms of fearful temperament (i.e., inhibition). The lack of consistency in linking 5-HTTLPR and inhibition likely occurs because the 5-HTTLPR polymorphism is only one of several genes related to temperamental fearfulness and, importantly, the expression of the 5-HTTLPR polymorphism seems to be sensitive to environmental circumstances.

During early childhood, children's social environment is largely created by parents. The quality of parents' social interactions with their children creates contexts that may be psychologically stressful or psychologically supportive. Supportive parenting requires parents to actively modify their behavioral and emotional responses to their children's cues in ways that compliment and challenge their children's reactivity and regulatory skills. In other words, supportive parents tailor their responses to children's distress in ways that facilitate and support emotional and behavioral regulation (Bates, 2012). While specific parenting responses may vary across situations and settings as well as for individual differences in children's characteristics,

supportive parenting generally involves parent behaviors that promote children's autonomy and facilitate children's development of adaptive regulation (Scaramella & Leve, 2004). In contrast, harsh parenting interferes with children's ability to self-regulate because such parenting is psychologically stressful and increases children's emotional arousal to levels that interferes with children's ability to autonomously regulate emotions and behaviors (e.g., Scaramella & Leve, 2004).

Temperamentally fearful children may be affected more heavily by the presence of supportive or harsh parenting than less fearful children. For instance, novel contexts are potentially more stressful for fearful children than less fearful children. Supportive parenting scaffolds new experiences, allowing children to set the pace for entry into novel activities. Such parenting has been linked to reductions in temperamental fearfulness among toddler age of children over-time (Rubin, Burgess, & Hastings, 2003). In contrast, harsh parenting interferes with children's ability to control their entry into a novel situation either by controlling and restricting children's efforts to approach the novel situation (e.g., overprotection) or by pushing or forcing children into a novel situations (e.g., under-protection; McLeod, Wood, & Weisz, 2007). Parents who respond with over-protection reinforce children's belief that the environment is overwhelming, while parents who respond with under-protection may increase children's anxiety by failing to assist their children in managing distress (Hudson & Rapee, 2001).

How parents respond to their children's fearful responses may affect the stability of observed fearfulness because parental responses may interact with children's genetic vulnerabilities. That is, the quality of the parent-child relationship has been found to interact with the 5-HTTLPR polymorphism to predict changes in fearful temperament during early childhood. Although not measuring harsh parenting per se, children carrying at least one short allele of the

5-HT and who were classified as insecurely attached were found to demonstrate increases in fear and negative emotionality from 4 months through 69 months (Pauli-Pott, Friedl, Hinney, & Hebebrand, 2009). Theoretically, insecure attachment occurs as a result of exposure to less responsive and supportive parenting; the poor quality of caregivers' responses may teach children that their caregiver is an inadequate source of support and comfort (Ainsworth, 1973).

In summary, the short variant of the 5-HTTLPR polymorphism is likely associated with variations in level of fearful temperamental characteristics during early childhood. However, risk associated with the 5-HTTLPR polymorphism may be moderated by the parenting environment. The current understanding of how parenting interacts with the 5-HTTLPR polymorphism to predict changes in fearful and inhibited temperament in early childhood relies on measures of attachment as a proxy for parenting behavior. Theoretically, attachment classification is affected by parenting behavior, attachment classification is based on how children respond to parenting behavior and is inherently dyadic. In the current study, parenting behavior, net of children's responses, is observed in an attempt to isolate the parenting environment to which children are exposed.

Hypotheses

The current study will empirically evaluate the following study hypotheses:

1. The homozygous short genotype *(s/s)* of the 5-HTTLPR polymorphism will be associated with elevated and sustained levels of parent-reported fearful temperamental characteristics across the early childhood period (i.e., child age 3, 4, and 5) as compared to the homozygous long genotype of the 5-HTTLPR polymorphism. The role of the heterozygous genotype (*s/l)* will be explored.
2. Observed parenting measured when children were 3 and 4 years of age will moderate the relationship between the homozygous short genotype of the 5-HTTLPR

polymorphism and trajectories of parent reported fearful temperamental characteristics from child age 3 to 5.

a. Supportive parenting will be associated with a decrease in temperamental fearfulness from child age 3 to 5.
b. Harsh parenting will be associated with stability of temperamental fearfulness from child age 3 to 5.

Alternative Competing Models: Diathesis-stress model versus differential susceptibility

The present study considers the moderating effect of the parenting environment on the relationship between the 5-HTTLPR genotype and fearful temperamental characteristics. Genotype x environment interactions can be interpreted from different theoretical frameworks, such as a diathesis-stress approach or a differential susceptibility model. As an alternative explorator hypothesis, the present study will consider if any GxE interactions provide support for diathesis-stress or differential susceptibility.

The diathesis-stress model is an additive model where individuals experience poor physical and mental health outcomes when combined with adversity in the environment (Monroe & Simmons, 1991). In the context of a supportive outcome, genetic risk may not be realized and the risk for maladaption would be reduced. In terms of the present study, the *s/s* 5-HTTLPR genotype is considered a risk factor for fearful temperament, but based on the diathesis-stress model, individual should only be at increased risk if they are also experiencing environmental risk, in this case, more harsh parenting.

In contrast, the differential susceptibility model suggests that vulnerability factors actually reflect a sensitivity to the environment; an adverse environment may confer risk for poor psychological and mental health outcomes, while an enhanced or supportive environment promotes more adaptive outcomes (Belsky, Bakermans-Kraneburg, & van IJzendoorn, 2007). In

other words, a differential susceptibility model indicates that individuals with genetic susceptibility are affected by the environment for better and for worse (Belsky & Pleuss, 2009). In terms of the present study, in order to support the differential susceptibility hypothesis, the *s/s* 5-HTTLPR genotype would be associated with more fearful temperamental characteristics when experiencing more harsh parenting and less fearful temperamental characteristics when experiencing more supportive parenting.

Methods

Participants

Data come from the Family Transitions Project, a prospective, longitudinal study of over 557 families living in the Midwest. In 1989, the Family Transitions Project began with 451 families with two biological parents (first generation, G1), their "target" adolescent enrolled in the seventh grade (second generation, G2), and a sibling within 4 years of the target adolescents. In 1991, an additional 106 mother-headed households who had recently experienced a divorce were added to the sample. These families included the mother, a target adolescent in the ninth grade and a sibling within 4 years of the target adolescent's age. Families were visited in their homes by trained interviewers and completed annual assessments with all family members through the target adolescent's senior year of high school. The last complete family assessment (1994) occurred when the target adolescent was approximately 18 years of age.

After 1994, annual assessments continued, but the focus shifted from the target's relationships with their family of origin to their emerging family of procreation. In keeping with this focus, beginning in 1997 the oldest biological child of the target participant (generation 3; G3) was recruited to participate when the child was 2 years of age. Annual assessments continued with the target, their romantic partner and their biological child. Over time, annual

assessments were replaced with biennial assessments because of funding constraints. All G3 participants completed an age 2 assessment, but after the age 2 assessment follow up interviews occurred on the same biennial schedule as the rest of the sample. As a result, there is a small portion of participants for whom only one or two assessments are available because of the timing of the biennial assessments. The proposed study will focus on data collected from the G2 targets and their partner and their G3 biological children when the G3 children were 3, 4, and 5 years of age. A total of 222 G2-G3 families have participated in at least one assessment when their child was 3, 4, or 5 years of age. Of these 222 families, 99 (44.6%) have data for all 3 age points, 66 (29.7%) have data for only 2 of the 3 assessments, and 57 (25.7%) have only one assessment.

Procedures

Trained interview teams visited G2 homes annually. Families were mailed questionnaires to be completed before the in home assessment. Relevant to the proposed study, target parents' and their partners' reports were used to measure children's temperamental characteristics and overall family demographic characteristics. During the home visits, families participated in a number of structured interactional tasks that were videotaped and later coded by trained coders. Relevant to the proposed study, G2 parent (target and their partner) separately completed a developmentally appropriate puzzle with their G3 child at each of the three assessments. Different puzzles were used for each parent and at each of the assessment waves. Parents were instructed to offer any assistance their G3 child may need to complete the puzzle, but to let the G3 child to solve the puzzle independently. Puzzles were selected to be too difficult for children to complete independently, therefore encouraging parents to assist the child during the interaction.

Between November 2007 and August 2012 participants were sent Oragene O-250 self-collection kit. G2 and G3 participants provided saliva samples using the Oragene kits at home

and mailed samples to the research site. Parents were instructed to spit into the tube. Saliva was collected from the G3 children by swabbing the inside of the children's cheek and inserting the swabs in the kit. Saliva kits were stored in a sub-zero freezer and transported to the University of Colorado, Boulder for assaying.

