The Role of the Serotonin Transporter Gene, Brain Structure and Family Environment in the Emergence of Depression during Adolescence

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ABSTRACT

Recent findings suggest that complex interrelations between genetics, brain structure and environmental contexts, including stressors and family processes, may have a role in the development of depressive disorders. The role of a functional variant in the 5-HT transporter promoter region polymorphism (5-HTTLPR) and its potential interaction with adverse, stressful life events in predicting depression has been the focus of considerable research attention. The validity of this gene-environment interaction, however, has been queried due to inconsistent findings. The current thesis aims to enhance current understanding of this interaction by considering how two different dimensions of environmental experience (threat versus deprivation) might interact with the serotonin transporter gene during adolescence, while also investigating potential underlying neurobiological mechanisms. Three interconnected studies were conducted that examined the interplay between the serotonin transporter gene, family environment, brain regions of interest and depression.

Study 1 examined whether 5-HTTLPR moderated associations between (1) high levels of negative, harsh, critical parenting behaviours (as an index of more threatening environments) and subsequent depression and (2) low levels of positive, supportive parenting behaviours (as an index of more deprived environments) and subsequent depression during adolescence. These GxE interactions were tested in adolescents from two independent longitudinal studies, the Australian Temperament Study (ATP, n=681) a population based sample that relied on questionnaire measures of environment and depression, and the Orygen Adolescent Development Study (ADS, n=174) a sample

enhanced for temperamental risk and resilience factors for internalising conditions, that drew on observational measures of the environment and semi-standardised clinical interview measures of depression. In both studies, adolescents carrying at least one copy of the S-allele appeared to be buffered against risk for depression in the context of low positive parenting, whilst adolescents in the L-homozygous group were at greater risk for depression with decreasing levels of positive parenting. Negative parenting did not interact with serotonin transporter genotype in either study.

S**tudy 2** was based on the ADS and examined the extent to which variation in hippocampus, amygdala, orbitofrontal cortex (OFC) and anterior cingulate cortex (ACC) volumes in early adolescence mediated a putative association between 5-HTTLPR genotype and first onset of Major Depressive Disorder (MDD) over a six year period. Increasing copies of S-alleles predicted smaller left hippocampal volume, which in turn was associated with increased risk of experiencing a first onset of MDD. Increasing copies of S-alleles also predicted both smaller left and right medial OFC volumes, although neither left nor right medial OFC volumes was prospectively associated with a first episode of MDD during adolescence.

Study 3 was also based on the ADS and employed an imaging-gene x environment (IGxE) framework to investigate whether the strength of the imaging genetics pathway involving the hippocampus that was identified in Study 2 differed as a function of parenting behaviour. Results were consistent with the presence of an indirect effect of the serotonin transporter S-allele on depression onset via smaller left and right hippocampal volumes that was significant only in family environments involving either higher levels of negative parenting or lower levels of positive parenting. The previously reported finding of S-allele

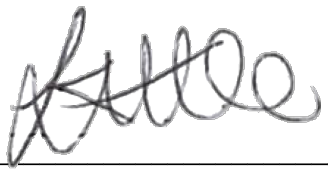
carriers' increased risk of depression in adverse environments may therefore be partly due to the effects of these environments on a neurobiological pathway from the serotonin transporter gene to depression onset that proceeds through variation in hippocampal volume.

It is hoped that approaches that aim to integrate genetic, environmental and neurobiological factors such as those utilised in this thesis will improve the likelihood of developing more targeted prevention and intervention opportunities for individuals at risk of or already experiencing clinical depression.

DECLARATION OF ORIGINALITY

This is to certify that:

1. The thesis comprises only my original work towards the PhD, except where indicated in the preface
2. Due acknowledgement has been made in the text to all other material used,
3. The thesis is less than 100,000 words in length, exclusive of tables, maps, bibliographies and appendices.
4. Human participants were treated in an ethical manner.

Signed: 

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This thesis is dedicated to my mother.

