The developmental origins of a helplessness endophenotype in children

Katherine O’Donnell
Department of Psychiatry, McGill University, Montreal
June, 2011

A thesis submitted to McGill University in partial fulfillment of the requirements of the degree of Master of Science

Supervisor: Professor Michael J. Meaney

© Katherine O’Donnell, 2011
# TABLE OF CONTENTS

1 TABLE OF CONTENTS........................................................................................................ 2

2 ABSTRACT .......................................................................................................................... 5

3 RÉSUMÉ ............................................................................................................................ 6

4 ACKNOWLEDGEMENTS .................................................................................................. 7

5 INTRODUCTION ............................................................................................................... 9

6 LITERATURE REVIEW .................................................................................................... 11

   6.1 Helplessness ............................................................................................................. 11
       6.1.1 Helplessness: Measurement in the present study .............................................. 15

   6.2 Serotonin Transporter-Linked Polymorphic Region (5-HTTLPR) ....................... 16
       6.2.1 5-HTTLPR: Theory and evidence ................................................................. 16
       6.2.2 5-HTTLPR: Intermediate phenotypes and psychopathology .................. 17
       6.2.3 5-HTTLPR: A candidate gene ................................................................. 18

   6.3 Attachment and Early Environment ..................................................................... 19
       6.3.1 Early environment: Theory and evidence .................................................... 19
       6.3.2 Early environment: Animal studies ............................................................ 20
       6.3.3 Early environment: Human studies ............................................................ 21
       6.3.4 Attachment ................................................................................................. 22
       6.3.5 Attachment: A candidate measure of early environment .......................... 24

   6.4 5-HTTLPR x Environment ....................................................................................... 25
       6.4.1 5-HTTLPR x environment: Theory and evidence ....................................... 25
       6.4.2 5-HTTLPR x environment: Animal research .............................................. 26
       6.4.3 5-HTTLPR x environment: Human studies of anxiety, depression and their endophenotypes ................................................................. 26
       6.4.4 Helpless and negative thinking styles: An endophenotype of anxiety and depression ................................................................. 27

6.5 Summary .................................................................................................................... 28

7 GENERAL METHODS .................................................................................................... 30

   7.1 The Maternal Adversity Vulnerability and Neurodevelopment (MAVAN) ........ 30

   7.2 Participants ............................................................................................................. 30

   7.3 Materials ................................................................................................................ 32
       7.3.1 Attachment security: The Strange Situation .............................................. 32
<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>7.3.2 Genotyping</td>
<td>34</td>
</tr>
<tr>
<td>7.3.3 Child helplessness</td>
<td>34</td>
</tr>
<tr>
<td>7.3.4 Strengths and Difficulties Questionnaire (SDQ)</td>
<td>35</td>
</tr>
<tr>
<td>7.3.5 Dominic/que-R</td>
<td>35</td>
</tr>
<tr>
<td>7.3.6 Statistics</td>
<td>36</td>
</tr>
<tr>
<td>7.4 Procedure</td>
<td>36</td>
</tr>
<tr>
<td>8 RESPONSE TO CHALLENGE PUZZLES</td>
<td>38</td>
</tr>
<tr>
<td>8.1 Materials</td>
<td>38</td>
</tr>
<tr>
<td>8.2 Self-report</td>
<td>38</td>
</tr>
<tr>
<td>8.2.1 Self-report: Procedure</td>
<td>38</td>
</tr>
<tr>
<td>8.3 Helplessness Behavior Coding</td>
<td>40</td>
</tr>
<tr>
<td>8.3.1 Behavior coding: Materials</td>
<td>40</td>
</tr>
<tr>
<td>8.3.2 Behavior coding: Procedure</td>
<td>40</td>
</tr>
<tr>
<td>8.4 Heart Rate</td>
<td>44</td>
</tr>
<tr>
<td>8.4.1 Heart rate: Materials</td>
<td>44</td>
</tr>
<tr>
<td>8.4.2 Heart rate: Procedure</td>
<td>45</td>
</tr>
<tr>
<td>8.4.3 Summary of measures</td>
<td>45</td>
</tr>
<tr>
<td>9 RESULTS</td>
<td>47</td>
</tr>
<tr>
<td>9.1 Self-report</td>
<td>47</td>
</tr>
<tr>
<td>9.1.1 Self-evaluation</td>
<td>48</td>
</tr>
<tr>
<td>9.1.2 Hopefulness</td>
<td>51</td>
</tr>
<tr>
<td>9.1.3 Motivation</td>
<td>52</td>
</tr>
<tr>
<td>9.1.4 Summary of self-report data</td>
<td>52</td>
</tr>
<tr>
<td>9.2 Behavioral Data</td>
<td>53</td>
</tr>
<tr>
<td>9.2.1 Latency of response</td>
<td>56</td>
</tr>
<tr>
<td>9.2.2 Summary of behavioral data</td>
<td>58</td>
</tr>
<tr>
<td>9.3 Heart Rate Data</td>
<td>59</td>
</tr>
<tr>
<td>9.3.1 Summary of heart rate data</td>
<td>61</td>
</tr>
<tr>
<td>9.4 Correlations Among Helplessness Measures</td>
<td>61</td>
</tr>
<tr>
<td>9.4.1 Summary of correlations between dimensions of helplessness</td>
<td>62</td>
</tr>
<tr>
<td>9.5 Correlations with SDQ and Dominic/que-R</td>
<td>63</td>
</tr>
<tr>
<td>9.5.1 Summary of correlation between dimensions of helplessness and rating of psychopathology</td>
<td>64</td>
</tr>
</tbody>
</table>
10 DISCUSSION ........................................................................................................................................ 65
  10.1 Summary of Study Findings ............................................................................................................. 65
  10.2 Study Strengths and Limitations .................................................................................................... 69
  10.3 Clinical Implications ...................................................................................................................... 71
  10.4 Future Directions ............................................................................................................................ 73
  10.5 Conclusion ...................................................................................................................................... 74

11 REFERENCE LIST .............................................................................................................................. 75

12 APPENDIX ........................................................................................................................................ 94
2 ABSTRACT

Research has demonstrated the link between helplessness cognitions and the development of anxiety and depression. However, there is a paucity of research on the origins of such thinking styles in children. There is mounting evidence for an interactive influence between the polymorphism within the promoter region of the serotonin transporter gene (5-HTTLPR) and early life adversity on the risk for anxiety, depression and their intermediate phenotypes. We hypothesized that children with one copy of the S allele of the 5-HTTLPR gene and an insecure attachment would manifest increased helplessness and stress reactivity when faced with a challenge. The data are drawn from a sub-sample of mother-child dyads from the MAVAN study. The pairs completed the laboratory measure of attachment security and buccal cheek swabs taken when the child was 18 and 36 months, respectively. At 60 months, the child performed a Response to Challenge Puzzle (RCP) task. The task was designed to assess the child’s response to “failure”, as some puzzles were impossible to solve. The child’s self-report, behavioral response and average heart rate were assessed during the RCP task. Multivariate analyses revealed significant effects of attachment and 5-HTTLPR on multiple dimensions of helplessness. The results suggest a potential pathway between child genotype, environment and risk for anxiety and depression.
3 RÉSUMÉ

La littérature scientifique endosse un lien entre les cognitions qui accompagnent un sentiment de résignation et le développement de l’anxiété et la dépression. Toutefois, il existe peu d’études sur les origines de telles cognitions chez les enfants. De plus en plus, les recherches au sein de ce champ d’investigation suggèrent que l’interaction entre le gène transporteur de sérotonine, qui comporte une région promotrice dont le siège est un polymorphisme fonctionnel (5-HTTLPR), et l’adversité vécue durant l’enfance sont associés avec un risque accru pour l’anxiété, la dépression et leurs phénotypes intermédiaires. Dans la présente recherche, nous avons émis l’hypothèse que les enfants portant une copie de l’allèle court du gène 5-HTTLPR et ayant un attachement insécure manifesteront davantage de résignation et de réactivité au stress lorsque confrontés par un défi, comparés aux enfants ne possédant aucune de ces caractéristiques. Les données proviennent d’un sous-échantillon de dyades mère-enfant issues du projet MAVAN. Chaque paire a complété la mesure d’attachement en laboratoire à 18 mois. Des échantillons d’ADN furent recueillis par l’entremise d’une serpillère de joue (prélèvement buccal) à 36 mois. À 60 mois, l’enfant exécuta un Casse-Tête Impossible (CI). Cette tâche, qui comporte des casse-têtes insolubles, fut conçue dans le but d’évaluer la réaction de l’enfant à “l’échec”. L’auto-évaluation, les réponses comportementales ainsi que la fréquence cardiaque de l’enfant furent mesurés lors du CI. Des analyses multivariées démontrèrent des effets significatifs de l’attachement et du 5-HTTLPR pour diverses composantes de la résignation. Les résultats suggèrent une avenue potentielle entre le génotype de l’enfant, l’environnement et le risque pour l’anxiété et la dépression.
4 ACKNOWLEDGEMENTS

Over the past five years, I have had the fortunate opportunity to work for the MAVAN project and it has been an incredibly enriching experience. I will be forever grateful that I was given the chance to join this team and learn so much.

First, I would like to take this opportunity to express my sincere gratitude to my supervisor Dr. Michael Meaney. I am very thankful for the thorough feedback and guidance I have received throughout this Master’s thesis. I am also thankful to have had a supervisor that has pushed me to think about my research in new ways and provided me numerous opportunities to develop as a researcher. It has been an honor.

I would also like to thank the people who run the MAVAN project, the project coordinator Hélène Gaudreau as well as Amber Reider and Josie Diorio. I would like to especially acknowledge Hélène for all her support and endless encouragement over the past three years, as well as her feedback on this manuscript and project design. Her role was critical in helping me realize my thesis. I would also like to thank Dr. Leslie Atkinson and his team for their coding of the Strange Situation and Dr. James Kennedy and his laboratory for the genetic analysis. I would like to thank my committee member Dr. Ashley Wazana for his valuable advice, support and generously sharing his knowledge with me. I would like to make a special thank you to Dr. Klaus Minde whose presence at our weekly Journal Club meetings has been a source of immense knowledge, inspiration and laughter.

I am very thankful to the devoted research assistants both past and present for their diligent work in collecting the data. Specifically, I would like to thank Fellah Mercier, Lyne Duchaine, Anne-Marie Dufour and the Hamilton team for administering the puzzle task. I would like to thank Dana Elsaleh for her tireless work on the reliability coding of the videos. I would like to thank David Brownlee, Pablo-Edouardo Moreno, Vincent Jollivet and Jonathan Desrosiers for their work on the database, technical support and assistance. I would like to thank Jean Paquet for assistance with the analyses. I would also like to thank Sara Colalillo and Vanessa Iacono for help in translating the abstract. I would like to make a special acknowledgement to Angélica Moroch who passed away during the time I worked on my thesis. She played a very important role for my project not only in collecting data but also in helping
organize and translate the puzzle protocol. Angel is greatly missed by myself and the MAVAN team as a whole, but her presence is still felt through cherished memories and her high quality work.

I would also like to acknowledge my fellow students in the MAVAN project Estelle Lawrence, Andrée-Anne Bouvette-Turcot, the staff with the MAVAN project and the members of the Meaney lab group. I would like to thank Magdalena Zbedik for her guidance and feedback on my project and Kieran O’Donnell for being a source of support, knowledge and reviewing my thesis.

I would also like to take this opportunity to acknowledge people who have influenced me a great deal. I am extremely grateful to the late Dr. John Abela and his incredible team especially Randy Auerbach and Claire Starrs who first taught me about helplessness and the cognitive vulnerability model of depression. I would also like to thank Dr. Susan Pawlby, Dr. Carmine Pariante and the team from the SPI lab at the Institute of Psychiatry in London who hosted me on an internship during my time as a Master’s student. I learned so much during my time at the IoP and felt so welcome.

I must thank the people who have been absolutely critical in helping me achieve this goal, but were my support “behind the scenes”. First, I must thank my family, especially my parents Laurie and Kevin and brother James for their love and support throughout this process. I have been blessed to have parents that instilled in me a love of learning and who supported me unconditionally. Importantly, years after telling them “no matter what I do, I know I don’t want to work in science”, never saying “I told you so”. I would like to thank the Hudson girls for their encouragement and friendship. Lastly, but certainly not least, I would like thank my partner, love and probably the engineer who knows more about “helplessness” than any other, Renaud Daenzer. I want to thank Renaud for being an enormous source of support, reading and helping format my thesis, listening to ideas and being my rock when things were difficult. I am so grateful to have a best friend who would go so above and beyond to help me realize my dreams.

Finally, I would like to thank the mothers and children that participate in the MAVAN project for so generously giving their time.
5 INTRODUCTION

Negative cognitions such as helplessness and low self-evaluation are important contributors to the development of anxiety and depression (Chorpita & Barlow, 1998). Therefore, understanding the origins of a helpless thinking style is imperative for a complete understanding of the etiology of affective and anxiety disorders. However, there is a paucity of research on the early manifestations of helplessness, especially in children. In the present study we explored the developmental antecedents of a helpless thinking style in children. We examined the role of two candidate factors, both of which associate with the risk for affective illness: the functional polymorphism in the promoter region of the serotonin transporter (5-HTTPLR) gene and attachment security.

To our knowledge this is the first study to prospectively assess the early manifestations of a negative or helpless cognitive style in children from this perspective. We used an ecologically valid measure of response to failure on a puzzle task to assess helplessness. The assessment included measures of 1) child reported self-evaluation, motivation and hopefulness, 2) child’s behavioral manifestations of helplessness and 3) heart rate reactivity to capture the underlying physiological response to failure.