Measures

Genotyping. Saliva samples were obtained from target participants with Oragene™ (DNA Genotek, Ontario, Canada) collection kits. Genotyping was conducted at the University of Colorado's Institute for Behavioral Genetics. Genomic DNA was isolated with Agencourt DNAdvance™ DNA Isolation Kits (Beckman Coulter, Brea, CA) using a Beckman-Coulter Biomek® FX workstation according to company protocols. The present study focuses on the 5-HTTLPR gene. The genotype is coded into three separate variables with 1 indicating presence of the genotype and 0 indicating the absence. In European decedents, it is expected that approximately half of the sample will have the heterozygous genotype (*s/l)* with heterozygous long (*l/l*) being the next most common with heterozygous short (*s/s*) being the least common (Lesch et al., 1994). Consistent with these expectations, the frequency of the 5-HTTLPR gene can be found in Table 1. The most common genotype is the heterozygous genotype *(s/l)* n=146 (44.9%), followed by homozygous long *(l/l)* n=124(38.20%), followed by homozygous short *(s/s)* n=55 (16.9%).

Table 1

Descriptive Statistics for Children's Fearful Temperament, Children's Genotype, and Parenting

	Range	Min-Max	Mean(SD)	Skew(SE)	Kurtosis(SE)
Age 3 CBQ Fear	1.00-7.00	1.50-6.09	3.67(.83)	-.12(.18)	.06(.35)
Age 3 CBQ Shyness	1.00-7.00	1.00-6.46	3.38(1.11)	.14(.18)	-.18(.35)
Age 3 CBQ Soothability	1.00-7.00	2.36-6.92	4.79(.69)	-.09(.18)	.65(.35)
Age 3 CBQ Inhibitory Control	1.00-7.00	2.67-5.85	4.18(.68)	.10(.18)	-.16(.35)
Age 4 CBQ Fear	1.00-7.00	1.91-5.92	3.93(.81)	-.20(.22)	-.40(.43)
Age 4 CBQ Shyness	1.00-7.00	1.15-6.38	3.50(1.21)	.13(.22)	-.54(.43)
Age 4 CBQ Soothability	1.00-7.00	2.25-6.31	4.73(.64)	-.42(.22)	1.09(.43)
Age 4 Inhibitory Control	1.00-7.00	1.92-6.00	4.37(.74)	-.16(.22)	.50(.43)
Age 5 CBQ Fear	1.00-7.00	1.25-5.80	3.95(.82)	-.27(.22)	.31(.45)
Age 5 CBQ Shyness	1.00-7.00	1.00-6.23	3.21(1.08)	.33(.22)	-.22(.45)
Age 5 CBQ Soothability	1.00-7.00	3.00-6.92	4.76(.73)	.12(.22)	.30(.45)
Age 5 CBQ Inhibitory Control	1.00-7.00	2.50-6.15	4.48(.69)	-.19(.22)	-.22(.45)
Mother Supportive Parenting	1.00-9.00	2.00-7.75	4.98(1.13)	-.11(.17)	-.31(.34)
Mother Harsh Parenting	1.00-9.00	1.00-5.40	1.58(.72)	2.42(.17)	7.89(.34)
Father Supportive Parenting	1.00-9.00	1.38-8.06	4.86(1.17)	.02(.18)	.32(.36)
Father Harsh Parenting	1.00-9.00	1.00-5.40	1.61(.80)	2.33(.18)	6.63(.36)

Fearful Temperament. Fearful temperament was defined as children's level of distress, inability to settle down when distressed, and inhibition in the context of novel or social situations. The Child Behavior Questionnaire (CBQ; Rothart, et al., 2001) was used to measure children's temperament at age 3, 4, and 5. The CBQ measures children's behavioral and emotional reactions to a variety of situations in which parents' likely witness. Parents think about their children's behavior during the past 6 months and rate each statement in terms of how true it was of their children using a 7 point Likert scale (1 = extremely untrue; 7 = extremely true). The 195 items were used to create 12 different subscales reflecting different temperamental dimensions. Only four dimensions related to fearful temperament were used as indicators of fearful temperament. These indicators included: fear, inhibitory control, falling reactivity and soothability and shyness subscales. Each of the four scales contained 12 to 13 items and had adequately published inter-rater reliability (.52-.58) and internal consistency (.70-.92; Putnam, & Rothbart, 2006; Rothbart, et al., 2001). When available, the mothers' and fathers' reports were averaged to create a single score. If only one parent report was available, only that parent report was used.

The *fear* subscale (12 items) measured the intensity of children's negative affect associated with the anticipation of pain, distress, and potentially threatening situations (e.g., is afraid of the dark). In general, parents reported moderate levels of fear for their children (Table 1; age 3, M = 3.67, SD =.83; age 4, M = 3.93, SD = .81; age 5, M = 3.95, SD = .82).

The *shyness* subscale consists of 13 items and evaluates the extent to which children are slow, inhibited, and distressed in social situations (e.g., is sometime shy even around people s/he has known a long time). In general, parents reported moderate levels of shyness for their children (Table 1; age 3, M = 3.38, SD = 1.11; age 4, M = 3.5, SD = 1.21; age 5, M = 3.21, SD = 1.08).

The *Falling Reactivity and Soothability* subscale includes 13 items and measures rate of recovery from peak distress. In general, parents reported moderate high levels of soothability (Table 1; age 3, $M = 4.79$, SD = .69; age 4, $M = 4.73$, SD = .64; age 5, $M = 4.76$, SD=.73).

The *inhibitory control* subscale includes 13 items and measures children's capacity to suppress approach when instructed or when exposed to novel or uncertain situations (e.g., takes a long time approaching new situations). In general, parents reported moderate levels of inhibitory control (Table 1; age 3, $M = 4.18$, SD = .68; age 4, $M = 4.37$, SD = .74; age 5, $M = 4.48$, SD = .69).

The four indicators of fearfulness were correlated (see Table 2). Interestingly, the four indicators were only modestly correlated within and across assessment point. Greater convergence occurred within the temperamental characteristic. For instance, correlations across fearfulness ranged from .50 to .69 across the three points in time, but correlations among the temperamental dimensions ranged from -.33 to .39 within the 3 year old assessment. Given the lack of convergence across indicators of fearful temperament, temperamental constructs were considered separately in tests of study hypotheses.

Table 2

Correlations among Children's Fearful Temperament, Children's Genotype, and Parenting

	1	2	3	4	5	6	7	8	9	10	11	12
1. Age 3 CBQ Fear												
2. Age 3 CBQ Shyness	.26**											
3. Age 3 CBQ Soothability	-.33**	-.10										
4. Age 3 CBQ Inhibitory Control	-.11	.06	.39**									
5. Age 4 CBQ Fear	.69**	.12	-.32**	-.16								
6. Age 4 CBQ Shyness	.20*	.67**	-.12	.05	.17							
7. Age 4 CBQ Soothability	-.32**	-.02	.67**	.40**	-.40**	-.15						
8. Age 4 Inhibitory Control	-.07	.22*	.46**	.71**	-.18*	.05	.55**					
9. Age 5 CBQ Fear	.50**	.12	-.37**	-.03	.54**	.16	-.43**	-.18				
10. Age 5 CBQ Shyness	.11	.68**	-.07	.16	.09	.78**	-.15	.09	.21*			
11. Age 5 CBQ Soothability	-.36**	-.04	.63**	.30**	-.38**	-.04	.69**	.55**	-.39**	-.11		
12. Age 5 CBQ Inhibitory Control	-.06	.11	.38**	.60**	-.17	-.02	.41**	.71**	-.05	.06	.49**	
13. Mother Supportive Parenting	-.23**	-.01	.13	.16*	-.15	.01	.15	.20	.02	.01	.06	.14
14. Mother Harsh Parenting	.07	-.02	-.07	-.16*	.14	-.05	-.13	-.19	.04	-.07	-.06	-.08
15. Father Supportive Parenting	-.14	-.13	.13	.23**	-.03	-.18	.06	.13	.00	-.05	.10	.19
16. Father Harsh Parenting	.07	-.03	-.11	-.25**	.13	-.02	-.13	-.14	-.02	-.02	-.04	-.20*
17. 5-HTTLPR *s/s*	-.13	-.16*	.19*	-.02	-.14	-.03	.10	.01	-.08	-.09	-.04	-.17
18. 5-HTTLPR *s/l*	.18*	.05	-.05	-.02	.19*	-.07	-.11	-.08	.05	.07	-.15	.01
19. 5-HTTLPR *l/l*	-.07	-.11	-.11	.04	-.08	.09	.03	.08	.01	-.01	.19*	.13

Note: p<.05 *; p<.01**; p<.001***

Table 2 Continued

	13	14	15	16	17	18
1. Age 3 CBQ Fear						
2. Age 3 CBQ Shyness						
3. Age 3 CBQ Soothability						
4. Age 3 CBQ Inhibitory Control						
5. Age 4 CBQ Fear						
6. Age 4 CBQ Shyness						
7. Age 4 CBQ Soothability						
8. Age 4 Inhibitory Control						
9. Age 5 CBQ Fear						
10. Age 5 CBQ Shyness						
11. Age 5 CBQ Soothability						
12. Age 5 CBQ Inhibitory Control						
13. Mother Supportive Parenting						
14. Mother Harsh Parenting	-.37**					
15. Father Supportive Parenting	.45**	-.21**				
16. Father Harsh Parenting	-.37**	.36**	-.36**			
17. 5-HTTLPR *s/s*	-.03	.16*	-.18*	.10		
18. 5-HTTLPR *s/l*	.03	-.02	.15	-.05	-.41**	
19. 5-HTTLPR *l/l*	-.01	-.11	.01	-.04	-.35**	-.71

Note: p<.05 *; p<.01**; p<.001***

Supportive and Harsh Parenting. Supportive parenting was defined as parenting which is positive and child-centered, specifically, parenting that is focused and responsive to the children's needs and interests and also warm and supportive of the children. In contrast, harsh parenting was defined as parenting that is parent-focused, which ignores children's need for autonomy and instead focuses on controlling the completion of the task in an emotionally harsh or negative way. Both parenting dimensions are measured from trained observational coders ratings of each parents' behavior directed towards the G3 child during the 5 minute interaction task at each of the assessment waves. Importantly, separate coders rated mothers' and fathers' parenting behaviors. Each behavioral code was rated on a 9-point Likert scale (1 = not at all characteristic; 9 = mainly characteristic) which assessed how well each behavioral code represented or characterized the parents' actions during the teaching task. Separate clusters of codes were used to measure supportive and harsh parenting.