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ABREVIATIONS

5-HT = 5-Hydroxytryptamine = Serotonin

5-HTT = 5-Hydroxytryptamine transporter = Serotonin transporter

5-HTTLPR = 5-Hydroxytryptamine transporter linked polymorphism = serotonin transporter-linked polymorphism

ADHD = Attention Deficit Hyperactivity Disorder

ACC = Anterior Cingulate Cortex

ACTH = corticotropin

BA = Brodman's Area

BDNF = brain-derived neurotrophic factor

BPD = Borderline Personality Disorder

CBT = cognitive behavioural therapy

CES-D = Centre of Epidemiologic Studies Depression Scale

CNS = central nervous system

CRF = corticotropin-releasing factor

d-ACC = Dorsal Anterior Cingulate Cortex

DLPFC = Dorsolateral Prefrontal Cortex

d-PaC = Dorsal Anterior Paracingulate Cortex

DSH = differential susceptibility hypothesis

DSM-5 = Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition

EATQ-R = Early Adolescent Temperament Questionnaire – Revised

GWAS = Genome-wide association studies

GxE = Gene x Environment

HPA = Hypothalamic-Pituitary-Adrenal

IGxE = imaging gene-environment interaction

MDD = Major Depressive Disorder

MDE = major depressive episode

MRI = Magnetic Resonance Imaging

NIMH = National Institute for Mental Health

OCD = Obsessive Compulsive Disorder

OFC = Orbitofrontal Cortex

PFC = prefrontal cortex

PTSD = Post-Traumatic Stress Disorder

r-ACC = Rostral Anterior Cingulate Cortex

RDoC = Research Domain Criteria

ROI = Region of Interest

r-PaC = Rostral Anterior Paracingulate Cortex

SLC6A4 = serotonin transporter gene

SNPs = single nucleotide polymorphisms

SSRIs = selective serotonin reuptake inhibitors

VBM = Voxel Based Morphometry

VNTR = variable-number-of tandem-repeats

PREFACE

I acknowledge the contribution of the following co-authors to the published and unpublished work in **Studies 1-3** (CHAPTER 4, CHAPTER 5, CHAPTER 7 and CHAPTER 9). A description of the contribution of each author is outlined below.

Study 1 is presented in CHAPTER 4 and CHAPTER 5. Work comprising CHAPTER 4 has been accepted at *Child Development.* I was lead (first) author, and I conceptualised the study and conducted the analyses, with consultation from co-author George J. Youssef, who assisted with preparation of the figures. I wrote the manuscript and prepared the response to reviewers and manuscript revisions with my co-authors Craig A. Olsson, Sarah Whittle, Jacqui A. Macdonald, Lisa B. Sheeber, George J. Youssef, Julian G. Simmons, Ann V. Sanson, Debra L. Foley and Nicholas B Allen providing feedback and editorial assistance. George J. Youssef assisted with preparation of the figures in CHAPTER 5.

Study 2 is presented in CHAPTER 7. Work comprising CHAPTER 7 (Little et al., 2014) has been published by *Translational Psychiatry.* I was lead (first) author, and I conceptualised the study, conducted the analyses, wrote the manuscript, and prepared the response to reviewers and manuscript revisions, with my co-authors Craig A. Olsson, Sarah Whittle, George J. Youssef, Michelle L. Byrne, Julian G. Simmons, Murat Yucel, Debra L. Foley and Nicholas B Allen providing feedback and editorial assistance.

Study 3 is presented in CHAPTER 9. Work comprising CHAPTER 9 (Little et al., 2015) has been published by *Journal of Abnormal Psychology*. I was lead (first) author, and I conceptualised the study, conducted the analyses, wrote the manuscript and prepared the response to reviewers and manuscript revisions, with my co-authors Craig A. Olsson, Sarah Whittle, George J. Youssef, Julian G. Simmons, Murat Yucel, Lisa B. Sheeber, Debra L. Foley and Nicholas B Allen providing feedback and editorial assistance.

Adam Pettit, Doctoral student, Department of Psychology, University of Oregon assisted with work in CHAPTER 3, section 3.3.1. He was the second reviewer of the titles and abstracts of papers to determine whether studies were appropriate to include in a review based on systematic review guidelines contained in this chapter. His contribution is also noted on page 104 of this manuscript.