In the first chapter of this thesis I discuss the role of the early environment, parent-child relationship and the 5-HTTLPR in the development of anxiety and depressive disorders, and their intermediate phenotypes. The methods of the present study are described in chapter two and detailed descriptions of the helplessness measures are provided in the third chapter. In the fourth chapter the association between child genotype and attachment will be assessed for each
dimension of helplessness measured, as well as their inter-relationship. Lastly, we assess if helplessness is in fact an appropriate intermediate phenotype of anxiety and depression in children. We examine the relationship between the measures of helplessness and both parent and child reported psychopathology. The thesis concludes in the fifth chapter with a discussion including a summary of the study’s findings, clinical implications and future directions of research in this field.

The first aim of the current study is to assess whether our two factors of interest, attachment measured at 18 months and child 5-HTTPLR genotype, associate with helplessness in children. The second aim is to create a comprehensive assessment of child helplessness, one that measures multiple dimensions of emotion regulation. Our goal was to capture a multifaceted assessment of risk for psychopathology by measuring self-report, behavioral and physiological reactivity in response to impossible puzzles. The third aim was to assess how our measures of helplessness associate with risk for psychopathology. This final step is critical in validating the measure as an endophenotype of anxiety or depression.
6 LITERATURE REVIEW

6.1 Helplessness

Helplessness is the set of beliefs that one has no control or ability to prevent the occurrence of negative outcomes. This cognitive style is associated with feelings of distress, anxiety and low mood. Laboratory animals exposed to repeated uncontrollable aversive stimuli display explicit signs of a stress response such as weight loss and gastric lesions (Weiss, 1968) as well as escape failure and immobility (Overmier & Seligman, 1967; Prince & Anisman, 1984). Controllable stressors of the same physical intensity produce few such effects. An analogous pattern of responses to uncontrollable stress, including loss of motivation and distress, is observed across the lifespan in infant, child and adult human samples (Cohen, Evans, Krantz, & Stokols, 1980; Lewis & Ramsay, 2005; Miller & Seligman, 1975).

Increased stress reactivity predicts the risk for depression in prospective studies (Wichers, et al., 2009). Individual differences in stress responses are attributable, in part, to psychological factors. Several researchers have put forward theories about different negative psychological factors or styles that are associated with an increased physiological and emotional response to stress. In pioneering work on patients suffering from depression, Beck determined that underlying cognitive distortions such as low self-regard are associated with depressive illness (Beck, 1963; Kovacs & Beck, 1978). Based on interviews with patients he determined that it is the negative automatic thoughts or “erroneous conceptualizations” (Beck, 1963, p. 44) of the self that lead to the development of depression. Similar theories include the reformulated learned helplessness theory where negative thinking styles are defined as attributing negative events to internal (due to the self), global (pervasive) and stable (over time) causes (Abramson, Seligman,
This theory was further developed to include hopelessness, a proximal and sufficient cause of depression (and specifically, hopelessness depression) (Abramson, Metalsky, & Alloy, 1989). Helplessness is thought to be the cognitive precursor of hopelessness, and part of the shared cognitive etiology of both anxiety and depressive disorders.

These theories have generated considerable research showing that negative cognitive distortions increase the risk for psychopathology. For example, the large-scale Temple-Wisconsin Cognitive Vulnerability to Depression (CVD) project is a prospective, multi-wave study developed to test the association between the cognitive vulnerability, depressive symptoms and clinically significant depressive episodes. Individuals with a negative cognitive style are more likely to suffer from episodes of depression (Alloy, et al., 2000). It is now believed that the thinking styles act as a diathesis for increased depression or anxiety symptoms when the individual is faced with adversity. For example, 3rd and 5th graders with a negative attributional style experienced increase in depressive symptoms in times of increased negative life events (Abela, 2001).

However, despite the evidence suggesting the role of cognitive styles in the development of psychopathology, there are a few studies that assess the developmental origins of pathogenic thinking styles. The study of the developmental origins of negative thinking has mostly focused on the role of traumatic events such as parental loss or separation (Beck, Sethi, & Tuthill, 1963), maltreatment (Gibb, Abramson, & Alloy, 2004; Gibb, et al., 2001) or other major stressors. Cross-sectional, retrospective report of early experiences such as harsh parenting style (Alloy, et al., 2001; McGinn, Cukor, & Sanderson, 2005) are also reported to influence the development of negative thinking styles. Research by Ingram, Overbey and Fortier shows that even low maternal bonding is associated with offspring thinking styles (Ingram, Overbey, & Fortier, 2001). Again,
using a cross-sectional design and retrospective report of parental care, higher parental bonding associates with increased positive thinking and decreased negative self-statements. Parent-child interactions need not be adverse in the extreme to affect thinking style.

Few studies of negative thinking style in children have been reported. This is despite substantial evidence that anxiety and depression are significant disorders of childhood (Cartwright-Hatton, McNicol, & Doubleday, 2006; Costello, Mustillo, Erkanli, Keeler, & Angold, 2003; Shaffer, et al., 1996), even in preschool aged children (Cote, et al., 2009). Moreover, early indicators of future illness may be present very early in life (Caspi, Moffitt, Newman, & Silva, 1996; Chronis-Tuscano, et al., 2009). This failure to study children is partly due to the longstanding belief that negative thinking styles could not be reliably assessed in children. As the child develops, his or her sense of self becomes crystallized. Yet, the representations of the self are not necessarily stable until late in childhood. Thus, on the one hand, studies of the “vulnerability-stress” models of attributional style show that attributional style may not present as a stable diathesis in early childhood, but reflect a response to current stressors (Nolen-Hoeksema, Girgus, & Seligman, 1992; Turner & Cole, 1994). On the other hand, prospective longitudinal research shows that helplessness present in children between five and six years of age is significantly stable two weeks, one and five years later (Ziegert, Kistner, Castro, & Robertson, 2001).

This discrepancy may in part be explained because self-report measures of helplessness are developmentally inappropriate for younger children. However, building on animal studies we know that observable behavior may shed light on individual differences in stress responses. Moreover, key paradigms in child development studies such as the Ainsworth Strange Situation (Ainsworth, Blehar, Waters, & Wall, 1978) use behaviors as a measure of underlying cognitive
schema. Thus, the behavior of children in the face of stress may reflect an evolving helplessness thinking style.

There is additional evidence for the idea that negative thinking styles may become evident after the induction of low mood. For example, even in adults suffering from depression, a low mood is necessary to evoke the underlying negative cognitive style (Miranda, Gross, Persons, & Hahn, 1998). In light of these findings researchers use challenging, or impossible tasks to observe the cognitive style in younger children. Early studies on mastery versus helplessness motivation in children found that helplessness in the face of a difficult task manifests itself in children through delayed initiation into a difficult task, lower expectancy for success and more negative self-related statements (Diener & Dweck, 1978). In a naturalistic experiment, children exposed to air traffic noise in their schools displayed more helplessness (giving up prematurely) in response to a challenging puzzle task, than their non–noise-exposed counterparts (Cohen, Evans, Krantz, & Stokols, 1980). More recently, Murray and colleagues administered a rigged card game between a child and his or her friend (Murray, Woolgar, Cooper, & Hipwell, 2001). On some of the trials the child won, on others the friend gained the lead. The authors evaluated the number of negative or hopeless utterances, particularly in the “losing trials” as a measure of cognitive schema. They found that children exposed to maternal depression during their lifetime, specifically through increased hostile care, displayed more negative statements, indicating more negative self-schemas.

Perhaps the most compelling research is that of Cole and colleagues on helplessness in children aged five using an impossible puzzle task (Cole, et al., 2007). This study shows that children with parents who reported themselves as more controlling, as well as children exposed to more negative events displayed more helplessness (i.e. increased negative affect and lower
self-reported performance) during failure. This study highlights the role of the early care-giving experience in shaping a child’s perception of ability and performance in the face of failure.

Another way that stress responses are observed is through autonomic reactivity to stress. Measuring a child’s physiological response could shed light to underlying cognitive reactivity. Increased average heart rate in face of a challenge has been observed in samples with pediatric anxiety disorders (Monk, et al., 2001). Similarly, Evans and colleagues found that exposure to the uncontrollable stress of noise pollution, not only influences the children’s performance on a challenging task, but also increases average heart rate reactivity to a mild stressor (Evans, Lercher, Meis, Ising, & Kofler, 2001). Conversely, attention and engagement in the task, behaviors seen in mastery-oriented children, lead to heart rate deceleration (Lewis, Kagan, Campbell, & Kalafat, 1966). Therefore, one way to buttress the findings of helplessness in children would be to couple measures of negative cognitive reactivity and behavioral manifestations with measures of the heart rate response. No such study to date has examined all three dimensions in children, especially under the age of eight years.

6.1.1 Helplessness: Measurement in the present study

In the present study we used a Response to Challenge Puzzle (RCP) task to measure underlying helplessness cognitions in children aged five. This task was modified from the paradigm used by Cole and colleagues with children of the same age (Cole, et al., 2007). The RCP measure consists of puzzles, the first and last of which are possible to solve while the middle three were impossible. We measured three dimensions of child response to the RCP task: self-reported evaluations after each puzzle, child’s behavioral response, and average heart rate. A detailed description of this task is found in Chapter 4 of this thesis.
6.2 Serotonin Transporter-Linked Polymorphic Region (5-HTTLPR)

6.2.1 5-HTTLPR: Theory and evidence

Serotonin (5-hydroxytryptamine or 5-HT) is a monoamine neurotransmitter associated with the regulation of sleep, appetite, cognition as well as mood. Neurobiological evidence demonstrates that serotonin is largely produced in the raphé nuclei of the brainstem, the neurons of which project widely to limbic and cortical regions of the brain. Serotonin levels in the amygdala and prefrontal cortex increase in response to stress (Kawahara, Yoshida, Yokoo, Nishi, & Tanaka, 1993). Moreover, selective serotonin reuptake inhibitors (SSRI), medication used to treat anxiety (Stein, et al., 1998) and depression (Kugaya, et al., 2003), moderate of the re-absorption of serotonin, increasing overall levels of serotonin in the brain. SSRI medication also decreases negative attributional bias in patients with General Anxiety Disorder (Mogg, Baldwin, Brodrick, & Bradley, 2004). Serotonin has been linked to depression through tryptophan depletion studies. Acute tryptophan depletion temporarily lowers serotonin and is associated with low mood in non-depressed males (Young, Smith, Pihl, & Ervin, 1985) and increased depressive symptoms in women in remission of depression, as well as cognitive reactivity (Booij & Van der Does, 2007).

Critically, serotonin release is associated with behavioral depression or learned helplessness in animals (Maier & Watkins, 2005). Exposure to uncontrollable stressors activates serotonin neurons in the dorsal raphé nucleus, whereas stressors signaled to be controllable by the medial prefrontal cortex do not evoke the same serotonin increase (Amat, et al., 2005). This activation sensitizes serotonergic neurons, which in turn makes the animal more likely to manifest behavioral depression upon future exposure to stress. Taken together there is strong
evidence suggesting that serotonin is implicated in the pathogenesis of mood and anxiety disorders.

The efficacy of the serotonergic system is dependent, in part, on the function of the serotonin transporter. The transporter is an important membrane protein encoded by the \textit{SLC6A4} gene and located on chromosome 17q11.1-q12. The 5-HT transporter (5-HTT) terminates the action of serotonin in the synaptic cleft, through presynaptic reuptake, to be recycled to the presynaptic neuron. In the serotonin transporter-linked polymorphic region (5-HTTLPR) located in the proximal 5’ flanking regulatory region of the gene there is a repeat sequence that encodes two functionally different allelic forms. First, the short (\textit{S}) allele has 14 copies of a 20–23 base pair repeat unit and second, the long (\textit{L}) variant comprises 16 copies. These differences in base pair repeat unit copies are associated with lower transcriptional efficiency of the transporter and rate of serotonin reuptake in the short carriers versus the long (OMIM, 2011).

Studies with non-human subjects report that variations in serotonin transporter expression associate with anxiety-like behaviors (Holmes, Murphy, & Crawley, 2003). For example, serotonin transporter knockout mice display more fearfulness including decreased exploratory behaviors in the elevated plus-maze and open field tests, as well helpless-like behaviors in the forced swim test (Carroll, et al., 2007) and exposure to the tail suspension test (Zhao, et al., 2006).

\textbf{6.2.2 5-HTTLPR: Intermediate phenotypes and psychopathology}

The 5-HTTLPR is a popular candidate gene in the study of anxiety and mood disorders in humans (Ressler & Nemeroff, 2000). Intermediate phenotypes, or endophenotypes, of mood and anxiety disorders such as neuroticism (Lesch, et al., 1996), increased amygdala reactivity to
threat (Hariri, et al., 2002) and coupling between the amygdala and the ventromedial prefrontal cortex (Heinz, et al., 2005) are associated with at least one copy of the \( S \) allele. This association is found in endophenotypes measured in children, such as negative emotionality (Nobile, et al., 2004) and an attention bias to threat in (Perez-Edgar, et al., 2009).

Few studies assess the role of 5-HTTLPR in relation to negative thinking styles, and even fewer in children. Undergraduates with at least one copy of the \( S \) allele demonstrate more negative performance evaluation cognitions than those homozygous for the \( L \) allele (Whisman, Johnson, & Smolen, 2011). In another sample of undergraduates, those with two copies of the \( S \) allele endorse more negative cognitions following a sad mood induction than those with at least one copy of the long allele (Beevers, Scott, McGeary, & McGeary, 2009). Children aged seven years homozygous for the \( S \) allele demonstrate greater negative self-referent processing than those homozygous for the \( L \) allele (Hayden, et al., 2008).