Supportive parenting was measured with 8 global scales that measure parents' level of sensitivity/child centeredness, warmth, and communication/responsiveness behaviors observed during the puzzle task. *Sensitivity/child centeredness* ratings measured how well parents' interactions show an awareness of the children's needs, moods, interests, and capabilities. Supportive/child centered parenting includes parental responses to their children that are well timed and paced with their children's behavior and mood. *Warmth/support* ratings measure the frequency and intensity of parents' nonverbal warmth (e.g., physical affection, smiling) and verbal behavior (e.g., expressed liking, appreciation, praise, care, concern, or support for the child). *Escalate warmth* measures the extent to which parents followed one indicator of warmth/support with another indicator of warmth/support. *Reciprocate warmth/support* measured parents' responded to children's warmth/support with warmth/support. *Prosocial* measured the frequency and intensity of parents' cooperation, sensitivity, helpfulness, and

willingness to comply with the needs and wishes of others (e.g., allowing the child to decide which piece to try next). *Assertiveness* indicates parental confidence and forthrightness while expressing encouragement and patience with the responses of the other (e.g., first, let's find all of the straight edged pieces). *Listener responsiveness* indicates the level and intensity of parents' ability to acknowledge and validate their children's verbalizations (e.g., I like your idea). *Communication* reflects parents' ability to verbally explain the rules or task requirements as well as their own needs/wants in a neutral or positive manner (e.g., we should put together the outside of the puzzle first because that is easier).

At each of the three time points, scores on the 8 subscales were averaged separately for each parent. Scores indicated that both mothers and fathers displayed moderate levels of supportive parenting (see Table 1; mothers, $M = 4.98$, SD = 1.13; fathers, $M = 4.86$, SD = 1.17). Supportive parenting scores were correlated across parents (see Table 2). Although statistically significant, mothers' and fathers' supportive parenting were only modestly correlated (e.g., supportive parenting: $r = .45$). Consequently, the impact of mothers' and fathers' supportive parenting on change in fearful temperament were evaluated separately.

Harsh parenting was created by averaging 6 codes measuring the extent to which parents' were observed to be overly controlling and harsh during their interactions with their children. *Parent harshness* measures the extent to which parents' evidence excessive control or inflexibility in completing the task. In other words, the code evaluates the extent to which parents appear to be overly concerned with completing the task (i.e. puzzle) without allowing their children the ability to explore the puzzle at their own pace. *Parent hostility* measures verbal and non-verbal indicators of harsh, angry, critical, disapproving, and rejecting behavior or statements directed towards their children during the task. *Escalate harsh* rates the extent to which parents' follow a harsh behavior with more hostility (e.g., gets on a hostility roll).

Reciprocate harsh rates the frequency and intensity of parents' harsh responses to children's negative affect. *Angry coercion* measures parents' use of hostility to achieve goals or attempts to control or change the behavior of the child (e.g., threatening harsh punishment for misbehavior). *Antisocial* measures parents' level of resistance, defiance, or inconsiderate actions as well as general uncooperative and unsociable behavior (e.g., Fine! I'm not going to play with you anymore).

At each of the three time points, scores on the 6 subscales were averaged separately for each parent. Scores indicated that both mothers and fathers displayed low levels of harsh parenting (see Table 1; mothers, $M = 1.58$, SD = .72; fathers, $M = 1.61$, SD = .80). Supportive parenting scores were correlated across parents (see Table 2). Although statistically significant, mothers' and fathers' harsh parenting were only modestly correlated (e.g., supportive parenting: $r = .36$). Consequently, the impact of mothers' and fathers' harsh parenting on change in fearful temperament were evaluated separately.

Data Analytic Plan. Before testing study hypotheses preliminary analyses will be computed in which all study constructs will be checked for violations to normality assumptions. Next, correlations across study constructs will be evaluated for consistency with expectations. Finally, hierarchical linear modeling (HLM) will be used to test study hypotheses. First, fearful and inhibited temperament is the outcome variable and age will be entered as a level 1 predictor. To test the first hypothesis, two of the 5-HTTLPR variables *(s/s & s/l)* will be entered as a level 2 indicator to examine if trajectories of fearful and inhibited temperament vary as a function of the 5-HTTLPR genotype. To test the second hypothesis, each of the parenting indicators will be added to the model independently along with the interaction term of 5-HTTLPR and parenting. Interaction terms were created by centering the independent variables and multiplying them together. These models were estimated separately for mothers and fathers. Any statistically

significant interaction of 5-HTTLPR genotype, parenting, and age will be decomposed.

Results

First, study constructs were evaluated to ensure that all constructs met normality assumptions. Harsh parenting scores were positively skewed, indicating that most parents showed very low levels of harsh parenting with few showing high levels on harsh parenting. The harsh parenting score was not transformed because a positively skewed distribution represents the typical distribution of harshness in a community sample.

Correlation Analyses. Next, bivariate correlations were computed among study constructs to evaluate the pattern of associations (see Table 2). The 5-HTTLPR homozygous short genotype *(s/s)* was negatively correlated with shyness at age 3 (r = -.16; p < .05) and positively correlated with soothability (r = .19; p < .05) at age 3, suggesting that 3-year old children with the *(s/s)* genotype were less fearful and calmed more quickly after becoming distressed. The heterozygous genotype *(s/l)* was positively associated with temperamental fear at age 4 (r = .19; p < .05), indicating that 4-year old children with the *(s/l)* genotype were more likely to be rated as fearful at age 4. Finally, the homozygous long genotype *(l/l)* was positively correlated with soothability at age 5 (r = .19; p < .05), indicating that 5-year old children with the (*l/l)* genotype were able to settle more quickly after becoming distressed. There were no other significant correlations between the 5-HTTLPR polymorphisms and fearful and inhibited temperamental characteristics.

Relatively few statistically significant associations emerged for parenting and indicators of fearful temperament. With regard to supportive parenting, mothers supportive parenting was negatively associated with fearful temperament at age 3 (r = -.23; p < .001) and positively associated with inhibitory control at age 3 (r = .16; p < .05), suggesting that more sensitive maternal parenting was associated with less fearfulness and more inhibitory control at age 3.

Fathers supportive parenting was only positively correlated with inhibitory control at age 3 (r = .23; $p < .001$), indicating that more supportive parenting from fathers was associated with more inhibitory control at age 3. Considering harsh parenting, both mothers' and fathers' harsh parenting was associated with less inhibitory control at age 3 (mother, r = -.16; $p < .05$; father; r = -.25, $p < .001$). At age 5, only fathers harsh parenting was negatively correlated with inhibitory control (r = -.20; $p < .05$). There were no other significant correlations between the parenting constructs and temperamental fear and inhibition.

Only two statistical association emerged between genotype and parenting. Specifically, the *(s/s)* genotype was positively associated with mothers harsh parenting (r = .16; $p < .05$) and negatively associated with fathers supportive parenting (r = -.18; $p < .05$). Children with the *(s/s)* risk allele received more harsh parenting from their mothers and less supportive parenting from their fathers. The following sections summarizes the results of the hypothesis testing.

Hypothesis 1: The homozygous short 5-HTTLPR genotype will be associated with higher trajectories of fearfulness. HLM was used to evaluate the relationship of the 5-HTTLPR genotype each indicator of fearful and inhibited temperamental characteristics. The first set of analyses estimated the direct effect of the 5-HTTLPR genotype on the initial intercept and change in dimensions of fearful temperament (i.e., fear, shyness, inhibitory control, and soothability). Four separate models were estimated, one for each dimension of fear. In each model, the specific indicator of fearful temperament was entered as the dependent variable with age as a level 1 indicator. The 5-HTTLPR polymorphism was entered as the level 2 indicator to estimate the direct effect of the 5-HTTLPR polymorphism on the slope and intercept of the indicator of fearful temperamental characteristic. Results are summarized in Table 3.