PUBLICATIONS AND PRESENTATIONS

Peer Reviewed Publications Arising Directly From This Thesis

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Little, K., Olsson, C. A., Whittle, S., Youssef, G. J., Byrne, M. L., Simmons, J. G., Yücel, M., Foley, D. L., Allen, N. B. (2014). Association between serotonin transporter genotype, brain structure and adolescent-onset major depressive disorder: a longitudinal prospective study. *Translational Psychiatry, 4*(9), e445. doi:10.1038/tp.2014.85

Little, K., Olsson, C. A., Youssef, G. J., Whittle, S., Simmons, J. G., Yucel, M., Sheeber, L.B., Foley, D.L. & Allen, N. B. (2015). Linking the serotonin transporter gene, family environments, hippocampal volume and depression onset: A prospective imaging gene x environment analysis. *Journal of Abnormal Psychology, 124*(4), 834-849. doi:10.1037/abn0000101

Conference Presentations Directly Arising from this Thesis

Little, K., Olsson, C.A., Youssef, G.J., Whittle, S., Simmons, J.G., Yücel, M., Sheeber, L., Foley, D.L., & Allen, N.B. (2015). Linking the Serotonin Transporter Gene, Family Environments, Brain Structure and Depression: Biological Bases of Differential Susceptibility. Paper presented as part of the Symposium "Neuro-Sensitivity to the Early Environment: Neurobiological Markers of Differential Susceptibility" at the *Society of Research in Child Development Biennial Meeting*, March, 2015.

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Little, K., Allen; N.B., & Olsson, C.A. (2013). Sometimes it's good to be short: 5HTTLR, parenting and depression in two Australian longitudinal studies. Paper presented as part of the Symposium "Adolescents and Their Environments: A Matter of Match or

Mismatch?" at the *International Society for Research on Child and Adolescent Psychopathology Sixteenth Scientific Meeting*, June, 2013. #

Little, K., Olsson, C.A., Whittle, S., Foley, D.L., Simmons, J., Byron, K., & Allen, N.B. (2013). Associations between 5-HTTLPR, brain structure and vulnerability to depression during adolescence: A longitudinal, prospective study. Poster presented at the *Society of Research in Child Development Biennial Meeting*, April, 2013.

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Related Peer Reviewed Publications During Candidature

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PROLOGUE

Evidence indicates that depressive disorders, the incidence of which increases dramatically during adolescence, are a significant contributor to negative outcomes, both during adolescence and later in life. Recent findings suggest that complex interrelations between genetics, brain structure and environmental contexts, including stressors and family processes, may have a crucial role in the development of depressive disorders. The purpose of this thesis is to increase understanding of these complex relationships as they relate to depression during this developmental period.

The search for candidate genes for depression has focused on the serotonin system in light of multiple lines of evidence implicating serotonin in affective disorders, including findings that selective serotonin reuptake inhibitor drugs are efficacious for the treatment of clinical depression (Blier & de Montigny, 1999; Nemeroff & Owens, 2002). Special attention has been paid to the serotonin transporter gene *(SLC6A4),* a key regulator of serotoninergic neurotransmission (Aslund et al., 2009). Within the promoter of the gene is a polymorphism with two common alleles – a short (*S*) variant and a long (*L*) variant. A number of *gene-environment* investigations have found the S-allele of serotonin transporter gene linked polymorphic region (5-HTTLPR) to interact with major life stress or adverse environments to predict depression (Caspi et al., 2003; Kendler, Kuhn, Vittum, Prescott, & Riley, 2005). However, results of further attempts that aim to replicate and extend such an interaction have been inconsistent, with recent meta-analyses providing evidence that carriers of an *S* allele have an elevated risk of depression following experiences of adversity compared individuals homozygous for the *L* allele (Karg, Burmeister, Shedden, & Sen, 2011; Sharpley, Palanisamy, Glyde, Dillingham, & Agnew, 2014) and another two meta-

analyses obtaining negative results (Culverhouse et al., 2017; Munafò, Durrant, Lewis, & Flint, 2009; Risch et al., 2009).

Certainly, the meta-analysis by Sharpley and colleagues (2014) highlighted both the number of studies that failed to identify any significant interactions (approximately one-quarter of studies included) as well as those that implicated the L-allele in an interaction with the environment that predicted depression (approximately one-tenth of studies). Debates surrounding the presence of a 'true' gene-environment effect involving the serotonin transporter gene are therefore ongoing, with concerns that variation in findings in the published literature might reflect a number of different factors, including underpowered analyses, a publication bias that has favoured significant findings implicating the S-allele, and discrepancies in the quality of methodology across studies (Caspi, Hariri, Holmes, Uher, & Moffitt, 2010; Dick et al., 2015; Duncan & Keller, 2011).

Somewhat surprisingly, one explanation for the discrepancies in interaction findings across studies that has received very little consideration is the possibility that this could reflect important variations in the *type* of environment assessed. Studies that focus on developing a more nuanced understanding of the *environmental* contribution to this gene-environment interaction by considering how different aspects of the environment might influence findings could make an important and unique contribution to the literature.