### 6.2.3 5-HTTLPR: A candidate gene

Beck proposed that the next stage for research in the field of cognitive distortions is to assess the role of genes, specifically those implicated in the development of anxiety and depression (Beck, 2008). 5-HTTLPR is a promising candidate gene in relation to the development of helplessness based on the above-mentioned evidence of a gene-to-disorder association. There are different views in the literature as to how the gene should be assessed. Research findings suggest that the 5-HTTPLR polymorphism functions in dominant-recessive manner, whereby possessing one copy of the \( S \) allele is associated with increased risk. Lesch and colleagues found that compared to those possessing two copies of the \( L \) variant, those with at least one copy of the \( S \) allele have lower basal level serotonin activity, up to two times less efficient uptake of labeled serotonin and lower steady-state serotonin transporter mRNA
expression (Lesch, et al., 1996). However, other groups show that individuals with two copies of the \( S \) allele have an increased susceptibility to environmental stress (Kendler, Kuhn, Vittum, Prescott, & Riley, 2005). Despite these mixed findings, to date, the most promising evidence, especially in relation to anxiogenic or depressogenic cognitions, is found by grouping those with at least one copy of the \( S \) allele together and comparing them with individuals homozygous for the \( L \) allele.

Further characterization of the 5-HTT gene has resulted in a more sophisticated resolution of the 5-HTT-LPR polymorphism. There are two functional variants on the \( L \) allele at position six of the first of two 22-bp imperfect repeats of the 16-repeat \( L \) allele. These two functional variants of the \( L \) allele result from a single nucleotide polymorphism (A>G, rs25531) upstream of the 5-HTT-LPR coding region. In several studies the \( L_G \) polymorphism is equivalent in its effects on transporter expression to the \( S \) allele. An almost three-fold difference in basal transcription is apparent in patients suffering from Obsessive Compulsive disorder between carriers of the \( S \) and \( L_A \) allele, but no significant difference between the \( L_G \) and the \( S \) (Hu, et al., 2006). Differences in serotonin transporter density and binding potential in the putamen also associate with the \( L_G \) and \( L_A \) triallelic format (Praschak-Rieder, et al., 2007), which also predicts treatment responses to anti-depressants (Hu, et al., 2007; Kraft, Slager, McGrath, & Hamilton, 2005).

### 6.3 Attachment and Early Environment

#### 6.3.1 Early environment: Theory and evidence

Early life experience significantly influences development. Infants are completely dependent on their caregivers for nourishment, shelter and care. According to the life history theory (Bateson, 1994; Belsky, Steinberg, & Draper, 1991; Chisholm, et al., 1993; Meaney,
2010), the offspring receives a significant amount of information about the safety of the environment through signals reflected in the nature of early parental care. For example, if food resources are low and the mother spends a large amount of time away from the child searching for food, her absence signals to the child a certain amount of threat in the environment. It is therefore possible that the child exposed to a caregiver who is not consistently available (emotionally, or physically) could develop expectations about the stability environment. In other words, from an early age children may develop ideas about the degree to which adversity is controllable.

6.3.2 Early environment: Animal studies

There are many elegant examples demonstrating the importance of early environment and how normal variations in caregiving influence developmental outcomes in non-human subjects. Seminal research by Harlow highlights the importance of warmth and comfort from caregiver on child emotional development (Harlow, 1958). The nonhuman primate field has continued this research showing that Rhesus Macaques reared in peer groups without maternal contact (as a measure of sub-optimal rearing conditions) display more anxiety-like behaviors, alcohol consumption, as well as enhanced physiological and behavioral reactivity to stress compared with maternal-reared animals (Higley, Hasert, Suomi, & Linnoila, 1991). The importance of control in the early environment on emotional development has also been evaluated by testing the effects of controllability of access to food and water (Mineka, Gunnar, & Champoux, 1986). Monkeys in the “non-control” group demonstrate more fear in face of a novel object and less exploratory behavior. Equally, monkeys with control display less anxious behaviors and are better able to distinguish safety from threat cues in their environment.
In studies with rats the importance of the early environment is apparent through studies on the effects of variations in maternal care on behavioral and physiological responses to stress (Francis, Diorio, Liu, & Meaney, 1999). Dams lick and groom their offspring. This behavior occurs within a normal distribution, but also shows considerable variation. Interestingly, pups exposed to higher levels of maternal licking and grooming display less anxiety-like behaviors than those pups reared by low licking mothers (Caldji, et al., 1998). Postnatal handling, a manipulation that increases the frequency of maternal licking/grooming, renders the offspring less susceptible to learned helplessness (Costela, Tejedor-Real, Mico, & Gilbert-Rahola, 1995).

### 6.3.3 Early environment: Human studies

There is a large corpus of literature on the role of extreme rearing environments such as maltreatment, orphanage rearing, and severe stressors in the pathophysiology of affective illness in humans. Parallels between maternal care in animal models and human childrearing practices can be drawn. For example, maternal sensitivity and responsivity (i.e. attunement, warmth, appropriate response to child’s cues) is associated with reduced anxiety and more modest stress responses in the offspring (De Wolff & van IJzendoorn, 1997). Conversely, harsh, controlling parenting, sometimes referred to as “affectionless control” is associated with increased anxiety. Maternal reported expression of anger and negative feedback to failure interacted with child's negative life events to predict greater negative thinking styles in a sample of 289 fifth grade students followed prospectively (Mezulis, Hyde, & Abramson, 2006). In contrast, responsive and sensitive childcare engenders a sense of control or security in the child (Bigelow, et al., 2010; Kobak, Cole, Ferenz-Gillies, Fleming, & Gamble, 1993).
**6.3.4 Attachment**

One way to bridge the research between parenting, control and the development of anxiety and depression is through the study of attachment (Chorpita & Barlow, 1998). Attachment to the primary caregiver is defined early in life. Attachment is an “innate behavioral–motivational system” (Del Giudice, 2009, p. 2) that ensures protection and care from the caregiver. In the first years of life the child regulates his or her emotional state through the assistance of the caregiver. This period of dyadic co-regulation is critical for the emotional development of the child. The child develops expectations of the caregiver that persist from “cradle to grave” (Ingram, 2003, p. 79). Current evidence suggests that attachment security is somewhat stable from infancy into adulthood (Waters, Merrick, Treboux, Crowell, & Albersheim, 2000).

Attachment schemas provide internal working models of the primary caregiver and are activated in times of stress (Bowlby, 1982). The accumulated histories of the interactions between primary caregiver and offspring engender the development schema. Parental sensitivity is associated with child attachment security (De Wolff & van Ijzendoorn, 1997; Higley & Dozier, 2009). Evidence from nonhuman primate research suggest that sensitive care versus standard peer-rearing is associated with a more organized attachment and higher scores on the Bayley scales of cognitive development (van IJzendoorn, Bard, Bakermans-Kranenburg, & Ivan, 2009).

The primary means of assessing attachment security in early childhood is through the Strange Situation paradigm (Ainsworth, et al., 1978). The Strange Situation paradigm is a series of increasingly stressful separation and reunion episodes between the child and his or her primary caregiver. In some episodes a “Stranger” is present (see Chapter 3 and Appendix for
further details). The patterns of behavior associated with attachment security classification can be assessed in children by inducing a mild stressor. The child’s stress response is thought to activate attachment schema and related internal working models. The four attachment categories are secure, insecure-ambivalent, insecure-avoidant and disorganized. A secure attachment is associated with sensitive parenting and parent-child interactions characterized by reciprocity, and warmth. In the Strange Situation paradigm a secure child may or may not be distressed by the departure of the caregiver. However, upon the return of the caregiver the child is relieved, seeks physical contact with their caregiver and is then able to further explore the environment.

Children without a secure attachment have mothers that have “disregarded their signals, or have responded to them belatedly or in a grossly inappropriate fashion…” (Ainsworth, 1979, p. 933). The child feels a loss of control over the environment and increased anxiety as he or she cannot count on the mother to be available or to behave in a manner that signals a safe environment. There are two categories of insecure attachment styles 1) insecure-ambivalent and 2) insecure-avoidant.

An insecure-ambivalent attachment is associated with inconsistent, unreliable parenting (Isabella, 1993). An ambivalent attachment manifests in displays of extreme distress upon separation from the caregiver (Ainsworth, 1979). However, when the parent returns the child is upset despite the presence of the mother. This behavior reflects the inability of the presence of the mother to effectively signal a safe environment. Insecure-avoidant attachment style is thought to be associated with emotionally unavailable, distant parents. Therefore, the child does not to display explicit emotional distress when separated from the caregiver. An insecure-avoidant child learns that such behaviors will not increase contact or comfort from the caregiver.
In the Strange Situation paradigm insecure-avoidant children ignore or shun their caregiver on return (Cassidy & Berlin, 1994).

The final, and more recent addition to the categories is the disorganized attachment category (Main & Solomon, 1990). Children are thought to develop a disorganized attachment pattern if they are maltreated or exposed to “frightened or frightening” caregivers (i.e. abused or abusive) (Lyons-Ruth, Bronfman, & Parsons, 1999). A disorganized pattern of attachment manifests itself through erratic behaviors in the Strange Situation paradigm. Children freeze or behave in an incoherent manner. This lack of organized pattern of response is associated with increased risk for psychopathology (van IJzendoorn, Schuengel, & Bakermans-Kranenburg, 1999). However, its validity as a measure of attachment security and not a measure of psychopathology is debated within the field (van Ijzendoorn & Bakermans-Kranenburg, 2003). To reconcile this debate many researchers look at the association with outcomes in children with secure versus “not secure” (as we refer to in this study “insecure”) attachment, grouping avoidant, ambivalent and disorganized attachment together.

6.3.5 Attachment: A candidate measure of early environment

An insecure attachment is associated with an increased risk for depression in adolescents (Sund & Wichstrom, 2002) and insecure and disorganized attachment is associated with externalizing behavior (Fearon, Bakermans-Kranenburg, van IJzendoorn, Lapsley, & Roisman, 2010). Child attachment security is also associated with measures of heart rate in response to a stressor (the Stranger Situation) (Spangler & Grossmann, 1993) and child self-image (Cassidy, 1988; Verschueren, Marcoen, & Schoefs, 1996). Therefore, research suggests that attachment security is associated with the development of psychopathology.
Attachment security also fulfills the criteria for a solid measure of the early rearing environment. Well-validated, unbiased measures are necessary to optimize the assessment of the early environment in relation to a given genotype (Moffitt, Caspi, & Rutter, 2005). Attachment measured through the Strange Situation paradigm is a developmentally appropriate and age-specific measure. The test eliminates many reporting biases as the categories are based on observation rather than parent or child report. The primary caveat of using attachment security is that it is not a direct measure of parental care. However, a secure attachment can be considered a proxy measure for a set of behaviors such as warm, sensitive parenting (Kochanska, Philibert & Barry, 2009). Conversely, a “not secure” attachment can be considered a proxy measure for a set of behaviors such as inconsistent, harsh care and potentially, at an extreme, maltreatment (Kochanska, Philibert & Barry, 2009).

6.4 5-HTTLPR x Environment

6.4.1 5-HTTLPR x environment: Theory and evidence

Despite compelling evidence for the role of the short allele of the 5-HTTLPR in the development of anxiety, depression and their intermediate phenotypes, the S allele is very common in the population and the mere presence or absence of the S allele alone does not strongly predict the incidence of affective illness. In a large representative sample 43 % of individuals possessed at least one copy of the short allele (Lesch, et al., 1996). The 5-HTTLPR genotype is a risk factor that associates with affective illness under particular environmental conditions. Research has thus shifted focus from genotype – phenotype relations to examine the interplay between genetic and environmental factors or the “behavioral effects due to interdependence between a specific identified variation in the DNA sequence and a specific measured environment” (Moffitt, Caspi, & Rutter, 2006, p. 6).
6.4.2 5-HTTLPR x environment: Animal research

In the male rat, the quality of the postnatal environment interacts with the SLC6A4 gene to effect acoustic startle and locomotor activity, two behaviors related to a fearful response (Belay, et al., 2011). A length variation homologous to the human 5-HTTLPR is found in Rhesus Macaques (rh5-HTTLPR). Macaques homozygous for the $L$ allele show improved emotion regulation on repeated neonatal assessments relative to those with at least one copy of the $S$ allele. Moreover, macaques with one copy of the $S$ allele and raised in nursery care showed poorer attentional orientation compared to those in the mother-reared condition (Champoux, et al., 2002). The interaction is also observed among female macaques, such that animals bearing one copy of the $S$ allele and raised in a peer-rearing environment (without maternal care) show increased adrenocorticotropic (ACTH) responses to stress (Barr, et al., 2004). Similarly, juvenile macaques with one copy of the $S$ allele that were peer-reared display more agitation after separation than those mother-reared or with two copies of the $L$ allele. These findings suggest that those individuals with one copy of the $S$ allele are differentially influenced by environmental adversity.

6.4.3 5-HTTLPR x environment: Human studies of anxiety, depression and their endophenotypes

Caspi and colleagues show in a prospective birth cohort, the Dunedin Multidisciplinary Health and Development study, that the risk for depression, as well as for suicidality, is associated with the interaction between genotype and exposure to adversity (Caspi, et al., 2003). Specifically, individuals with at least one copy of the short allele of the 5-HTTLPR and exposed to maltreatment during childhood are more likely to develop depression. Individuals with two copies of the long allele did not show an increase in depressive episodes as a function of childhood maltreatment.
In the past decade several groups have attempted to reproduce these results with mixed outcomes (Fergusson, Horwood, Miller, & Kennedy, 2011). Meta-analyses fail to unequivocally reconcile the inconsistent findings (i.e. Karg, Burmeister, Shedden, & Sen, 2011; Munafo, Durrant, Lewis, & Flint, 2009; Risch, et al., 2009). Such inconsistent findings linking either genotype or genotype by environment interactions to disease states are common and have led to an emphasis on the study of intermediate or endophenotypes (i.e. Lau & Pine, 2008). Endophenotypes are less complex, more easily quantifiable than psychopathology, and are present in individuals with or without active illness. Endophenotypes may shed light on the pathway between risk and psychopathology. Recent research finds support for the 5-HTTLPR by environment interactions for intermediate phenotypes such as of emotional regulation in children (Kochanska, Philibert, & Barry, 2009), heart rate reactivity to hospital admission in depressed patients (Shinozaki, Romanowicz, Kung, & Mrazek, 2011). Moreover, children with one copy of the \( S \) allele and whose mothers receive less social support have an increased risk for behavioral inhibition than children with two copies of the \( L \) allele (Fox, et al., 2005).