Table 3

Effect of the 5-HTTLPR Genotype on Fearful Temperamental Characteristics from 3 to 5 Years Old

	b (SE)	*t-value*
Fear		
Intercept	3.59(.09)***	38.53
5-HTTLPR Intercept *(s/s)*	-.13(.19)	-.71
5-HTTLPR Intercept *(s/l)*	.26(.14)	1.85
Slope Intercept	.14(.05)**	2.89
5-HTTLPR Slope *(s/s)*	.08(.10)	.77
5-HTTLPR Slope *(s/l)*	-.10(.08)	-1.22
Shyness		
Intercept	3.55(.13)***	27.80
5-HTTLPR Intercept *(s/s)*	-.41(.25)	-1.66
5-HTTLPR Intercept *(s/l)*	-.07(.18)	-.40
Slope Intercept	-.08(.07)	-1.12
5-HTTLPR Slope *(s/s)*	.18(.09)	1.57
5-HTTLPR Slope *(s/l)*	-.02(.09)	-.19
Soothability		
Intercept	4.71(.09)***	51.38
5-HTTLPR Intercept *(s/s)*	.36(.14)*	2.50
5-HTTLPR Intercept *(s/l)*	.06(.11)	.55
Slope Intercept	.12(.05)	2.54
5-HTTLPR Slope *(s/s)*	-.29(.07)***	-3.99
5-HTTLPR Slope *(s/l)*	-.17(.06)**	-2.84
Inhibitory Control		
Intercept	4.20(.08)***	50.29
5-HTTLPR Intercept *(s/s)*	-.03(.16)	-.20
5-HTTLPR Intercept *(s/l)*	-.06(.11)	-.52
Slope Intercept	.21(.04)***	5.06
5-HTTLPR Slope *(s/s)*	-.17(.07)*	-2.39
5-HTTLPR Slope *(s/l)*	-.01(.06)	-.11

Fear. The first model estimated considered the impact of the 5-HTTLPR genotype on the intercept and slope of the fear subscale (see Table 3). First, both the intercept and slope of fear was statistically significant. That is, the initial level of fear was statistically and significantly different from 0 and children varied in their rate of change over time. The slope of fear was positive, indicating that fear scores increased over time. Second, in contrast to expectations, the *s/s* genotype was not statistically and significantly associated with either the intercept or slope of fear. While no predictions were made with regard to the *s/l* genotype, this genotype also was not statistically significantly associated with the intercept or slope of fear.

Shyness. With regard to temperamental shyness, only the shyness intercept was statistically significant, indicating that initial level of shyness was different from 0. The intercept of the slope was not statistically significant, suggesting that there was not a significant amount of change in average shyness from age 3 to age 5. Contrary to expectations, the *s/s* genotype was not significantly associated with the intercept or slope of shyness. Like fear, the *s/l* genotype also was not statistically significantly associated with the intercept or slope of shyness.

Soothability. Considering soothability, only the intercept was statistically significant, but no statistically significant change in the slope of soothability from age 3 to age 5 emerged. Interestingly, children with the *s/s* genotype had a higher initial levels of soothability (b = .36; SE = .14, $p < .05$, see Table 3), indicating that parents rated children with the *s/s* genotype as more soothable at age 3 as compared to children with the *s/l* or *l/l* genotypes. The 5-HTTLPR genotype also significantly affected the trajectory of soothability. For both the *s/s* and *s/l* genotypes as compared to the *l/l* genotype, soothability decreased from age 3 to age 5 (*s/s*; b = -.29, SE = -3.99, $p < .05$; *s/l*; b = -.17, SE = -2.84, $p < .05$; see Table 3). In other words, children with the *s/s* and *s/l* genotypes became less soothable from age 3 to age 5.

Inhibitory control. The slope and the slope intercept of inhibitory control were both

statistically significant, indicating that initial levels of inhibitory control and the average rate of change in inhibitory control was significantly different from 0 from age 3 to age 5. Next, the 5-HTTLPR genotype was not associated with initial levels of inhibitory control at age 3. Instead, the *s/s* genotype did significantly impact the slope of inhibitory control (b = -.17; SE = -2.39; $p <$ .05, see Table 3). Although levels of inhibitory control did not vary across those with the *s/s* versus *s/l* and *l/l* genotype, the rate of change over time for children with the *s/*s genotype is significantly different than the *s/l* and *l/l*. Parents' ratings of inhibitory control for children with the *s/s* genotype decreased from age 3 to age 5 but not for children with the *s/l* genotype as compared to children with the *l/l genotype*.

Hypothesis 2: Observed parenting at child age 3 to 4 years of age moderates the relationship between the *s/s* genotype of the 5-HTTLRP polymorphism and trajectories of parent reported fearful temperamental characteristics from child age 3 to 5.

To evaluate this next hypothesis, separate HLM equations were estimated for sensitive (see Table 4) and harsh (see Table 5) parenting and for mothers and fathers for each of the temperamental characteristics considered. For each indicator of temperament, results will first be discussed for supportive parenting, mothers' supportiveness followed by fathers' supportiveness, and then for harsh parenting, mothers' harshness followed by fathers' harshness.

Fear: Mothers' supportive parenting. First, considering mothers' supportive parenting (see Table 4). Mothers' supportive parenting did not have a direct effect on the intercept (b = -.14, SE = .09, $p >$ .05) or slope (b = .09, SE = .05, $p >$ .05) of fear. In addition, mothers' supportive parenting did not moderate the relationship between the 5-HTLPR genotype and fear intercept (*s/s*, b = -.21, SE = .18, $p >$.05; *s/l*, b = .23, SE = .18, $p >$ .05) or slope (s/s, b = .03, SE = .10, p > .05; s/l, b = -.08, SE = .08, p > .05).

Table 4

Interaction of Observed Supportive Parenting and the 5-HTTLPR Polymorphism on Trajectories of Temperamental Characteristics from Child age 3 to 5

	Mothers		Fathers	
Fear	*b* (SE)	*t*-value	*b* (SE)	*t*-value
Intercept	3.63(.09)***	38.62	3.70(.06)***	57.79
Parenting Intercept	-.14(.09)	-1.66	-.11(.08)	-1.45
5-HTT Intercept s/s	-.21(.18)	-1.17	-.22(.18)	-1.25
5-HTT Intercept s/l	.23(.14)	1.61	.22(.14)	1.53
Parenting x 5-HTT Intercept s/s	-.20(.18)	-1.12	.12(.19)	.63
Parenting x 5-HTT Intercept s/l	-.03(.13)	-.25	-.10(.13)	-.81
Slope Intercept	.13(.05)*	2.52	.11(.04)*	2.59
Parenting Slope	.09(.05)	1.89	.03(.04)	.80
5-HTT Slope s/s	.11(.11)	1.06	.14(.12)	1.13
5-HTT Slope s/l	-.10(.08)	-1.24	-.10(.08)	-1.24
Parenting x 5-HTT Slope s/s	.03(.10)	.29	-.01(.09)	-.05
Parenting x 5-HTT Slope s/l	-.08(.08)	-1.03	.05(.07)	.70
Estimation of Variance Intercept	□□(114)=417.31***		□□(105)=347.04***	
Estimation of Variance Slope	□□(114)=179.23***		□□(105)=153.37**	
Shyness	*b* (SE)	*t*-value	*b* (SE)	*t*-value
Intercept	3.52(.13)***	26.90	3.46(.08)***	40.76
Parenting Intercept	.10(.10)	1.00	-.14(.11)	-1.31
5-HTT Intercept s/s	-.47(.25)	-1.89	-.48(.27)	-1.79
5-HTT Intercept s/l	-.04(.19)	-.19	.03(.18)	.15
Parenting x 5-HTT Intercept s/s	-.15(.21)	-.72	.02(.29)	.08
Parenting x 5-HTT Intercept s/l	-.07(.15)	-.50	.02(.17)	.10
Slope Intercept	-.09(.08)	-1.16	-.05(.04)	-1.23
Parenting Slope	-.08(.07)	-1.27	-.06(.05)	-1.20
5-HTT Slope s/s	.23(.12)	1.94	.25(.12)*	2.02
5-HTT Slope s/l	.01(.10)	.11	-.01(.10)	-.08
Parenting x 5-HTT Slope s/s	.13(.13)	1.03	.12(.11)	1.11
Parenting x 5-HTT Slope s/l	.12(.09)	1.39	.17(.08)*	2.06
Estimation of Variance Intercept	□□(114)=380.37***		□□(105)=293.41*	
Estimation of Variance Slope	□□(114)=113.52		□□(105)=102.07	

p<.05 *; p<.01**; p<.001***

Table 4 continued

	Mothers		Fathers	
Soothability	*b* (SE)	*t*-value	*b* (SE)	*t*-value
Intercept	4.67(.09)***	50.96	4.84(.05)***	95.22
Parenting Intercept	.16(.08)*	2.03	.11(.07)	1.62
5-HTT Intercept s/s	.40(.15)**	2.73	.32(.15)	2.16
5-HTT Intercept s/l	.09(.11)	.81	-.01(.11)	-.10
Parenting x 5-HTT Intercept s/s	-.10(.16)	-.67	-.13(.13)	-1.02
Parenting x 5-HTT Intercept s/l	-.16(.10)	-1.70	-.04(.09)	-.49
Slope Intercept	.13(.05)**	2.67	-.01(.03)	-.50
Parenting Slope	.01(.04)	.33	.04(.04)	.97
5-HTT Slope s/s	-.33(.07)***	-4.31	-.32(.08)***	-4.23
5-HTT Slope s/l	-.21(.06)**	-3.29	-.19(.06)**	-2.92
Parenting x 5-HTT Slope s/s	-.07(.07)	-.99	-.06(.07)	-.83
Parenting x 5-HTT Slope s/l	-.06(.05)	-1.02	-.07(.06)	-1.13
Estimation of Variance Intercept	□□(114)=371.45***		□□(105)= 338.11***	
Estimation of Variance Slope	□□(114)=134.07		□□(105)=125.49	
Inhibitory Control	*b* (SE)	*t*-value	*b* (SE)	*t*-value
Intercept	4.18(.08)***	51.84	4.20(.06)***	74.43
Parenting Intercept	.17(.08)*	2.22	.15(.08)*	2.00
5-HTT Intercept s/s	-.04(.16)	-.22	-.02(.17)	-.10
5-HTT Intercept s/l	-.07(.11)	-.62	-.15(.12)	-1.26
Parenting x 5-HTT Intercept s/s	-.21(.14)	-1.50	-.15(.13)	-1.16
Parenting x 5-HTT Intercept s/l	-.11(.11)	-1.02	-.02(.10)	-.16
Slope Intercept	.22(.04)***	5.01	.17(.03)***	5.78
Parenting Slope	.04(.04)	1.03	.02(.03)	.64
5-HTT Slope s/s	-.16(.07)*	-2.22	-.18(.08)*	-2.29
5-HTT Slope s/l	.01(.07)	.11	-.02(.07)	-.25
Parenting x 5-HTT Slope s/s	-.10(.08)	-1.34	.02(.07)	.25
Parenting x 5-HTT Slope s/l	.02(.06)	.29	-.01(.05)	-.07
Estimation of Variance Intercept	□□(114)=427.00***		□□(105)=438.49***	
Estimation of Variance Slope	□□(114)=150.86**		□□(105)=158.26***	

p<.05 *; p<.01**; p<.001***

Table 5

Interaction of Observed Harsh Parenting and the 5-HTTLPR Polymorphism on Trajectories of Temperamental Characteristics from Child age 3 to 5

	Mothers		Fathers	
Fear	*b* (SE)	*t*-value	*b* (SE)	*t*-value
Intercept	3.64(.09)***	39.88	3.69(.07)***	55.70
Parenting Intercept	.15(.11)	1.38	.26(.11)*	2.32
5-HTT Intercept s/s	-.20(.18)	-1.11	-.23(.19)	-1.23
5-HTT Intercept s/l	.21(.14)	1.49	.22(.14)	1.52
Parenting x 5-HTT Intercept s/s	-.28(.33)	-.86	-.20(.21)	-.93
Parenting x 5-HTT Intercept s/l	.18(.16)	1.15	.02(.17)	.14
Slope Intercept	.11(.04)*	2.34	.11(.04)**	2.84
Parenting Slope	-.13(.08)	-1.66	-.16(.05)***	-3.27
5-HTT Slope s/s	.11(.09)	1.25	.14(.11)	1.19
5-HTT Slope s/l	-.06(.08)	-.86	-.11(.08)	-1.37
Parenting x 5-HTT Slope s/s	.25(.17)	1.47	.09(.08)	1.20
Parenting x 5-HTT Slope s/l	.12(.12)	1.03	.12(.09)	1.33
Estimation of Variance Intercept	□□(114)=402.49***		□□(105)=333.35***	
Estimation of Variance Slope	□□(114)=178.86***		□□(105)=148.92**	
Shyness	*b* (SE)	*t*-value	*b* (SE)	*t*-value
Intercept	3.53(.13)***	26.61	3.47(.08)***	40.98
Parenting Intercept	.09(.19)	.48	-.09(.16)	-.55
5-HTT Intercept s/s	-.48(.26)	-1.86	-.44(.26)	-1.71
5-HTT Intercept s/l	-.05(.19)	-.27	.01(.18)	.07
Parenting x 5-HTT Intercept s/s	-.11(.47)	-.24	.05(.34)	.14
Parenting x 5-HTT Intercept s/l	-.14(.29)	-.50	.19(.25)	.75
Slope Intercept	-.08(.08)	-1.02	-.05(.04)	-1.19
Parenting Slope	-.01(.13)	-.05	.04(.08)	.56
5-HTT Slope s/s	.23(.12)	1.88	.21(.12)	1.72
5-HTT Slope s/l	-.01(.10)	-.11	-.02(.10)	-.24
Parenting x 5-HTT Slope s/s	-.06(.17)	-.33	-.04(.11)	-.33
Parenting x 5-HTT Slope s/l	-.02(.15)	-.14	-.10(.10)	-1.02
Estimation of Variance Intercept	□□(114)=379.24***		□□(105)=295.60*	
Estimation of Variance Slope	□□(114)=114.37		□□(105)=104.11	

Table 5 Continued

	Mothers		Fathers	
Soothability	*b* (SE)	*t*-value	*b* (SE)	*t*-value
Intercept	4.67(.10)***	48.06	4.83(.05)***	98.83
Parenting Intercept	-.03(.12)	-.23	-.24(.12)*	-2.04
5-HTT Intercept s/s	.38(.16)*	2.38	.33(.14)*	2.34
5-HTT Intercept s/l	.09(.12)	.80	-.03(.11)	-.25
Parenting x 5-HTT Intercept s/s	.14(.18)	.79	.26(.15)	1.76
Parenting x 5-HTT Intercept s/l	-.25(.14)	-1.72	.02(.15)	.13
Slope Intercept	.13(.05)*	2.61	-.02(.03)	-.63
Parenting Slope	.06(.14)	.43	.13(.05)**	2.71
5-HTT Slope s/s	-.32(.07)***	-4.40	-.26(.07)***	-.3.61
5-HTT Slope s/l	-.20(.06)**	-3.15	-.16(.06)*	-2.57
Parenting x 5-HTT Slope s/s	.14(.18)	-.44	-.20(.06)***	-3.52
Parenting x 5-HTT Slope s/l	-.02(.14)	.12	-.12(.07)	-1.76
Estimation of Variance Intercept	□□(114)=358.80***		□□(105)= 323.23***	
Estimation of Variance Slope	□□(114)=132.58		□□(105)=119.17	
Inhibitory Control	*b* (SE)	*t*-value	*b* (SE)	*t*-value
Intercept	4.14(.08)***	52.60	4.20(.05)***	79.84
Parenting Intercept	-.32(.12)*	-2.56	-22(.11)*	-1.99
5-HTT Intercept s/s	-.04(.17)	-.23	-.01(.16)	-.06
5-HTT Intercept s/l	-.03(.11)	.24	-.16(.11)	-1.43
Parenting x 5-HTT Intercept s/s	.56(.20)**	2.77	.17(.15)	1.09
Parenting x 5-HTT Intercept s/l	.11(.16)	.65	-.11(.15)	-.74
Slope Intercept	.21(.04)***	4.85	.17(.03)***	5.45
Parenting Slope	.08(.08)	1.02	.01(.04)	.27
5-HTT Slope s/s	-.17(.08)*	-2.12	-.19(.07)*	-2.55
5-HTT Slope s/l	-.01(.07)	-.04	-.01(.07)	-.08
Parenting x 5-HTT Slope s/s	-.06(.10)	-.54	.02(.07)	.28
Parenting x 5-HTT Slope s/l	-.09(.11)	-.84	-.01(.09)	-.06
Estimation of Variance Intercept	□□(114)=416.11***		□□(105)=422.99***	
Estimation of Variance Slope	□□(114)=153.56**		□□(105)=158.61***	

p<.05 *; p<.01**; p<.001***

Fear: Fathers' supportive parenting. Next, the direct and moderating effect of fathers' parenting on change in fear was examined (see Table 4). Like mothers' parenting, there was no main effect of fathers' supportive parenting on the intercept (b = -.11, SE = .08, p > .05) or the slope (b = -.11, SE = .08, p > .05). Fathers' parenting also did not moderate the relationship between the 5-HTTPR genotype and the intercept of fear (*s/s,* b = -.12, SE = .19, p >.05; *s/l,* b = -.10, SE = .13, p > .05) or the slope of fear *s/s, (*b = -.01, SE = .09, p >.05; *s/l,* b = .05, SE = .07, p > .05).

Fear: Mothers' harsh parenting. The main effect of mothers' harsh parenting was not associated with the initial levels or change in children's fear (see Table 5). Contrary to expectations, the mother harsh parenting x 5-HTTLPR genotypes (s/s or s/l) did not predict initial levels or rates of change in children's fear.