6.4.4 Helpless and negative thinking styles: An endophenotype of anxiety and depression

If an endophenotype is meaningfully associated with genotype, either in direct genotype – phenotype or within genotype by environment – phenotype relations, then variation across the population in the endophenotype should be heritable (Moffitt, et al., 2005). There is substantial evidence that negative thinking styles are heritable and thus potentially linked to genotype. This finding is consistent with data showing that both depression (Sullivan, Neale, & Kendler, 2000) and anxiety (Boomsma, et al., 2000; Hettema, Neale, & Kendler, 2001) disorders reveal modest heritability. Similarly, relevant endophenotypes that associate with depression and anxiety are
also heritable including anxiety sensitivity (Stein, Jang, & Livesley, 1999) and negative cognitive style in adolescents (Eley, et al., 2008; Lau & Eley, 2008).

One study to date has assessed the role of 5-HTTLPR and early adversity in association with negative cognitive reactivity (Antypa & Van der Does, 2010). Cognitive reactivity is a type of negative cognitive style that relates to the differential activation of negative thinking styles in the face of a low mood. Interestingly, undergraduate students homozygous for the \( S \) allele and exposed to less childhood emotional abuse were less likely to have a cognitive style reactive to low mood, in contrast to students with at least one copy of the \( L \) allele and exposed to more childhood emotional abuse (Antypa & Van der Does, 2010). Also pertinent to the current study, among adults, higher childhood socioeconomic status and having at least one copy of the \( S \) allele is associated with lower blood pressure and heart rate in the face of a mental challenge compared those with two copies of the \( L \) and lower SES (Williams, et al., 2008).

### 6.5 Summary

The research reviewed above suggests there are a number of studies that support a link between 5-HTTLPR genotype and early environment on the development of affective illnesses; however this evidence is not conclusive. The study of individual differences in endophenotypes of anxiety and depression may shed light on the nature of this association. Both disorders are characterized by cognitive styles that emphasize attention to negative outcomes, low self-evaluation and most importantly inability to control negative life events or outcomes. Likewise, variation in the 5-HTTLPR genotype associates with individual differences in the processing of threatening information as well as behavioral and endocrine responses to stress. Increased sensitivity to threat and enhanced stress reactivity both associate with an increased risk for affective illness. There is also considerable evidence for a link between parenting and both
depression and anxiety (Garber, Robinson, & Valentiner, 1997; Rapee, 1997). However, to date, we know little of the developmental pathways that lead to variation in such endophenotypes. Moreover, we also know little about the role of early environment and child genotype play in the developmental course of these intermediate phenotypes. Therefore, based on these findings we hypothesized that:

1) Children with at least one copy of the short allele of the serotonin transporter gene and an insecure attachment at age 18 months would display more negative self-report on the dimensions: self-evaluation, motivation and hopefulness on the Response to Challenge puzzle task than to children with one or neither factor.

2) Children with at least one copy of the short allele and an insecure attachment would display more helplessness behaviors and a longer delay in task initiation during the Response to Challenge puzzle task than to children with one or neither factor.

3) Children with at least one copy of the short allele and an insecure attachment would display an increased heart rate during the response to challenge puzzle, than to children with one or neither factor.

4) Children that displayed a greater helpless response on any of dimension of helplessness measures, would present with higher self or parent reported symptoms of psychopathology.
7 GENERAL METHODS

7.1 The Maternal Adversity Vulnerability and Neurodevelopment (MAVAN)

The MAVAN project is a prospective, longitudinal study of child development. It is a multidisciplinary, collaborative study that includes several laboratories from across Canada. The aim of the project is to assess the role of a myriad of factors such as maternal well-being and the mother-child interaction on child outcomes. The study has two recruitment sites, Montreal, Quebec and Hamilton, Ontario. The majority of mothers in the study were recruited during pregnancy. After birth the mother-child dyads were regularly assessed when the child was three, six, twelve and 18 months of age and then once yearly from age two years onwards. At each time-point, a battery of assessments is administered including assessments of the offspring emotional and cognitive functioning, maternal mood, and the interaction between the dyad. Biological measures such DNA were also taken. In the present study a selection of measures collected over the course of the study will be discussed.

7.2 Participants

Women were recruited during pregnancy from several hospitals across the island of Montreal, Quebec and Hamilton Ontario, Canada. Women were included in the study if they were 18 years of age and older and fluent in either English or French. Mothers were excluded from the study if they experienced any serious obstetric complications during the pregnancy or delivery of the child and if the child was born premature (≤37 weeks gestation) or with any congenital diseases. The Montreal cohort was selected from the general population. In Montreal sample recruitment was performed with an over-selection for children born small for gestational
age (SGA), as a proxy measure of fetal environment. The reference criteria for fetal growth was based on the “New and Improved Population-Based Canadian Reference for Birth Weight for Gestational Age”, where infants born SGA are in bottom 10th percentile of birth weight (Kramer, et al., 2001). The Hamilton sample was also recruited from the general population, with a high-risk group of women recruited from a mental health clinic (undergoing treatment for depression or anxiety).

Due to ongoing data collection and for completeness of the data analyzed, the current study is based on children from the Montreal sample, unless otherwise stated. Demographic information is listed below and based on the prenatal interview (Table 1). We chose to look at family characteristics during pregnancy as this is the period in which we have the most complete dataset, and has been shown to be critical for child future development (Rice, Jones & Thapar, 2006).

Seventy-six children were administered the helplessness outcome measure at age 60 months (Mean = 5.06, SD=0.08). Of these children 46 participated in the Strange Situation at age 18 months and 57 provided DNA samples. In the main analyses, we have complete data on 45 children (sample size will be specified for each set of analyses). Finally, on the secondary outcome measure of psychopathology, we have mother and/or father Strength and Difficulties Questionnaire for every child (N=75) and the majority of children completed the self-report Dominic/que assessment at age 72 months (N=70).
Table 1: Family Demographic Information

<table>
<thead>
<tr>
<th>Measure</th>
<th>Percentage or Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mother’s age at birth of child</td>
<td>28.8 year, (SD 4.9)</td>
</tr>
<tr>
<td>Mother ever smoked during pregnancy</td>
<td>18.7%</td>
</tr>
<tr>
<td>Mother ever consumed alcohol during pregnancy</td>
<td>41.3%</td>
</tr>
<tr>
<td>Previously pregnant</td>
<td>44.7%</td>
</tr>
<tr>
<td></td>
<td>31.4% One other child</td>
</tr>
<tr>
<td></td>
<td>8.6% Two other children</td>
</tr>
<tr>
<td></td>
<td>60% (Not live birth)</td>
</tr>
<tr>
<td>Child’s birth weight</td>
<td>3149.22 g (SD 437.9g)</td>
</tr>
<tr>
<td>Percentage born SGA</td>
<td>27.6%</td>
</tr>
<tr>
<td>Child gender</td>
<td>42 female (55.3%)</td>
</tr>
<tr>
<td>Child Ethnicity: Caucasian</td>
<td>65.7%</td>
</tr>
<tr>
<td></td>
<td>7.9%</td>
</tr>
<tr>
<td></td>
<td>4.5%</td>
</tr>
<tr>
<td></td>
<td>20.9%</td>
</tr>
<tr>
<td>Child language: French</td>
<td>67.6%</td>
</tr>
<tr>
<td></td>
<td>13.2%</td>
</tr>
<tr>
<td></td>
<td>19.1%</td>
</tr>
<tr>
<td>Child language: English</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td>Mother’s highest level of education achieved:</td>
<td></td>
</tr>
<tr>
<td>Primary school</td>
<td>9.2%</td>
</tr>
<tr>
<td>High School</td>
<td>7.9%</td>
</tr>
<tr>
<td>CEGEP, trade, college</td>
<td>35.5</td>
</tr>
<tr>
<td>University</td>
<td>46.1%</td>
</tr>
<tr>
<td>Father’s highest level education achieved:</td>
<td></td>
</tr>
<tr>
<td>Primary school</td>
<td>9.2%</td>
</tr>
<tr>
<td>High School</td>
<td>25%</td>
</tr>
<tr>
<td>CEGEP, trade, college</td>
<td>21.1%</td>
</tr>
<tr>
<td>University</td>
<td>39%</td>
</tr>
<tr>
<td>Missing (no partner, not answered)</td>
<td>5.3%</td>
</tr>
<tr>
<td>Family’s income</td>
<td>&lt; 20 000 $</td>
</tr>
<tr>
<td></td>
<td>14.7%</td>
</tr>
</tbody>
</table>

7.3 Materials

7.3.1 Attachment security: The Strange Situation

The Strange Situation is a standardized laboratory-based measure of infant attachment security (Ainsworth, et al., 1978). The paradigm consists of a series of eight increasingly stressful separation and reunion episodes between the child and his or her primary caregiver. In
the present study, the mother is the caregiver assessed. The assessment took place in the MAVAN laboratory in Douglas Mental Health University Institute, Verdun, Quebec.

The Strange Situation was videotaped and the infant’s behavior toward his or her mother was coded by the research team of Dr. Leslie Atkinson at Ryerson University in Toronto Ontario. Based on the recordings children were classified as secure (B), avoidant (A), resistant (C) (Ainsworth, et al., 1978) or disorganized (D) (Main & Solomon, 1990). The disorganized classification is coded either as a dimension, on a scale of 1 to 9, or categorically, as “D” or “not D”. The disorganized classification is considered orthogonal to the organized attachment groups and it is standard procedure to force classify D into the best-fitting alternative classification (A, B, or C; Main & Solomon, 1990).

Reliability for the coding was based on 27 randomly selected tapes, rated by two coders. Reliability for D as a dimension (rho = .64, p < .05 was significant,) but not as a category. The failure to obtain reliability on D is not uncommon (van IJzendoorn, et al., 1999). Therefore, we coded cases that first coded as D into the best-fitting alternative “organized” classification. The coders obtained 78% agreement (Cohen’s kappa (k) = .59, t = 3.68, p < .0005) on the three-way (A, B, C) classification and 81% agreement (k = .63, t = 3.34, p < .001) on the secure/insecure dichotomy. For the present analyses, due to our small sample size and evidence supporting the comparison of secure versus insecure/”not secure” as a measure of early environment (De Wolff, van IJzendoorn, 1997), we used the dichotomized secure versus insecure classification. Sixty-two children in the present study took part in the Strange Situation and had videos of codable quality. Approximately half the children were coded as insecure (46.8%) and secure (53.2%), which is consistent with other studies considering the high-risk nature of our sample (van IJzendoorn & Sagi, 1999).
7.3.2 Genotyping

Deoxyribonucleic acid (DNA) samples were extracted using buccal cheek swabs. Cheek swabs are an ideal method for collecting DNA from children, since taking blood samples is difficult with this sample. Samples were extracted using soft cotton Omniswab brushes. Four samples (two brushes for each cheek) were collected per child. After extraction, the samples were dried for 24 hours and then placed within one conic tube and shipped for analysis.

DNA extraction and genotyping for 5-HTTLPR was performed at the Center for Addiction and Mental Health (CAMH) in Toronto (Ontario, Canada) under the supervision of Dr. James Kennedy. A modified QIAGEN DNA Mini Kit was used to extract the DNA and Polymerase Chain Reaction (PCR) were employed to detect the 5-HTTLPR polymorphism. More specifically, the Single-Stranded Conformational Polymorphism (SSCP) technique, a novel PCR-RFLP detection method developed in the Kennedy laboratory was used. DNA sequencing was performed with an ABI Prism 310 Genetic Analyzer. Children were then categorized as having one or two copies of the $L_G, L_A$ and $S$ alleles. As previously mentioned, due to functional similarities, the $L_G$ variant was considered as an $S$. For our analyses children having one copy of the $S$ allele or the functional equivalent $L_G$ were grouped as $S$ allele together (from here on, called “S”).

7.3.3 Child helplessness

Child helplessness was measured with a “Response to Challenge” Puzzle task (RCP). The materials and procedure of this task are covered in detail in Chapter 4.
7.3.4 Strengths and Difficulties Questionnaire (SDQ)

The SDQ is a 25 item questionnaire that pertains to the child’s behavior between the ages of 3 and 16. For example, reporters (often the parent) are asked if the child is “Often unhappy, depressed or tearful”. Each question is answered using a 3-point likert scale from “Not true”, “somewhat true”, to “certainly true”. The questions may be compiled to create 5 sub-scores: emotional symptoms, conduct problems, hyperactivity/inattention, peer relationship problems, and prosocial behavior. The four first scores may also be summed to create a “total difficulties score”. The SDQ concludes by asking the parent whether they believe that the child has a problem, and to the extent the problem causes the child distress, social impairment, and burden to others and its chronicity. The questionnaire is widely used in clinical and research settings and has good psychometric properties (Stone, Otten, Engels, Vermulst, & Janssens). In a study of 10,438 5-15-year-olds the reliability coefficient indicated satisfactory internal consistency across scores (mean of 0.73) and impairment scores (0.8 or higher) (Goodman, 2001). The retest stability after 4 to 6 months was 0.62 months. The questionnaire in the MAVAN project was administered when the child was 60 months of age. The questionnaire was completed by the child’s mother and father. We have SDQ data from maternal and/or paternal report for all children who took part in the puzzles (N=76).

7.3.5 Dominic/que-R

The Dominic/que-R is a child self-report measure of symptoms of psychopathology. The measure consists of a set of 91 cartoons, depicting a child, Dominic/que-R in various scenarios. Each scenario represents various psychopathology symptoms, inter-mixed with normal behaviors. The sex of Dominique is ambiguous to allow for all children to identify with the character. The child is asked whether they are “like Dominique” in the picture. For example
“Are you afraid of heights, like Dominic?” which require either a yes or no answer. The measure takes between 15-20 minutes to administer. Scores are tabulated using DSM III-R diagnoses for depression, hyperactivity, general anxiety, social anxiety, phobia, and conduct problems. It should be noted that an electronic version of the Dominic/que-R exists, that tabulates DSM-IV diagnoses. However, based on the advisory of one of the measure’s creators (A. Wazana, personal communication), we selected the paper version as it is more developmentally appropriate (easier to comprehend) in children aged six.