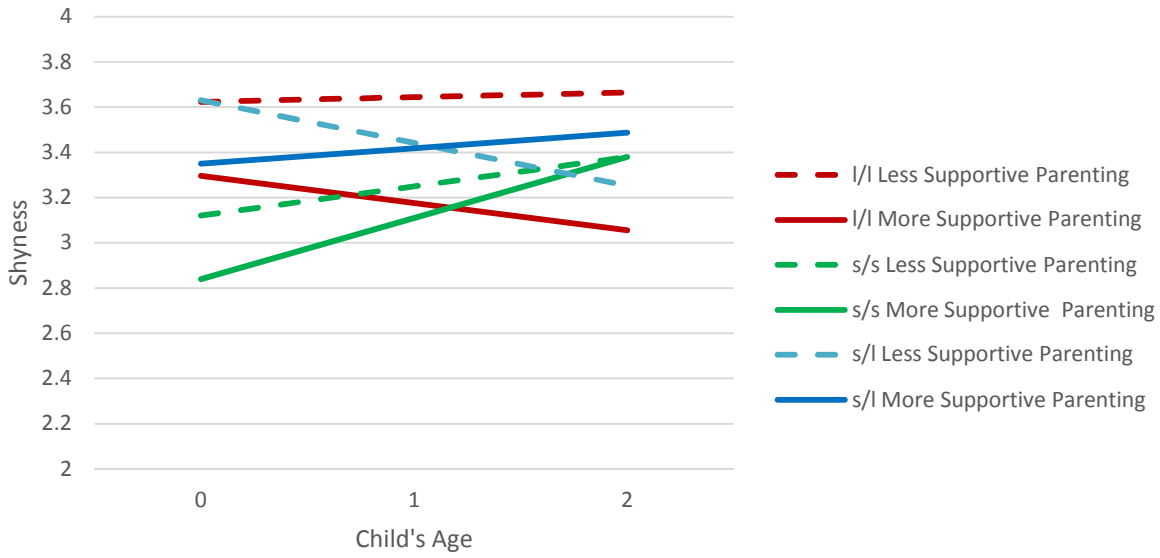
Fear: Fathers' harsh parenting. However, fathers' harsh parenting was associated with children's initial level of fear (see Table 5; b = .26, SE = .07, p < .05) as well as the rate of change in fear (b = -.16, SE = .05, p < .05). Regarding the initial level of harsh parenting, more harsh parenting from fathers was associated with higher initial levels of fearfulness at age 3. In contrast, less harsh parenting from fathers was associated with an increase in child fear ratings from age 3 to 5. Fathers' harsh parenting did not moderate the association between genotype and child fear.

Shyness: Mothers' supportive parenting. First, considering mothers' supportive parenting (see Table 4) mothers' supportive parenting did not directly predict the intercept of slope of children's shyness. Contrary to expectations, mothers' supportive parenting did not moderate the associations between the 5-HTTLPR genotype and children's shyness.

Shyness: Fathers' supportive parenting. While no main effect of fathers' supportive parenting on the intercept or trajectory of shyness emerged, the beta coefficient associated with

the fathers' supportive parenting and the *s/l* 5-HTTLPR genotype on the slope of shyness was statistically significant (b = .17, SE = .08, $p < .05$; see Table 4). In addition, after adding fathers' supportive parenting to the model, the main effect of the *s/s* genotype on the slope of shyness became significant (b = .25, SE = .12, $p < .05$). In other words, after controlling for the co-variance between fathers' supportive parenting and shyness, the 5-HTTLPR *s/s* genotype directly predicted the slope of shyness. Children with the *s/s* genotype became shyer over time. Figure 1 provides a decomposition of the statistical interaction between the 5-HTTLPR genotype and fathers' supportive parenting on shyness. More supportive parenting from fathers' was associated with increasing shyness for children with the *s/l* genotype. In contrast, less supportive parenting from fathers' was associated with decreasing shyness for children with the *s/l* genotype.

Figure 1. Visual depiction of the interaction of fathers' supportive parenting and children's 5-HTTLPR genotype on trajectories of shyness from age 3 to age 5 at 1 SD above and below the mean on supportive parenting



Note: Age was coded age 3=0, age 4=1, age 5=2

Shyness: Mothers' harsh parenting. For mothers' harsh parenting (see Table 5), mothers' harsh parenting did not directly predict the intercept or the slope of children's shyness. Similarly, and contrary to expectations, mothers' harsh parenting did not interactively, combined with 5-HTTLPR genotype, predict either the initial level or rates of change in children's shyness.

Shyness: Fathers' harsh parenting. Similarly, the beta coefficients associated with fathers' harsh parenting or fathers' harsh parenting x genotype did not account for statistically significant portions of the variance associated with the initial levels or rates of change in shyness (see Table 5).

Soothability: Mothers' supportive parenting. First, the beta coefficient associated with mothers' parenting accounted for statistically significant portions of the variance associated with the initial level of soothability ($b = .16$, SE = .08, $p < .01$; see Table 4) but not the slope, or change in soothability over time. While more supportive parenting from mothers' was associated with higher levels of soothability at age 3, mothers' supportive parenting did not impact change in soothability from age 3 to age 5. Mothers' supportive parenting did not interact with children's genotype to predict initial levels or change in soothability.

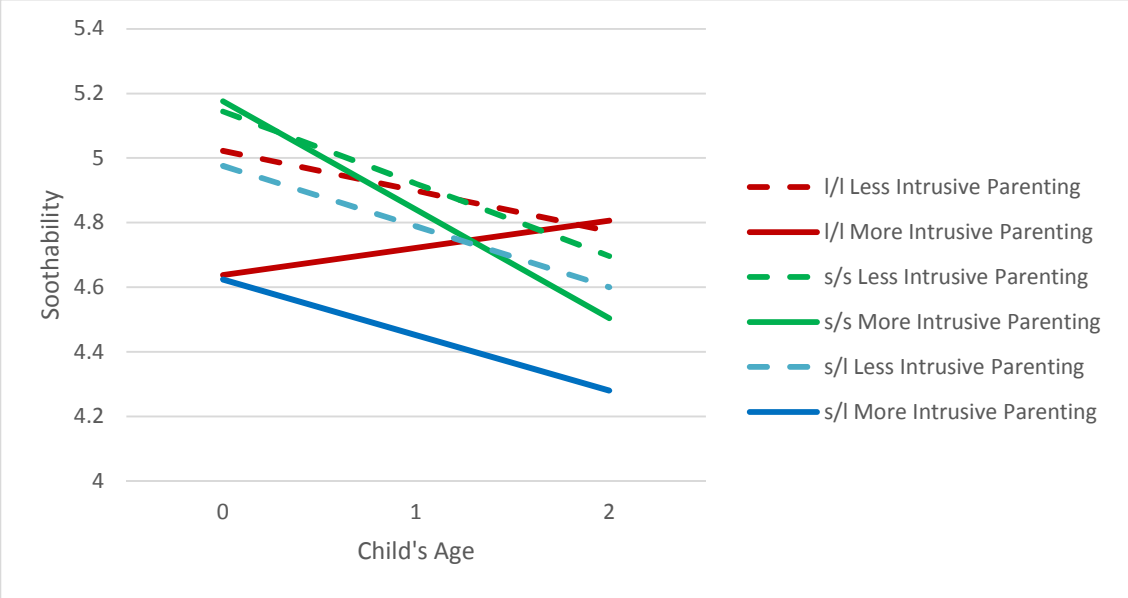
Soothability: Fathers' supportive parenting. In contrast to mothers, the beta coefficient associated with fathers' supportive parenting and the intercept and change of children's soothability was not significant. Similarly, the interaction between fathers' supportive parenting and the 5-HTTLPR genotype did not explain statistically significant portions of the variance associated with either the initial level or rate of change in soothability (see Table 4).

Soothability: Mothers' harsh parenting. Next, the impact of mothers' harsh parenting on the initial levels and rates of change in soothability was considered. In contrast to supportive parenting, the beta coefficient associated with mothers' harsh parenting did not account for a statistically significant portion of the variance associated with the intercept or rate of change in

children's soothability. Additionally, mothers' harsh parenting x 5-HTTLPR genotype did not account for statistically significant portions of the variance associated with children's initial level or rate of change in soothability (see Table 5).

Soothability: Fathers' harsh parenting. Far different results emerged for fathers' harsh parenting. First, considering the intercept of soothability, the beta coefficient associated with fathers' harsh parenting was statistically significant (b = -.24, SE = .12, $p < .05$; see Table 5), indicating that more harsh parenting from fathers' was associated with less soothability at age 3. The harsh parenting x genotype interaction terms did not explain statistically significant portions of the variance associated with the initial level of soothability. Next, considering the slope of soothability, the beta coefficient associated with fathers' harsh parenting was not statistically significant when predicting the slope of soothability. Instead, fathers' harsh parenting interacted with the *s/s* genotype to predict the slope of soothability (b = -.20, SE = .06, $p < .05$). Figure 2 presents a graphical representation of the decomposition of the statistical interaction between the *s/s* genotype and harsh parenting. Children with the *s/s* genotype who experience more harsh parenting became less soothable from age 3 to age 5. In contrast, children with the *l/l* genotype who experienced more harsh parenting became more soothable over time.

Figure 2. Visual depiction of the interaction of fathers' harsh parenting and children's 5-HTTLPR genotype on trajectories of soothability from age 3 to age 5 at 1 SD above and below the mean on harsh parenting



Note: Age was coded age 3 = 0, age 4 = 1, age 5 = 2

Inhibitory Control: Mothers' supportive parenting. First, the beta coefficient associated with mother's supportive parenting did statistically and significantly predict initial levels of inhibitory control (b = .17, SE = .08, p < .05), indicating that more supportive parenting from mothers' was associated with more inhibitory control at age 3. The beta coefficient of mothers' supportive parenting on the slope of inhibitory control was not statistically significant. In addition, mother's supportive parenting did statistically interact with children's genotype to predict either the initial level or the slope of inhibitory control.

Inhibitory Control: Fathers' supportive parenting. Next, the beta coefficient associated with fathers' supportive parenting was statistically and significantly associated with initial levels of inhibitory control (b = .15, SE = .08, p < .05; see Table 4), but not with the rate of change in inhibitory control over time. More supportive parenting from fathers' was associated with more inhibitory control at age 3. In addition, the beta coefficients associated with the fathers' supportive parenting x genotype interaction terms did not statistically and significantly predict the initial level or slope of inhibitory control from age 3 to age 5.

Inhibitory Control: Mothers' harsh parenting. Considering mothers' harsh parenting, mothers' harsh parenting predicted initial levels of inhibitory control (b = -.32, SE = .12, p < .05, see Table 5), but not the rate of change in inhibitory control. More harsh parenting from mothers' was associated with less inhibitory control at age 3, but unrelated to growth in inhibitory control. Furthermore, mothers' harsh parenting did not directly or interactively, with children's genotype, predict the slope of inhibitory control.

Inhibitory Control: Fathers' harsh parenting. Finally, considering fathers' harsh parenting, fathers' harsh parenting was significantly associated with initial levels of inhibitory control (b = -.22, SE = .11, p < .05, see Table 5), but not change in inhibitory control over time. Specifically, more harsh parenting from fathers' was associated with less inhibitory control at age

3. Harsh parenting did not directly or interactively, with children's genotype, predict the slope of inhibitory control from age 3 to age 5.

Discussion

Temperamentally fearful children, particularly children rated high on behavioral inhibition, are generally found to be shyer and more withdrawn during childhood (Kagan, et al., 1988), report feeling lonelier and more socially rejected during middle childhood (Boivin, Hymel, & Bukowski, 1995), and seem to be at increased risk for internalizing disorders during adolescence (Rubin, et al., 1995; Williams et al., 2009). Understanding the development of fearful temperament during early childhood may help to identify possible routes for preventative intervention during the early childhood period. The 5-HTTLPR gene may be related to fearful temperamental characteristics, but the findings linking the 5-HTTLPR polymorphism with fearful temperament have been inconsistent with some finding a relationship between the *s/s* genotype and more fearful temperament (e.g., Hayden et al., 2007) and others finding no relationship between the 5-HTTLPR polymorphism and fearful temperament (e.g., Schmidt et al., 2002). One possible explanation for these discrepant findings may be the moderating role of parenting on temperamental expression (Saudino, 2005).

The present study addressed this possibility by considering the role of the 5-HTTLPR polymorphism on fearful temperament as well as the potential moderating role of supportive and harsh parenting on fearful temperamental characteristics during the early childhood period. The following sections will first discuss the direct relationship between the 5-HTTLPR polymorphism on fearful temperamental characteristics in early childhood. Next, the direct and interactive effect of mothers' and fathers' supportive and harsh parenting will be discussed, with a particular focus on the unique effects of father's. Finally, the results will be discussed in terms of how well they fit the diathesis stress versus differential susceptibility.

5-HTTLPR Polymorphism and change in fearful temperament

The 5-HTTLPR polymorphism was hypothesized to directly predict initial levels and change in fearful temperamental characteristics such that children with the *s/s* genotype, and perhaps the *s/l* genotype, would demonstrate more change in fearful temperamental characteristics. This hypothesis was only partially supported, no relationship between the *s/s* genotype and fear and shyness emerged, but the *s/s* genotype was associated with declines in soothability and declines in inhibitory control over time. The 5-HTTLPR polymorphism likely influences fearful temperamental characteristics by moderating serotonin availability in fear relevant areas of the brain, such as the amygdala which is related to fear recognition and arousal (Azmitia & Gannon, 1986). Emotion regulation difficulties may underlie the association between the 5-HTTLPR genotype and internalizing disorders in adolescence. Difficulty managing negative emotions following a stressful life event has been found to mediate the relationship between stressful life events and mental health problems in adolescents such that experiencing more stressful life events was only an issue if the adolescent had a difficult time regulating their emotions (McLaughlin, Hatzenbuehler, & Phil, 2009). However, difficulty regulating emotional arousal is likely only an issue in the context of stressful events. Without experiencing distress, it is likely that an individual that has issues regulating negative emotions appears to be as well-adjusted as an individual adept at managing their emotions. Likewise, the 5-HTTLPR *s/s* genotype has only been associated with a higher risk for depression following stressful life events (Caspi et al., 2003).

It is unclear why the 5-HTTLPR genotype did not directly influence temperamental fear or shyness. In the childhood period, the *s* allele has been associated with temperamental shyness in some cases (e.g., Hayden et al., 2007), while others report no association between the 5-HTTLPR genotype and temperamental shyness (e.g., Schmidt et al., 2002). Both studies used mothers'

report of fearful temperamental characteristics during the preschool period. However, neither study considered characteristics of the environment as conditioning the impact of genotype on fearful temperament. Quite possibly, parenting may provide a stressful or supportive environment that moderates the expression of the 5-HTTLPR genotype on expressed fearfulness and shyness in early childhood. The next section explores parenting as a possible moderator of the 5-HTTLPR genotype on fearful temperament.

Parenting quality moderates associations between 5-HTTLPR and indicators of fearful temperament.

Parenting was hypothesized to condition the relationship between the 5-HTTLPR polymorphism and fearful temperamental characteristics, such that less supportive and more harsh parenting would predict increases in fearful temperamental characteristics only for children with the *s*/s variant of the 5-HTTLPR polymorphism. Before discussing the results associated with the hypotheses, several ancillary findings will be considered. Not surprisingly, mothers and fathers varied in their levels of observed supportive and harsh parenting. Both mothers and fathers were generally more supportive than harsh, but mothers' and fathers' ratings of supportive and harsh parenting were only modestly correlated. As a result, mothers' and fathers' parenting quality was evaluated separately. Theoretically, variations in parenting quality across mothers and fathers allows for an examination of the differential impact of mothers' and fathers' parenting on children's development of fearful temperamental characteristics. Important differences in the association of genotype and characteristics of fearful temperament emerged for mothers' and fathers' parenting.

Mothers and fathers uniquely contributed to the development of fearful temperamental characteristics during early childhood. In general, mothers' parenting quality did not moderate gene expression. Instead, mothers' supportive parenting predicted increases in soothability and

inhibitory control, and mothers' more harsh parenting predicted lower initial levels of inhibitory control, regardless of genotype. These results are consistent with previous studies that have linked mothers' parenting quality with indicators of soothability and inhibitory control (e.g., Dennis, 2006; Eisenberg, et al., 2005). Mothers' parenting quality was unrelated to indicators of fear or shyness.

Similarly, fathers' harsh parenting had a main effect on children's fear. However, contrary to the hypothesized relationship, harsh parenting was associated with more fear at age 3, but more harsh parenting from fathers' was actually associated with a decrease in children's fearfulness over time. These findings should be considered in the context of the amount of harsh parenting observed in this sample. It is possible that at lower levels, harsh parenting actually provides structure for the child, and fathers' may be firm about encouraging their children to confront fears. Fathers' who are somewhat harsh may be less likely to validate children's fears. Over-time, children who confront fears are more likely to have a decrease in their fearfulness over-time (Rubin et al., 2002).

Only fathers' parenting quality was found to moderate the impact of gene expression on indicators of fearful temperament, specifically children's shyness and soothability. First, considering shyness, the *s/s* genotype was not directly associated with higher levels of shyness. However, after accounting for father's supportive parenting, the magnitude of the relationship between the *s/s* genotype and shyness increased to become significant. The relationship between the *s/s* genotype and shyness seemed to be partially masked by the covariance of fathers' supportive parenting, and when placed in the same model, the *s/s* main effect emerged. Interestingly, there was a negative correlation between the *s/s* genotype and fathers' supportive parenting, meaning that children with the *s/*s genotype were receiving less supportive parenting from their fathers, but this does appear to be an evocative effect of shyness as children with the

s/s genotype were not rated significantly shyer than other children at age 3. Quite possibly children with the *s/s* genotype are rated more difficult on another dimension of temperament that is not considered in the present study.

While the role of parenting in moderating the impact of the s/l genotype on characteristics of fearful temperament were only exploratory, less supportive parenting was found to be associated with a declining shyness for children with the *s/l* genotype. Additionally, more supportive parenting was associated with stability of fearful characteristics for children with the *s/l* genotype. These results are quite perplexing, possibly father's supportive parenting promotes stability in shyness through over-protection (Rubin et al., 2002). Parenting that is over-protective validates children's fearful emotions and may signal that the environment is dangerous, therefore promoting stability in shyness (Rapee, Schniering, & Hudson, 2009).