The reliability of a Dominic/que-R-based diagnoses with clinical judgment based on DSM-III-R diagnoses ranged from 0.64 to 0.88 (Valla, Bergeron, Berube, Gaudet, & St-Georges, 1994). Within a large sample of children from France and Quebec, internal consistency across measures was high, for example 0.90 and 0.89 for internalizing and externalizing disorders respectively (Shojaei, et al., 2009).

7.3.6 Statistics

The statistical analyses described in this study were carried out using SPSS, version 16. A p<0.05 was our designated cut-off for statistical significance. Where appropriate, corrections for multiple testing were applied. These will be mentioned where applicable.

7.4 Procedure

Ethical approval for this study was obtained from the Douglas Mental Health University Institute (see Appendix). Women were recruited to take part in the MAVAN study during pregnancy from obstetric units across the island of Montreal. During the prenatal visit, mother’s well-being as well as family demographic information was collected. If accepted in the study, the mother-child dyads were assessed regularly during the first year of life. When the child was
eighteen months old, the dyad participated in a laboratory assessment. Upon arrival at the laboratory, the mother and child were greeted by a research assistant. Shortly after, the dyad participated in the Strange Situation. A detailed description of the Strange Situation laboratory procedure is outlined in the Appendix (Table 3).

At the three year laboratory visit DNA samples were extracted. A separate consent form was signed for the taking of the genetic samples. Prior to sampling mothers were instructed not allowed the child to eat or drink. The samples were taken either by the mother or the research assistant (depending on the child). Important information for genotyping (i.e. birth date) was also gathered.

When the child turned five years of age, several assessments of the child development were administered. The RCP were administered during a visit to the child's home. Detailed explanation of the task administration may be found in Chapter 4. At the same 60 month time point both the mother and father report child concurrent emotional or behavioural symptoms with the Strengths and Difficulties questionnaire. Finally, one year later children also report their own level of psychopathology symptoms at a home visit at six years of age using the Domine/que. Note, the families were compensated for each interview.
8 RESPONSE TO CHALLENGE PUZZLES

8.1 Materials

The protocol for the Response to Challenge Puzzles (RCP) was a modified version of a puzzle task previously used in five year old children, by Cole and colleagues (Cole, et al., 2007). The anagram puzzles were created using Binary Arts, Shape by Shape puzzles. The materials required to complete the task were the puzzle base, pieces and five images, the coding sheets and a stop watch. The puzzle base was bright green and 13cm x 13cm. The fourteen pieces are red and yellow and 1 cm in depth. The pieces are glued together to make eight, larger pieces (Appendix, Figure 11). The pieces were glued together ensure that some of the puzzles would be possible (and easy) and others would be impossible to solve. The images were yellow and red with a green trim to replicate the pieces and base. The 9.6 cm by 9.6 cm images were roughly the size of the puzzle base. The pictures were enlarged and laminated from the images provided by the Shape by Shape game. We pilot tested the ease of the puzzles with a group five year old children. The child completed puzzles and were asked to rate their performance.

8.2 Self-report

8.2.1 Self-report: Procedure

The puzzles were administered in the child’s home, as part of a larger battery of tests administered at the 60 month time-point (most data not included in this thesis, unless otherwise specified). A wrist-mounted heart rate monitor was placed on the child just prior to the start of the puzzle task. Next, the child was instructed how to use two age-appropriate rating scales to assess his or her performance during the puzzle task. First the child was explained how to use a happy face rating scale, containing 5 faces ranging from “very happy to “very
unhappy” (Appendix, Figure 10). To practice with this scale the child was asked, “How do you feel about doing the puzzles?”. Following, the child was shown a star rating scale with 5 columns of starts, with increasingly greater number of starts on each row (Appendix, Figure 9). This scale was used to measure performance appraisal. To practice with this scale the child was asked, “How well do you think you will do on the puzzles” and, “How well do you think the other children in the study will do on the puzzles?”. The child’s responses to these questions were considered practice trials. Before the start of the task the child was encouraged to speak out loud and this was demonstrated by the research assistant. The purpose of speaking out loud was to measure self-talk and negative statements.

The design of the task was a series of five puzzles after which the child was asked to rate his or her feelings about the task after each puzzle was complete. The main task started with a solvable puzzle, where the child had unlimited time to solve the task. To ensure that each child felt that he or she was successful in completing the task, the research assistants were instructed to provide help surreptitiously to the child if he or she could not complete the task alone. After the puzzle was completed the child was asked the three questions (similar to those at baseline): “How well do you think that you did on the puzzle?” , “How well do you think you will do on the next puzzle?” and “How do you feel about doing the next puzzle” to measure self-evaluation, hopefulness and motivation, respectively.

Following, the child was administered three impossible puzzles. As previously mentioned, the puzzles were super-glued together to ensure that the images were impossible to re-create. Before the start of the second puzzle the child was told that he or she only had two minutes to work on the puzzle, and that the image would be removed when the time passed. The child completed three impossible puzzles. The research assistants were instructed not to tell the
child the puzzles were impossible, and to encourage the child to complete the task. However, in the case that the child refused to do the puzzle, or showed real distress the puzzle was curtailed. Like with the solvable puzzles, the child was asked the three questions after each impossible puzzle.

The fifth and final puzzle was solvable and like the first, the child has unlimited time to work on the task. After completing the final puzzle the child answered the three questions. Finally, the child was told that there were no more puzzles and the child was asked to rate their overall appraisal of the task. At the end of the puzzle task the child was congratulated for his or her effort and allowed to select a prize. Anecdotal evidence from the research assistants suggest that most children stated that the overall puzzle task as enjoyable, once the RCP was complete.

8.3 Helplessness Behavior Coding

8.3.1 Behavior coding: Materials

The RCP task was video recorded and the tapes were coded to assess behavioral manifestations of helplessness during the task. The Noldus Observer XT 7, version 7.0.214 coding software was used to record the behaviors. An LG computer, powered by Windows 2003 was used for coding. To view the videos a second monitor was installed. The screen was a Samsung SyncMaster701N, 35 x 27cm in size. This allowed the coding scheme to appear on one screen and the video to be viewed clearly on the other.

8.3.2 Behavior coding: Procedure

To create the coding scheme all the videos were observed. Based on these observations the behaviors most frequently viewed were recorded. The behaviors listed in the manual provided by David Cole, as well as the behaviors counted by other similar studies were
considered (Diener & Dweck, 1978; Murray, et al., 2001). The coding scheme was created in the Noldus software. In order to have a more quantitative and thorough measure of behaviors, we created a novel coding scheme in which every four seconds the presence of each behavior was considered and recorded if present. The videos were coded by a coder blind to the genotype and attachment security of the child. The puzzle was coded for a maximum of two minutes, to ensure that each child’s behavior reflected the same exposure to stress. To count the presence of behaviors of interest, the X-keys USB Switch Interface was programmed so that each button represented one behavior.

To develop the coding scheme, 10 percent of the videos available were coded for over thirty behaviors including smiling, scratching forehead etc. Next, the data was visually scanned to determine which behaviors were most frequent and demonstrated the greatest variation across the task. We narrowed our behavior selection and refined our coding scheme to include a large range of positive and negative affect related behaviors. A list of the behaviors coded and their descriptions are found in Table 2. All videos were coded using this coding scheme. Inter-rater reliability was tested for 10 videos. We chose to select the behaviors with a minimum kappa coefficient of 0.6 or higher, unless the kappa was above 0.5 and the percent agreement between coders was over 90%. The behaviors retained for analyses are listed on Table 2.
### Table 2: Behaviors Coded in RCP

<table>
<thead>
<tr>
<th>Behavior</th>
<th>Description</th>
<th>Kappa value</th>
<th>% Agreement</th>
<th>Status</th>
</tr>
</thead>
<tbody>
<tr>
<td>Frown</td>
<td>Down-turned lips</td>
<td>0.18</td>
<td>60%</td>
<td>x</td>
</tr>
<tr>
<td>Growl</td>
<td>Aggressive vocalization, no content,</td>
<td>0.41</td>
<td>60%</td>
<td>x</td>
</tr>
<tr>
<td>Smile</td>
<td>Up-turned lips</td>
<td>0.64</td>
<td>95%</td>
<td>Retained</td>
</tr>
<tr>
<td>Request Assistance</td>
<td>Ask for help</td>
<td>0.73</td>
<td>97%</td>
<td>Retained</td>
</tr>
<tr>
<td>Move piece quickly</td>
<td>Moving pieces frantically on base, trying new pieces one after the other</td>
<td>0.34</td>
<td>90%</td>
<td>x</td>
</tr>
<tr>
<td>Negative Tone</td>
<td>Sad, irritated or aggressive tone of voice</td>
<td>0.56</td>
<td>95%</td>
<td>Retained</td>
</tr>
<tr>
<td>Negative Vocalization</td>
<td>Negative statements about the task or self.</td>
<td>0.68</td>
<td>98%</td>
<td>Retained</td>
</tr>
<tr>
<td>Lean Away</td>
<td>Moving body position away from the table.</td>
<td>0.2</td>
<td>87%</td>
<td>X</td>
</tr>
<tr>
<td>On task</td>
<td>Engaged in puzzle task, eyes on puzzle material</td>
<td>0.86</td>
<td>96%</td>
<td>Retained</td>
</tr>
<tr>
<td>Head on hands</td>
<td>Resting head on hands or resting on arms (if on the table)</td>
<td>0.79</td>
<td>98%</td>
<td>Retained</td>
</tr>
<tr>
<td>Positive vocalization</td>
<td>Positive statements (including tone and content)</td>
<td>0.53</td>
<td>98%</td>
<td>Retained</td>
</tr>
<tr>
<td>Sigh</td>
<td>Long inhalation or exhalation.</td>
<td>0.57</td>
<td>96%</td>
<td>Retained</td>
</tr>
<tr>
<td>Rough</td>
<td>Moving pieces in an overtly aggressive manner. Child may push away the table or throw image.</td>
<td>N/A</td>
<td>98%</td>
<td>Retained</td>
</tr>
</tbody>
</table>

In order to have another measure of helplessness, one that is less influenced by the expressiveness of the child, we also measured the delay in initiation of the task. Delaying the start of the task might also be indicative of underlying belief that one is not capable. Latency of response was determined to be the time elapsed between the start of the puzzle and the child starting the task. We operationally defined the child’s initiation of the task, as the moment the child placed the first piece flat on the base. We coded this by playing the video at one 20th speed and listening for the sound of contact. Inter-rater reliability was verified on 10 videos. Intraclass correlations were excellent (0.9).

The next step in creating a measure of helplessness was to determine the proportion of 4 second intervals in which a select behavior was observed. This gave percentages for each behavior over puzzle one to five. We were then interested to see whether these behaviors co-occurred to measured positive or negative coping styles. We hypothesized that the behaviors: negative tone, negative vocalization, head resting on hands, sigh, increased latency of response,
disengaging from the task and rough behavior would occur together to reflect helpless coping. It was hypothesized that positive vocalization and smiling would be considered positive coping.

To create a helplessness/negative affect score we performed a Principal Component Analysis (PCA) on each puzzle. First we tested for redundancy, and ensured that no variables, across puzzles correlated too highly. The highest correlation between behaviours was negative tone and negative vocalization at 0.77, 0.78, 0.8, 0.87 and 0.75 for puzzle one to five respectively. Both variables were retained for analyses. Next, we tested the distribution of the variables. Most variables were highly skewed. Therefore, applying the standard rule that 20% of values should not be one value or zero (in our case all were 48% or higher), all variables except disengagement for puzzle one to four and latency for puzzle one to five were dichotomized. Next, to normalize the remaining dimensional variables, a logarithmic transformation was applied to the latency and disengagement variables. These transformations were verified by visually appraising the P-P plots. It is important to note that the PCA was performed on our full dataset of children who took part in the puzzles (N=102). The children are excluded from the analyses due significant missing data. The decision to include all children for the PCA was to increase the sample, and thus the power of the PCA. For all factors created there were no significant differences between the children included or not included in this study.

Once all the data was prepared, the PCA were performed on each puzzle separately. Variables were retained for analysis if they loaded on to the factor with a minimum loading of 0.4. A varimax, orthogonal rotation was applied to each factor. We retained all factors accounting for a minimum of 20% of the variance. Note, for all PCAs only the first factor accounted for enough of the variance to be included. Variance accounted for by Factor 1 for puzzle one to five are 22.65%, 23.43%, 24.7%, 26.9 and 25.55%, respectively.
For puzzle one the determinant or test of multi-collinearity was a satisfactory .3 and the Kaiser-Meyer-Olkin (KMO) measure of sampling adequacy was above the minimum .5 cut-off (.6). Factor one reflected a helpless cluster of behaviors with sufficient factor loadings included: requesting assistance, negative tone and vocalization and sighing. For puzzle two, the determinant was 0.18 and the KMO was .56. As with the first puzzle, factor one from puzzle two reflected helplessness behaviors, and consisted of the behaviors resting head on hands, asking for assistance, negative tone and vocalization, and sighing. Puzzle three also met criteria for a principal component analysis with a determinant of .17 and KMO of .59. The first factor only included vocalization variables and thus could be considered a “helplessness/negative affect vocalization” factor. For puzzle four the determinant was .15 and KMO was .63. The factors that was created included the rough behavior, negative tone and vocalization and disengaging from the task. Finally, the PCA had a determinate of 0.15 and a KMO of .59 for puzzle five created another helplessness vocalization factor including the variables negative tone and vocalization and sighing.