Most notably, fathers' harsh parenting uniquely predicted trajectories of soothability dependent on the genotype. As expected, more harsh parenting moderated the relationship between the *s/s* genotype and soothability such that children became less soothable over-time. For children already experiencing difficulty managing their emotions, more harsh parenting responses likely overwhelm children, interfering with emotion regulation (Scaramella & Leve, 2004).

Quite unexpectedly, fathers' harsh parenting moderated the relationship between the *l/l* genotype and soothability, such that more harsh parenting from fathers' was associated with increases in soothability. Children with the *l/l* genotype became more soothable, possibly in an effort to experience less harsh parenting. These results mirror findings of callous and unemotional children where children with the *l/l* genotype who also live in lower SES neighborhood also are rated as more callous and unemotional (Sadeh et al., 2010). Emotional numbing has been found to mediate the relationship between trauma experienced in a low SES

environment and callous and unemotional traits (Kerig, Bennett, Thompson, & Becker, 2012). Children with the *l/l* genotype may respond to distressing environments by dampening their emotional responses rather than becoming more distressed.

Research studies often focus solely on mothers' parenting because mothers are assumed to assume most of the responsibility for raising children (e.g., Finley, Mira, & Schwartz, 2008). As compared to fathers, mothers' generally spend more time with very young children, resulting in more socializing and disciplinary opportunities (e.g., Manlove & Vernon-Feagans, 2002). In contrast, the amount of time fathers spend with their children is more variable (NICHD Early Child Care Research Network, 2000). Possibly, fathering moderates the expression of the 5-HTTLPR gene because children's temperamental characteristics may influence father involvement. For example, Meteyer & Perry-Jenkins (2010) reported that fathers of less soothable infants became more involved with their children during the infancy period, possibly in an effort to help the mother manage the stress that comes with having a difficult child. Children with the *l/l* genotype were reported to be less soothable at age 3, and even though children with the *s/s* genotype were relatively more soothable at age 3, soothability was declining from 3 to 4 years old, the time period where parenting was measured. Consequently, although not measured by the present study, fathers' may have been more involved in parenting in order to assist the mother with these challenging behaviors.

Both mothers' and fathers' have an impact on the development of fearful temperament in early childhood. Most unique to this study is that qualitative features of fathers' parenting should be considered when designing interventions for temperamentally difficult children. Although not addressed by the present study, fathers' may be more responsive with children who are especially difficult and fathers' parenting may amplify more negative temperamental traits. Interventions targeted at preschool children that do not include mothers' and fathers' may be missing an

important component in the etiology of internalizing disorders in early childhood.

Differential Susceptibility and Protective vs. Adaptive

Genotype x environment interactions can be interpreted from different theoretical frameworks, such as a diathesis-stress approach or a differential susceptibility model. The diathesis-stress model is an additive model where individuals experience poor physical and mental health outcomes when combined with adversity in the environment (Monroe & Simmons, 1991). In the context of a supportive outcome, genetic risk may not be realized and the risk for maladaption would be reduced. In contrast, the differential susceptibility model suggests that vulnerability factors actually reflect a sensitivity to the environment; an adverse environment may confer risk for poor psychological and mental health outcomes, while an enhanced or supportive environment promotes more adaptive outcomes (Belsky, Bakermans-Kranenburg, van IJzendoorn, 2007). In other words, a differential susceptibility model indicates that individuals with genetic susceptibility are affected by the environment for better and for worse (Belsky & Pluess, 2009). Two GxE interactions between the 5-HTTLPR genotype and parenting emerged. Previous research suggests that the *s/s* genotype is a "risk" genotype such that the responsivity of the *s/s* genotype is considered highest "risk", *s/l* is considered moderate "risk", and *l/l* is considered the lowest "risk" (Belsky & Pluess, 2009; Caspi et al., 2003) . The following sections will consider how the results fit with either the diathesis-stress or differential susceptibility best. Finally, additional considerations for conceptualizing genetic risk will be considered.

In the first GxE interaction, fathers' supportive parenting interacted with the 5-HTTLPR genotype to predict children's shyness. First, the *s/s*, or highest risk genotype was directly associated with increases in shyness, suggesting that the "risk" from the *s/s* genotype was so strong that no additional environmental stress was required to increase children's shyness. The intermediate "risk" however was only associated with increases in shyness in the context of more

supportive parenting, indicating that the presence of genetic risk and environmental conditions in combination predicted changes in shyness. More supportive parenting being associated with an increase in shyness is perplexing, however, overall, the relationship between the 5-HTTLPR genotype and the parenting environment was more consistent with the diathesis-stress model.

In the second interaction, fathers' harsh parenting moderated the relationship between the both the *s/s* and *l/l* genotype (see Figure 2). These results provide some support for both a diathesis-stress model and differential susceptibility model of vulnerability. In this case there was only a relationship between the genotype and change in soothability when fathers' harshness was high. Unexpectedly though, it was the highest "risk" and lowest "risk" in this case that were most responsive to environmental influence, with the moderate "risk" genotype not appearing to be affected by harsh parenting. What gene is actually considered at "risk" may change when conceptualizing genotype based on differential susceptibility.

There may be more than a single way to conceptualize genotype "risk". Functionally, the genotypes are graded where *s/s* is the least functional, meaning that the least number of SERT proteins are transcribed, and the *l/l* genotype is the most functional with the most number of proteins transcribed, with the *s/l* falling somewhere in the middle. However, from the perspective of differential susceptibility, the more extreme genotypes, in this case the *s/s* and *l/l*, are likely the most vulnerable and most responsive to environmental conditions while the most prevalent genotype, in this case the *s/l* genotype is relatively unaffected by the environment. Theoretically, when experiencing more stressful environments, individuals with the *s/l* are likely to function moderately well because they likely maintain enough 5-HT availability through moderate 5-HT re-uptake. However, in the context of stress, having too few SERT may result in a deficit in available 5-HT because not enough 5-HT is being re-used. Potentially, there is also a negative consequence of having many SERT proteins because in the event that 5-HT is released in a

stressful context, it may be removed quickly from the synaptic cleft resulting in a deficit of 5-HT in the synaptic cleft.

In conclusion, the results neither support nor refute the diathesis-stress or differential susceptibility hypothesis. Differential susceptibility requires finding a relationship between the independent variables and the dependent variable. Without a wide range of supportive and harsh environments, it is more difficult to find evidence of differential susceptibility. It is quite possible that because there was not a full range of parenting that it was not possible to find the full range of relationship between parenting and fearful temperament.

Strengths, Limitations, and Future Directions

These findings strengthen existing research examining trajectories of fearful temperament in early childhood by specifically considering the role of both 5-HTTLPR genotype and parenting on fearful temperamental characteristics in a number of ways. First, by examining trajectories of fearful temperament over time, the impact of genotype and parenting quality on the trajectories of fearful temperamental characteristics could be examined. Using linear modeling allowed for an understanding of how parenting interacted with children's genotype to change the trajectory of fearfulness in early childhood. Second, both mothers and fathers parenting were considered separately, which is unique, since most studies only consider mothers parenting. By considering mothers' and fathers' parenting separately, differential effects of fathers' parenting were found, highlighting the importance of father's influence on children's development in early childhood. Finally, this project considers multiple aspects of fearful and inhibited temperament rather than considering a single fearful factor which combines several aspects of fearful temperament. This is important because temperamental fear has multiple components and there were associations with some aspects of fearful temperament, but not others. First, the significant relationships may not have been found if the temperamental characteristics were combined. Second, examining the

parts of fearful temperament separately highlighted the importance of soothability rather than just the more broad dimensions of fear and shyness.

This study is not without limitations. First, there were missing time points due to the biannual assessments, and the missing data points were estimated using HLM. Ideally, all children would have been assessed at each time point. Second, although a strength in some ways, the observational tasks put parents into a situation where they must interact with their children when in reality they may not typically have the types of one on one interaction that is being used to measure their parenting. Finally, the amount of harsh parenting observed in this study is very low, making it difficult to interpret the meaning of high harsh parenting. It is likely that rates of harsh parenting is low because observational measures were used, and it is much less likely that parents would be highly harsh in a video-taped interaction because they are aware of being watched. However, it is expected that the rank order of harsh parenting would be maintained. Despite this, fathers' harsh parenting had a significant impact on children's soothability.

Despite these limitations, the results have important implications for future research. First, these findings highlight the unique effects of mothers' and fathers' parenting on children's development, but this is just one piece of the child's rearing environment. Future research should consider other areas of children's context such as other caregivers. Second, this study has important implications on the development of temperament, but does not directly address psychopathology. Theoretically, fearful temperamental characteristics put children at risk for internalizing disorders, but fearful temperament alone may not be enough to confer risk for psychopathology. Future research should consider these temperamental trajectories as possible pathways to psychopathology. Finally, more research on the impact of fathers' on children's fearful behavior needs to be explored. There were many uncertainties about what fathers' might be doing when interacting with their children to have an impact on children's fearful

temperamental characteristics. It appears that the role of fathering in the earl etiology of internalizing disorders an untapped dimension, and understanding fathers' role in the development of fear may provide new avenues for future interventions.

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