8.4 Heart Rate

8.4.1 Heart rate: Materials

To measure the child’s physiological response to the puzzle challenge, the child wore a heart rate monitor (HRM) for the duration of the task. The HRM had two components. The first component was a Polar Wearlink transmitter with an adjustable band, one inch in width, that was worn around the child’s chest. There are two electrodes on the transmitter that was placed on the front of the child’s chest. The second component was the Polar RS400 watch computer. The watch weighs 45g and was worn by the child or placed on the table. The Polar RS400 reports accuracy up to ±1beat per minute. Based on the recorded inter-beat interval the child’s average
heart rate was measured in five second intervals. The HRM data was uploaded using the Polar ProTrainer 5 software for storing the HRM data.

### 8.4.2 Heart rate: Procedure

Before placing the HRM on the child, the research assistant moistened the electrodes with water to facilitate transmission. The watch timer was activated and placed on the table or the child’s wrist. The instruction period was used as the baseline as this period was standard across each child. Then, to measure heart rate we used two measures: the average heart rates at baseline and each puzzle, as well as the change from baseline for each puzzle. In order to calculate the “reactivity” or change from baseline, the mean heart rate each puzzle was subtracted from the mean at baseline.

To determine the start times for the baseline and puzzles the timestamps from the video codes were taken from the Noldus behavior code outputs. These times were used to synchronize the behavior codes with the HRM files. The six events were identified on the videotape and their specific time was recorded, and matched with the corresponding HR interval. In order to synchronize the HR files with the video times the research assistants said a key phrase (“Let’s start”). Times between the seconds into the video, actual time, and time into the HRM were calculated. Inter-rater reliability for the timing of the “Let’s start” and each puzzle was calculated with excellent interclass correlation reliability coefficients of “Let’s start”, instruction and puzzle start times at 1.00, 0.96, and 0.9 respectively.

### 8.4.3 Summary of measures

In sum, there are three types of outcome measures of helplessness used in the following analyses. First, there are three measures of self-report: self-evaluation, hopefulness and
motivation, across each puzzle. Second, there are two types of behavioral data: the behavior factors from puzzle one to five and latency of response across puzzles. Third, there are two measures of heart rate: average heart rate collected at baseline and across puzzles one to five and the change of average heart rate across on puzzle one to five. Each measure of helplessness will be assessed in relation to child 5-HTTLPR genotype and attachment security.


9 RESULTS

The results are presented in relation to the main hypotheses on the interaction between attachment security and child 5-HTTLPR across three domains of helplessness: self-report, behavior and physiological reactivity. The sample size varies depending on the domain of helplessness assessed due to missing data. In light of the multiple demographic measures available and to avoid controlling for spurious associations, a factor was considered a potential confound if it correlated with at least 40% of the dimensions tested (for example, two out of the five puzzles). Prior to the start of our main analyses we tested whether there was a differential distribution of child genotype within the different attachment groups. Pearson chi² revealed no significant difference \( \chi^2(1, N = 50) = .73, p = .39 \).

9.1 Self-report

Seventy-six children of the Montreal cohort were administered the puzzle task. Data are missing at puzzle one due to error in administration (N=2), refusal on puzzle two (N=3), three (N=3) and four (N=8) and illness on puzzle four and five (N=1), therefore we had complete self-report data on seventy children. Due to missing attachment and child genotype data the total sample with complete data was N= 45, with N=16 Insecure/SS/SL, N= 4 Insecure/LL, N= 17 Secure/SS/SL and Secure/LL N=8.

The association between child self-report and potential covariates was verified. No relationship was observed between the self-report questions and most of the demographic variables of interest (Table 1). However, independent samples \( t \)-test revealed widespread gender differences on the self-report data. On the measure of motivation (question three) \( t(8)=2.89, \)
p<0.05] of the first puzzle boys (M= 4.39, SD=.93) rated their performance significantly lower than girls (M=4.78, SD=.58). On puzzle two for the measure of hopefulness \[ t(8)=2.89, p<0.05 \] boys (M=3.65, SD=1.37) rated themselves as significantly less hopeful than girls (M=4.49, SD=.95). On the second puzzle \[ t(8)=2.35, p<0.05 \], boys (M=3.32, SD=1.37) also rated themselves as less motivated for the next puzzle than girls (M=4.1, SD=1.2). On the third puzzle \[ t(8)=2.89, p<0.05 \] again, boys (M=2.68, SD 1.55) rated themselves as less hopeful than girls (M=3.88, SD=1.62). Conversely, there was a significant difference in self-evaluation in the opposite direction on puzzle four self-evaluation \[ t(8)=2.89, p<0.05 \], where girls (M= 2.35, SD=1.44) rated their performance lower than boys (M=3.12, SD=1.75). Therefore, in all our analyses of the child self-report gender was included as a covariate. In light of the pervasive gender differences, we also tested whether there was an association between gender and our predictor variables. There was no differences in attachment categorization \[ \chi^2(1, N = 62) = 2.51, p = .11 \] amongst males or females. There was no significant difference \[ \chi^2(1, N = 59) = .29, p = .59 \] in the distribution of child genotype between males and female.

The data were analyzed using a three-way repeated measures analysis of variance, with puzzle as the within-subject measure and gene and attachment as between-subjects variables. A modest Sidak correction for multiple testing was applied for all tests. We chose a modest correction due to the sample size, and the preliminary nature of these analyses (See section 6.2 for further discussion). To view the self-report trends for the entire sample for puzzle one to five see Figure 1.

### 9.1.1 Self-evaluation

There was an overall significant main effect \[ F(4, 160)=16.99, p<0.05 \] for puzzle number on the measure of self-evaluation. When testing for the simple effects, the Mauchly’s test was
significant and thus sphericity was not assumed. A Greenhouse-Geisser correction was applied. The analyses revealed a significant difference in the ratings of the “possible puzzles” versus the “impossible puzzles”. With both ratings in puzzle one and five significantly different than puzzle two to four p<0.05, but not each other p= 1.00. Similarly, the ratings on the impossible puzzles were not significantly different from each other.

![Child Self-Report Across Puzzle # 1-5](image)

**Figure 1: Child Self-Report Across Puzzle # 1-5**

There was a main effect [F(1,40)=5.39, p<0.05] of child genotype on child reported self-evaluation. Children homozygous for the L allele rated their performance higher than those with at least one copy of the S allele. There was also an overall interaction effect [F(1,40)=4.8, p<0.05] between 5-HTTPLR genotype and attachment. Pair-wise comparisons [F(1,40)=6.95, p<0.05] revealed that children with one copy of the S allele those with an insecure attachment rated their overall rating of performance as significantly lower than those with a secure
attachment. There was no difference \([F(1,40)=1.03, p=.317]\) by attachment security amongst those with two copies of the \(L\) allele. We also tested for effects as a function of their attachment security. No difference \([F(1,40)= 0.02, p=0.90]\) amongst children with a secure attachment on child reported self-evaluation as a function of the presence or absence of one copy of the \(S\) allele.

There was a significant interaction \([F(4,160)=2.53, p<0.05]\) between puzzle number and 5-HTTPLR genotype. Comparisons revealed a significant difference in self-evaluation ratings between genotype groups on puzzle two \([F(1,42) 4.87, p=0.05]\) and at trend level on puzzle four \([F(1,42)=3.91, p=0.06]\). In both cases, children with one copy of the \(S\) allele rated their performance lower than those with two copies of the \(L\) allele. Comparisons were made for each genotype group across puzzles. The analyses revealed that there were no significant differences in ratings of self-evaluation within the \(LL\) group across puzzle one to five. However, amongst the \(SS/SL\) group, scores of self-evaluation were significantly different between \([F(4, 39)=23.77, p<0.05]\) the possible and impossible puzzles.

A three-way interaction effect between puzzle, 5-HTTPLR genotype and attachment security \([F(4, 160)=3.16, p<0.05]\) on self-evaluation is shown in Figure 4.1. Two-way interactions were assessed across each puzzle. Analyses revealed amongst children with an insecure attachment, those with one copy of the \(S\) allele rated their performance significantly lower than homozygous for the long \([F(1,4)=16.57, p<0.05]\). There was no significant effect differences by 5-HTTPLR genotype in children with a secure attachment \([F(1,4)=.38, p=.54]\). We evaluated the simple effects to test our hypothesis that children with one copy of the \(S\) allele would be differentially susceptible to the environment. We found that amongst children with at least one copy of the \(S\) allele, those with a secure attachment rated their performance
significantly higher than those with an insecure attachment \([F(1,40)=14.5, p<0.05]\). An unexpected trend was also observed amongst children homozygous for the \(L\) allele with a secure attachment. These children rated their performance lower than those with an insecure attachment \([F(1,40)=3.97, p=0.053]\) (Figure 2).

![Attachment x 5-HTTLPR: Puzzle #4 Self-Evaluation](image)

**Figure 2: Attachment x 5-HTTLPR: Puzzle #4 Self-Evaluation**

### 9.1.2 Hopefulness

There was a significant main effect \([F(4,16)=3.46, p<0.05]\) for puzzle number on child rating of hopefulness. Multivariate comparisons revealed an interesting pattern of results. Ratings of hopefulness on the first puzzle were significantly different than the ratings at puzzle three and four. The mean ratings on the second puzzle were not significantly different from any other puzzle. The hopefulness ratings on puzzle three and four rating were only different from
the first puzzle ratings. Finally, hopefulness ratings at puzzle five were not different from any other puzzle.

There was also a trend for an attachment by puzzle interaction [F(4, 16) 2.26, p=0.065] on child reported hopefulness. Despite non-significant findings, exploratory pair-wise comparisons were performed to disentangle these effects, as the role of attachment security is critical to this study. Analyses revealed that there was no significant difference amongst the securely attached children for their ratings of hopefulness across the puzzles. There was a trend effect difference amongst the insecurely attached children between puzzle one and puzzle four [F(4, 37) 2.89, p=0.067]. There were no other main or interaction effects detected in these analyses.

9.1.3 Motivation

There was a main effect [F(4,16)= 3.46, p<0.05] for puzzle number within the motivation ratings. Multivariate comparisons revealed that the first puzzle mean ratings were different from puzzle two to four ratings. Ratings on the impossible puzzles (two to four) were only different from the first puzzle one, as puzzle five was not significantly different from any other the other ratings. No other main effects or interactions were significant.

9.1.4 Summary of self-report data

First, preliminary analyses revealed that males and females generally rated their performance similarly. However, on several occasions boys rated their performance lower than girls. This is interesting and in line with research that demonstrates gender differences in anxiety and depression onset is in adolescence. The analyses revealed that our hypothesis was supported for the self-report data on the ratings of performance evaluation. Overall, that there was an overall main effect of child genotype. The difference was seen at puzzle two and at trend level
for puzzle four. Children with at least one copy of the S allele rated their performance lower, suggesting they were more affected by the stress of failure. These effects were superseded by an interaction between attachment and genotype. Across, all puzzles our proposed “risk group” with an insecure attachment and at least one copy of the S allele rated their performance lower. Moreover, in a three-way interaction the fourth impossible puzzle showed the most pronounced group differences. Those with one copy of the S allele and an insecure attachment rated their performance lowest. In the measure of hopefulness child ratings decreased at trend level if they had an insecure attachment. Interestingly, children with a secure attachment scores did not seem to significantly decrease over the course of the puzzles. This finding suggests they were buffered from the effects of the repeated stress. Lastly, although there were differences across the sample in motivation ratings, there were no main effects or interactions with this data.

9.2 Behavioral Data

Based on the Principal Component Analyses mentioned in Chapter 3.0, the helplessness scores were assessed for each puzzle. To begin, all potential covariates were tested. No variables correlated with helplessness across all puzzles. This is consistent with previous studies of child negative cognitive style (Murray, Woolgar, Cooper, & Hipwell, 2001). Puzzle one helplessness data was available for 47 children. Amongst those children N= 21 insecure (17 of those had one copy of the S allele) and N=26 secure (18 with one copy of the S allele). A univariate ANOVA was performed on the helplessness factors of puzzle one. There was no main effect for attachment. There was a trend \( [F(1,3)=.19, \ p=0.081] \) for a main effect of child genotype (Figure 3). Children with one copy of the S allele displayed more helplessness than children with two copies of the L allele. No interaction for child genotype and attachment was observed.
Univariate ANOVAs were also assessed for helplessness on puzzle two to five. No significant main effects of child attachment, child genotype or their interaction were observed. In light of the findings of the uncertainty of the division of 5-HTTLPR, we also performed exploratory analyses looking at the three groups of child genotypes separately (i.e. SS vs. S/L vs. LL) by attachment security through graphs. For puzzle one, two and five the error within each group overlapped significantly. However, we observed effects in puzzle three (Figure 4) and four (Figure 5), where children with two copies of the SS allele tended to demonstrate more helplessness (vocalization factor for puzzle three) and an insecure attachment than those with at least one copy of the L and an insecure attachment.
Figure 4: Attachment x 5-HTTLPR: Puzzle #3 Helplessness Vocalization

Figure 5: Attachment x 5-HTTLPR: Puzzle #4 Helplessness Behavior
9.2.1 Latency of response

Since latency of response was a behavioral variable of particular interest, and itself did not load on to any factor in the PCA, it was assessed separately. First, the effects of any confounds were assessed. No child or parental demographic variables were associated with child response latency. As previous mentioned, the latency variables were not normally distributed but benefited from logarithmic transformation.

A three-way repeated measures ANOVA was performed to test the differences in latency across with the five-level within subject variables (puzzles one to five) by two level between subject gene (SS/SL vs. LL) and attachment (secure vs. insecure). There were N=42 children in this set of analyses. Specifically, there were N=14 children insecure with at least one copy of the S allele and N=4 children with an insecure attachment, homozygous for the L allele. There were N=24 children characterized as secure, N=16 of these children had at least one copy of the S allele.

A significant interaction between puzzle and attachment was found [F(4,152)=3.31, p<0.05] (Figure 6). Pair-wise comparisons of the simple effects were tested, with a Sidak correction. A significant difference between children with a secure versus insecure attachment style was seen at puzzle one [F(1,38)=10.55, p<0.05]. There was also a difference at trend-level between children categorized as secure and insecure on puzzle two [F(4, 38)=2.68, p=0.10]. In both cases children with an insecure attachment were slower to initiate the task than children with a secure attachment. The simple effects revealed that there was no significant difference in latency times of children with a secure attachment. However, amongst children with an insecure attachment times significantly decreased from puzzle one to puzzle four [F(1,38)= 3.23, p<0.05].
A significant interaction between puzzle number and child genotype was found [F(1,38)=4.43, p<0.05] (Figure 7). Multivariate simple effects were tested, and revealed that amongst the children with one copy of the $S$ allele versus those homozygous for the $L$ revealed that in their scores a significant [F(1,38)=2.61, p<0.05] difference at puzzle four. Multivariate pair-wise tests also revealed that there were no significant differences between children with one copy of the $S$ allele across the puzzles. However, there was a significant difference [F(1,35=2.44, p<0.05] amongst the children homozygous for the $L$ allele in scores on the latency to start puzzle four. Children homozygous for the $L$ allele were slower to initiate in the puzzle task at the first puzzle than the fourth.
9.2.2 Summary of behavioral data

There were two ways of assessing the behavioral manifestation of helplessness: helplessness/negative affective behaviors and latency of response. Our hypotheses were that children with at least one copy of the S allele and an insecure attachment would display the most helplessness behaviors was partially supported. In the analyses based on the PCA helplessness factors there was a trend level effect of child genotype on puzzle one. This effect was in the predicted direction, as children homozygous for the L allele displayed less helplessness that the children with at least one copy of the S allele.

The latency of response data showed two different patterns of results. On the one hand, there was an interaction between puzzle and attachment. The difference in attachment was significant only at the first puzzle, showing that insecure children were more inhibited at the start of the task. Pair-wise comparisons revealed that secure children’s score did not change over the course of the puzzles and that the significant different in the insecure group was between puzzle one and four. On the other hand, there was another interaction between puzzle and child
genotype. This interaction was driven by the S group having shorter response time than LL by puzzle four. Another interesting finding from these results is that despite taking part in a task that became increasingly more stressful, children were engaged throughout attempting the task across every puzzle.

9.3 **Heart Rate Data**

There were N=35 children with usable data for the heart rate analyses. Data was not available due to administration errors (N=5), child refusal (N=2), and HRM malfunction (N=10). Partial data (i.e. data for some puzzles, but not all) was available because of HRM malfunction (N=4) and refusal in the middle of the task (N=2). Of the 35 children in the analyses, 14 children were insecurely attached with at least one copy of the S allele, four children were insecurely attached with two copies of the L, N=16 had a secure attachment and at least one copy of the S allele and 8 had a secure attachment and two copies of the L allele.

A repeated measures ANOVA was performed on child heart rate from baseline to puzzle five. The mean heart rates for baseline (M= 98.48, SD 9.66), and puzzle one (M=99.69, SD 9.23) two (M= 100.96, SD 8.97), three (M=101, SD 10.01), four (M=101.8, SD 9.62), and to five were (M=101.6 SD 9.49) are in line with published norms for this age group (Fleming, et al., 2011). The data was looked at in two ways. First, a repeated measures ANOVA was performed on the average heart rate from baseline and puzzle one to five. These analyses did not reveal any significant main effects or interactions. Next, a repeated measures ANOVA of the change in average heart rate (difference from baseline scores) was performed. Again, there were no significant main effects or interaction in the child’s change in heart rate. Due to the small sample size, it was not surprising that no effects were detected. Therefore, to explore the nature of the data, and the hypothesis that children with at least one copy of the S allele would be more
reactive to the environment, the change scores were plotted with a scatter plot, by child genotype (Appendix, Figure 11-15). Children with one copy of the S allele’s change in heart rate from baseline have a wider distribution in both positive and negative directions.

Therefore, it was decided to graph the differences in group means of children with and without a secure attachment by genotype (Figure 8). These graphs demonstrate that in some conditions, error bars do not overlap suggesting potential effects. Over time, the graph shows that in the impossible puzzles there is a difference in the heart rates of children with one copy of the S allele. This effect seems to be driven by the difference amongst children with an insecure attachment. The most salient differences between the four groups are at puzzle three.

![Attachment x 5HTTLPR: Change in Heart Rate Across Puzzle # 1-5](image)

Figure 8: Attachment x 5HTTLPR: Change in Heart Rate Across Puzzle # 1-5
9.3.1 Summary of heart rate data

Although the analyses did not reveal any statistically significant results, exploratory analyses suggest an effect of genotype. Scatter plots of the data organized by genotype suggest heart rate variation in children with at least one copy of the $S$ allele, compared to a relatively homogenous distribution in children with two copies of the $L$ allele. This is consistent with the theory that the short of allele of the 5-HTTLPR is associated with differential susceptibility to the environment. The differences between the four groups of interest are even more striking when the change in mean heart rate are viewed over time. Specifically, the change in heart is most pronounced in children with a $S$ allele and an insecure attachment at puzzle 3. However, in line with the theory that it is in adverse conditions that differences in phenotypes emerge, differences within children with an insecure attachment are the most prominent across puzzle one to four.

9.4 Correlations Among Helplessness Measures

Exploratory analyses were performed to see if, or how, any of the measures of helplessness were related to each other. For a correlation to be considered a robust association it was decided that the two variables had to correlate across at least 40% of the measures (i.e. two out of the five self-evaluation questions) or in a meaningful pattern to avoid making false associations based on spurious effects.

We first tested which variables related to the self-report questions. There were no significant correlations between self-report questions and the helplessness factor scores from puzzle one to five. There were significant correlations between child self-evaluation on puzzle four and latency of response on puzzle two $r(67)=-.26$, $p<0.05$, three $r(66)=-.26$, $p<0.05$ and four $r(64)=-.25$, $p<0.05$. There was a surprising correlation between puzzle one $r(55)=.28$, $p<0.05$.
and puzzle two average heart rate and child motivation on the fourth puzzle $r(54)=.29$, $p<0.05$. The results suggest that children with higher average heart rates at the beginning of the task reported themselves as more motivated to do the next puzzle after the third impossible puzzle.

The next set of correlations were performed to test the relationship between helplessness behaviors and the other measures. There were no significant correlations between child helplessness measures and the heart rate data (average or change scores). There were significant correlations between helplessness displayed at puzzle two and latency on puzzle one $r(68)=.-25$, $p<0.05$, and five $r(67)=.-24$, $p<0.05$ and a trend at puzzle two $r(67)=.-22$, $p<0.05$. These correlations suggest that higher levels of helplessness after the first impossible puzzle were associated with faster latency of response for the subsequent puzzle. Lastly, child response latency was correlated with measures of heart rate. The most pronounced findings were in the overall average heart rate. The response latency on the fourth puzzle was correlated with the average heart rate at baseline $r(51)=.-31$, $p<0.05$, puzzle one $r(51)=.-21$, $p<0.05$, puzzle two $r(50)=.-26$, $p=0.06$, puzzle three $r(49)=.-32$, $p<0.05$, puzzle four $r(49)=.-27$, $p=0.058$ and puzzle five $r(48)=.-25$, $p=0.076$. In other words, the higher the child’s heart rate through the puzzle task, the faster the child’s response latency on puzzle four.

### 9.4.1 Summary of correlations between dimensions of helplessness

Correlations were performed between the multiple dimensions of helplessness measured during the RCP task. No clear pattern emerged across measures. These findings suggest that each measure may capture a unique dimension of helplessness. The results from the latency and heart rate in particular also suggest that less obvious measures of stress response may be important, especially when considering risk for internalizing disorders.
9.5 Correlations with SDQ and Dominic/que-R

The last stage of the analyses was to test correlations between the three domains of helplessness with child and parent reported anxiety and depressive symptoms. We compared the three domains of helplessness measured with the “total difficulties” and “emotional symptoms” scales of the SDQ for the mother and father. We also compared the measures of helplessness with child reported depressive, phobia, generalized anxiety and separation anxiety symptoms from the Dominic/que-R.

The first set of correlations performed was with the self-report data. The most compelling set of correlations was between mother’s rating of child total difficulties with correlations on puzzle three motivation $r(68) = -0.25$, $p<0.05$, and puzzle five self-evaluation $r(68) = -0.22$, $p=0.07$ and hopefulness $r(68) = -0.24$, $p<0.05$. These results suggest that children who rate themselves lower on these questions were rated as having more overall difficulties by their mothers. There was no significant pattern of results with the fathers ratings, however it is noteworthy that the highest correlation in this data was between father rating of offspring emotional problems and child motivation on puzzle five $r(56) = -0.38$, $p<0.05$.

On the self-evaluation question for the first puzzle higher ratings were associated with higher child-reported general anxiety symptoms $r(65) = 0.2$, $p=0.1$ and phobia symptoms $r(65) = 0.27$, $p<0.05$. A different pattern of results emerged once the child started to complete impossible puzzles. For example, self-evaluation on puzzle one was correlated with higher separation anxiety symptoms $r(67) = 0.33$, $p<0.05$ and trend-level lower phobia symptoms $r(67) = -0.22$, $p=0.07$. Furthermore, child ratings of self-evaluation $r(68) = -0.22$, $p<0.05$ and hopefulness $r(66) = -0.37$, $p<0.05$ on puzzle five correlated with depressive symptoms. Similarly, ratings of hopefulness on puzzle three $r(66) = -0.24$, $p=0.051$, as well as self-evaluation $r(66) = -0.25$, $p<0.05$
and hopefulness \( r(66)=-.34, p<0.05 \) on puzzle five correlated with child symptoms of separation anxiety.

There were no significant correlations between the helplessness behavior factors of puzzle one to puzzle five and any child or parent ratings of psychopathology. Similarly, latency of response times did not significantly correlate with any measures of child psychopathology. Lastly, the heart rate data were correlated with the measures of psychopathology. While most correlations were not significant, a trend emerged between child ratings of phobia and average heart rate across all time-points: baseline \( r(52)=.22, p=0.11 \), puzzle one \( r(52)=.25, p=0.07 \), puzzle two \( r(51)=.26, p=0.06 \), puzzle three \( r(49)=.25, p=0.07 \), puzzle four \( r(47)=.3, p<0.05 \) and puzzle five \( r(48)=.29, p<0.05 \).

### 9.5.1 Summary of correlation between dimensions of helplessness and rating of psychopathology

The correlations between the three dimensions of helplessness with parent and child ratings of psychopathology yielded partial support for the hypothesis that increased display of helplessness on the three domains measured would be associated with increased symptoms of psychopathology. Maternal reported total difficulties were correlated with measures of child self-reports. There was no correlation between father ratings expect on puzzle five end emotional symptoms. SDQ total scores are most associated with diagnosis and so it is a good indicator of risk for psychopathology (Goodman & Goodman, 2011). The most compelling results are the correlations between child ratings on the puzzle five, after repeated uncontrollable stress and child ratings of depression.
10 DISCUSSION

10.1 Summary of Study Findings

We examined the effects of child attachment security measured at 18 months and 5-HTTLPR genotype on the development of a helpless cognitive style. Across multiple dimensions of helplessness individual differences in response to failure were observed in children five years of age. There were two key findings from this study. First, as predicted, children with either one or two copies of the $S$ allele of the promoter polymorphism of the serotonin transporter gene and an insecure attachment were most affected by exposure to a failure task in the domains of self-evaluation. Second, our helplessness measures, particularly when measured after the child was exposed to two impossible puzzles were associated with increased symptoms of psychopathology.

The most compelling results from the self-report data were obtained from the question of self-evaluation. We found that across all five puzzles children with at least one copy of the $S$ allele and an insecure attachment rated their performance lower than children with a secure attachment. There were no differences in the ratings of children with two copies of the $L$ allele. This result was most apparent in puzzle four (the third and final impossible puzzle), arguably the period of greatest stress. Our findings are consistent with the theory of “differential susceptibility to environmental influences”. The $S$ allele is believed to be associated with reactivity to environmental influences (Belsky, Bakermans-Kranenburg, & van IJzendoorn, 2007, p. 300). Conversely, children homozygous for the $L$ allele appear less influenced by environmental context. Our results are consistent with Biological Sensitivity to Context (Boyce & Ellis, 2005) theory as children with two copies of the $L$ allele did not differ in their ratings as a
function of their attachment security. In contrast, among children with an S allele ratings differed significantly between the secure and insecure groups.

An important issue concerning the self-evaluation data is whether or not children are “helpless” if they evaluate their performance as poor when they have failed the task. In fact, they might be considered to be the most realistic in assessing their performance. However, there is evidence for the idea that children who are not mastery oriented (i.e. helpless), show low self-evaluation, give up more quickly in the face of challenge and have poorer performance after failure (Dweck, 1975). Moreover, it could be adaptive for young children to believe that they are able to succeed, even when evidence does not support this claim. First, it is posited that positive illusions, even when false, are essential to mental health (Taylor & Brown, 1994). Second, in light of the rapid development during the early years, it is possible that tasks performed that are at first impossible, become possible with the development of new cognitive abilities and continued effort. Giving up in the face of impossible tasks may compromise neural development by limiting experience.

We did not find the same pattern of results with the hopefulness or motivation questions as was found with the self-evaluation question. This was an unexpected finding as Cole and colleagues found the same patterns of results across all three questions (Cole, et al., 2007). Previous research has found that children categorized as helpless have lower self-evaluation (Dweck, 1975). In an intervention study targeting children identified as having a poor response to failure, the helpless children continued to rate their self-evaluation low, even after a cognitive restructuring session in which they learned to interpret failure in terms of effort rather than ability (Dweck, 1975). Since there is little research on cognitive styles in such young children, further research is required to understand this pattern of results.
We saw a similar pattern of results from the self-evaluation data in the behavior data. Although our analyses did not meet statistical significance, graphic representations of the findings suggest that children with two copies of the $S$ allele and an insecure attachment displayed more helplessness behaviors than children with a secure attachment. We also observed that children homozygous for the $L$ allele did not display an elevated level of our helplessness measure compared to children with at least one copy of the $S$ allele. Due to the nature of the data, we cannot fully test the measure of biological sensitivity to context since we were unable to make a factor of “positive coping” with our Principal Component Analysis. However, in five year old children it is likely unrealistic to expect to observe significant positive behaviors during a failure task. As mentioned in chapter three, many of our coded behaviors were dichotomized to account for highly skewed distributions. It is possible that coping, as well as engagement in the task is simply associated the absence of “helplessness” behaviors and a display of neutral rather than positive affect.

We did not find any significant effects of attachment or child genotype in relation to average heart rate or reactivity. In light of our small sample size this was not surprising. However, when graphically visualizing the heart rate averages seems that again, the same pattern as the behavior and self-report data emerged. Interestingly, visual inspection of the data suggest that children with one copy of the $S$ allele and an insecure attachment displayed the greatest increases in heart rate. This finding is consistent with recent findings amongst healthy adult females where those with two copies of the $S$ allele had increased blood pressure and heart rate in response to a psychosocial stress (Way & Taylor, 2011). Moreover, treatment with SSRIs in a sample of patients with PTSD showed stabilization of heart rate variability at baseline (Cohen, Kotler, Matar, & Kaplan, 2000).
The absence of significant effects in measures of heart rate could be due to other factors. In a small group of championship chess players, hopefulness was assessed in relation to heart variability (Schwarz, Schächinger, Adler, & Goetz, 2003). In retrospective reporting of mood during the chess match, participants' ratings of increased "helplessness/hopelessness" were associated with decreased heart rate frequency and variability. Conversely, changes towards optimism during the match were associated with increased heart rate. To our knowledge, only one other study has assessed average heart rate during a challenge task in children. Children exposed to excessive community noise (stress) displayed higher average heart rate (Evans, et al., 2001). It is therefore not clear whether heart rate increase is associated with increased stress or excitement about the current task. These mixed results highlight the need for further investigation, particularly with children.

A second important finding from this study is that multiple measures of helplessness were associated with both parent and child ratings of psychopathology symptoms. These associations provide preliminary evidence that helplessness is an intermediate phenotype of anxiety and depression in children. We found several correlations between parental reported SDQ “total difficulties” and “emotional problems” scores with child ratings on the impossible puzzles section of the Response to Challenge Puzzles task. The total difficulties score is particularly important as it associates with diagnoses (Goodman & Goodman, 2011). Similarly, ratings of children during the impossible puzzles were also significantly correlated with child self-ratings of depression and separation anxiety on the Dominic/que-R. Although both the Dominic/que-R and the puzzle ratings are both based on child ratings, measures were obtained one year apart and within very different contexts. Moreover, taken in conjunction with the parental ratings these data suggests a relationship between the child’s response to failure and
future risk for increased symptoms of psychopathology. It is also interesting that child ratings in the face of challenge are specifically associated with child self-ratings of separation anxiety, and not other measures of anxiety such as generalized anxiety or phobias as was predicted. This finding is interesting in light of the association with attachment security. Given epidemiological evidence of the heterotypic developmental trajectory of psychopathology from childhood into later in life, especially of separation anxiety, follow-up with this sample is crucial (Costello, et al., 2003).

10.2 Study Strengths and Limitations

The following issues should be considered when interpreting the results. The present research is drawn from a hypothesis-driven, prospective longitudinal study. However as this study is based on data from an on-going study, at present the sample size is small. Therefore, these analyses should be considered as preliminary results, upon which we will expand as more children take part in the puzzle task. It is noteworthy that despite the small sample we have been able to replicate a similar pattern of results across several dimensions of response to challenge. Moreover, our results are consistent with previous findings of the interaction between environment and 5-HTTLPR (Caspi, et al., 2003; Kochanska, et al., 2009). If our results persist within a larger sample, it would suggest that our measure of response to a failure is a promising candidate endophenotype for anxiety and depression.

The next caveat in this study is our measure of parental care. The attachment categorization is yielded from well-validated coding of the Strange Situation. However, there are two issues to be considered when considering the results from this measure. The first is the methodological issue, that like other groups, we failed to achieve reliability in the coding of the disorganized attachment group. Future direction for the study might include re-training and re-
coding of these videos in order to have ratings that include the dimension of disorganized attachment.

Second, these categorizations are not direct measures of parenting. Attachment security is a measure of the child’s felt security and can considered a proxy measure for parental sensitivity, emotional availability and control. Since attachment is measured based on behaviors in the child it is plausible that underlying genetic factors influence both attachment security and helplessness. To counter this argument we were able to show that there was no differential distribution of child 5-HTTLPR genotype by attachment security groups, these results have also been found in adults (Reiner & Spangler, 2010). Also, attachment security has been used in other studies as a measure of early environment in relation to child negative emotionality (Pauli-Pott, Friedel, Hinney, & Hebebrand, 2009) and emotion regulation (Kochanska, et al., 2009). For example, attachment measured at 15 months interacts with child 5-HTTLPR to predict emotion regulation (Kochanska, et al., 2009). Consistent with our results, these authors found a differential response among children with at least one copy of the S allele as a function of their attachment security compared to children with two copies of the L allele.

Previous research on the development of negative thinking styles relies on retrospective report of adult offspring, or parental report of their own parenting style. We have been able to replicate these findings with prospective measures of parenting. Moreover, the Strange Situation is a well-validated measure, based on observer report and therefore not confounded by the mood or bias of the subject. Additionally, we had a priori reasons, based on attachment theory, to believe that attachment schemas are associated with the development of helplessness cognitions. Nonetheless, future research may use direct measure of parenting such as sensitivity, attunement and control to help clarify this issue.
Finally, our measure of average heart rate data is a basic measure of reactivity. Many studies with a focus on stress reactivity include measures of respiration with their heart rate data. In our study, it was observed that most children remained seated and quite immobile throughout the duration of the puzzle task. Moreover, the fact that group differences occur despite these unrefined measures speaks to the robustness of the findings. However, a significant heart rate data were lost due to HRM malfunction and misuse.

The greatest strength of this study is the multi-faceted measurement of helplessness and child symptoms of psychopathology. We have been able to show differences in response to failure across the multiple dimensions assessed and these differences suggest a helplessness style. Most studies have not attempted to measure underlying cognitive style in such a young sample. We have also used multiple informants to report on child symptoms of psychopathology (i.e. mother, father and child). Although the algorithm for the Dominic/que-R yielded a level of symptoms based on DSM-III-R criteria, it has allowed us to add in the child’s perspective on symptoms. Moreover, taken together with the parent ratings of psychopathology, our data suggest that our helplessness measures may be associated with future emotional symptoms and difficulties.

10.3 Clinical Implications

Early indicators of risk for anxiety and depression are present in very young children. Many would argue that prevention of the disorder would be the optimal target for intervention, avoiding the impairment caused by the disorder in those affected. The results from the present study confirm that differences in response to failure and helplessness are observable in children five years of age. Moreover, in light of the association between measures of helplessness and both parent and child reported psychopathology, our data demonstrates that these differences in
response to failure are meaningful for symptoms of mental illness. Perhaps the most important clinical implication of these findings is the role of attachment in the development of helplessness. Attachment-focused interventions in families with maltreating parents have been successful at reducing emotional and behavior problems in children (Tarabulsy, et al., 2008). Prevention programs could target mother-child relations, and specifically work to increase the mother’s role as a “secure base” and feelings of control and stability in the child could prevent the development of helplessness in these children. Ideally, early intervention could prevent the cascade of cognitive and physiological changes that lead to increased risk for psychopathology.

The implications for this research may also extend to other groups exposed to repeated “uncontrollable” stress such as children with learning disabilities and people suffering from chronic pain. For example, baseline helplessness/hopelessness is associated with better outcomes (i.e. disease-free survival) in patients undergoing breast cancer treatment (Watson, Homewood, Haviland, & Bliss, 2005). Interventions with “at-risk” groups that target feelings of security and support may also be beneficial for both their emotional and physical well-being.

Another way in which the Response to Challenge Puzzles may be useful in a clinical context is as a tool for assessment. It is difficult to assess internalizing symptoms in young children. Moreover, as the DSM V is being prepared, the field of psychiatry is moving towards a dimensional approach of measuring symptoms of psychopathology. The degree to which the child responds with helplessness in the face of failure may be a good indicator of coping behaviors. The RCP is an ecologically valid tool and may provide the clinician with insight into the child’s behavior in the “real world”, as well tailor interventions to the child’s needs.
10.4 Future Directions

The study of gene-environment interactions is a burgeoning field of research. The 5HTTLPR polymorphism specifically, has been particularly well investigated in relation to psychopathology, as well as intermediate phenotypes. One next step in determining the developmental origins of negative thinking styles is to explore the role of other candidate genes associated with mood and anxiety disorders genes. In their review of the literature, Belsky and Pluess highlight the multiple genes that are associated with differential susceptibility to the environment including dopamine receptor D2 and D4 (Belsky & Pluess, 2009). They propose that future research should also incorporate not only gene-environment interactions, but gene x gene x environment interactions. This approach has been tested with a three-way interaction between Brain-derived neurotrophic factor (BDNF) genotype, 5-HTTLPR, and maltreatment history in predicting depression (Kaufman, et al., 2006), but yet to be tested in relation to cognitive style.

Another important direction for future studies will be to follow children over time, to determine the stability of the children’s response to challenge. The results from the present study suggest that at age five, differences in response to failure may be observed. With this data we cannot answer the question of the stability of helplessness or whether our measures of helplessness act as a “diathesis” to increase anxiety or depressive symptoms in times of stress. Future investigation could include the assessment of negative cognitions over time, starting in young children. Factors such as child temperament, negative life events and even gender may be investigated in relation to the stability of a helpless cognitive style.

The Maternal Adversity Vulnerability and Neurodevelopment study follows children over the course of development. We will be able to answer many questions concerning the
interaction between genotype and other early environmental factors on child helplessness, such as prenatal environment or maternal depression. We will also continue to follow these children to test the predicted validity of the helplessness measures. This approach will become even more interesting as the children reach puberty, where there is a marked increase in the prevalence of anxiety and depressive disorders. Ultimately, the goal will be not only to better understand the mechanism through which children develop maladaptive response to stress, but in what ways these early signs of helplessness are associated with future development.

10.5 Conclusion

We provide preliminary evidence that child attachment measured at 18 months and child 5-HTTLPR interact to predict child helplessness in children aged five. We found that child response to failure was also associated with ratings of psychological symptoms. Taken together, our data suggest that response to challenge may be a candidate factor for an endophenotype of anxiety and depression in pediatric samples.
11 REFERENCE LIST


glucocorticoid receptor mRNA expression, corticosterone, and behavior in adult male rats. *Behav Neurosci, 125*(2), 150-160.


during chronic antidepressant administration. *Neuropsychopharmacology, 28*(2), 413-420.


12 APPENDIX

This table describes the Ainsworth Strange Situation procedure (Chapter 3, Materials)

Table 3: Strange Situation Episode. Taken and Modified from Ainsworth et al., 1978, p. 37.

<table>
<thead>
<tr>
<th>Episode</th>
<th>People in the Room</th>
<th>Duration</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Mother, Baby, Research Assistant</td>
<td>No set duration (&lt; 1 minute)</td>
<td>Enter experimental room, introduction and explanation of lab</td>
</tr>
<tr>
<td>2</td>
<td>Mother and Baby</td>
<td>3 minutes</td>
<td>Start of Strange Situation M and BB are alone in room</td>
</tr>
<tr>
<td>3</td>
<td>Mother, Baby, Stranger</td>
<td>3 minutes</td>
<td>A) S enters the room, sits on a chair and reads B) S interacts with Mother C) try to join in with BB’s play</td>
</tr>
<tr>
<td>4</td>
<td>Stranger, Child</td>
<td>3 minutes</td>
<td>M discreetly leaves without saying goodbye</td>
</tr>
<tr>
<td>5</td>
<td>Mother, Baby, Stranger</td>
<td>3 minutes</td>
<td>M re-enters the room S discreetly leaves the room</td>
</tr>
<tr>
<td>6</td>
<td>Mother and Baby</td>
<td>3 minutes</td>
<td>2nd separation M says “bye-bye” and leaves handbag</td>
</tr>
<tr>
<td>7</td>
<td>Stranger and Baby</td>
<td>3 minutes</td>
<td>-S returns and minimally greets BB, sits in chair if BB is not too distressed</td>
</tr>
<tr>
<td>8</td>
<td>Mother and Baby</td>
<td>3 minutes</td>
<td>-M returns for 2nd reunion and comforts BB S discreetly leaves the room</td>
</tr>
</tbody>
</table>

*** If baby shows too much distress separation is shortened
The following two figures are the two rating scales (star rating scale and happy face rating scale) used to assess child self-report in the RCP (Chapter 4, Self-report).

Figure 9: Star Rating Scale
Figure 10: Happy Face Rating Scale

1 Very Happy
2 Unhappy
3 Neutral
4 Happy
5 Very Happy
This image displays the RCP puzzle pieces and the final “possible puzzle” image the children created (Chapter 4, Materials).

Figure 11: RCP puzzle pieces
The following scatterplot graphs represent the change in heart rate averages (from baseline) for puzzle one to five.
Figure 13: Scatterplot of Change in Heart Rate by Child 5HTTLPR: Puzzle #2
Figure 14: Scatterplot of Change in Heart Rate by Child 5HTTLPR: Puzzle #3
Figure 15: Scatterplot of Change in Heart Rate by Child 5HTTLPR: Puzzle #1
Figure 16: Scatterplot of Change in Heart Rate by Child 5HTTLPR: Puzzle #1