Moreover, studies that have capacity to reveal underlying neurobiological mechanisms by which interactions might occur may be in a better position to determine the validity of interaction results. Some initial progress in this regard has come from *imaging genetics* studies, which have concentrated their efforts on identifying the contributions of candidate genes to neuroanatomy or the activity of brain regions that had been shown to

have a role in psychopathology. The emerging literature in this research domain suggests that the serotonin transporter gene may exert an influence on regions such as the hippocampus, amygdala, anterior cingulate cortex (ACC) and orbitofrontal cortex (OFC) though variation in findings have also been noted (see Scharinger, Rabl, Sitte, & Pezawas, 2010 for a review), but it remains unclear whether the variance in these regions that is specifically attributed to differences in serotonin transporter genotype is linked with meaningful variation in depression outcomes.

An additional challenge of studying neurobiological mechanisms of depression in clinical populations (i.e., individuals who present with active illness) is that it remains unresolved as to whether detected neurological abnormalities represent a cause of the disorder (primary pathology) or reflect the consequences of the disorder (secondary pathology). Prospective, longitudinal studies that examine individuals prior to the onset of depression may have greater opportunity to shed light on these processes. An integration of gene-environment and imaging genetics methodologies into an overarching imaging gene-environment (IGxE) framework may enhance understanding of pathways from among genes, environments and the brain to psychopathology (Hyde, Bogdan, & Hariri, 2011).

The specific aims of this thesis were therefore threefold. The first aim was to evaluate the extent to which genotype for the serotonin transporter-linked polymorphism (5-HTTLPR) might moderate the risk for depressive symptoms and the emergence of Major Depressive Disorder (MDD) during adolescence that was posed by different dimensions of environmental experiences during early adolescence, namely (1) lower levels of positive parenting, considered to represent more deprived environments and (2) higher levels of negative parenting, considered to represent more threatening environments. The second aim

is to evaluate the extent to which hippocampal, amygdala, anterior cingulate cortex (ACC) and orbitofrontal cortex (OFC) volumes might serve as intermediate phenotypes that mediate a relationship between 5-HTTLPR genotype and first onset of MDD. The third aim was to simultaneously model the effects of the serotonin transporter gene, brain structure (hippocampal volume specifically), and the different parenting influences on onset of MDD to evaluate the benefits of this more integrated yet complex IGxE approach.

This thesis will begin with an overview of depression during adolescence, including a definition of the disorder, a review of relevant epidemiology literature and some of the key theories regarding etiology (0). This will be followed by a critical appraisal of the literature regarding a putative interaction between the serotonin transporter gene and life-stress/adversity in predicting depression and the current paradigms that have influenced this research (CHAPTER 2). CHAPTER 2 will also provide an in-depth consideration of underlying traits or characteristics that might be associated with different serotonin transporter genotypes as well as the different dimensions that might underlie various environmental experiences, and based on this research, a new theory, named the differential capability hypothesis, will be proposed. This will be followed by a comprehensive review of available evidence that would support the family environment and specific aspects of parenting as potential candidate environments that, according to the differential capability theory, might plausibly interact with serotonin transporter gene to predict depression (CHAPTER 3). The results from the first of three empirical investigations are presented in two chapters (Study 1, CHAPTER 4 and CHAPTER 5) in the current thesis, which centres on gene-environment analyses, and are interpreted in the context of this literature.

The focus of the thesis will then shift to incorporating an understanding of the potential role that neurobiology (in particular brain structure) might play in risk relationships involving the serotonin transporter gene, environments and depression. This begins with an evaluation of the literature suggesting four brain regions of interest (ROIs) could be intermediate phenotypes for depression, and that their genetic underpinnings could include the serotonin transporter gene (CHAPTER 6). The results from the second empirical investigation which is based on the literature of the preceding chapter and draws on an imaging genetics framework, will then presented (Study 2, CHAPTER 7). The rationale and possible methodological frameworks for combining gene-environment and imaging genetics investigations are then outlined (CHAPTER 8), followed by the results of the third empirical investigation which provides an illustration of the application of relevant imaging gene-environment (IGxE) frameworks based on both prior literature reviewed in the current thesis and specific findings from the two previous empirical investigations (Study 3, CHAPTER 9). Finally, a general discussion is presented, which integrates the findings across the three empirical investigations, suggests areas for future research and provides a conclusion (CHAPTER 10).

CHAPTER 1: THE DEVELOPMENT OF DEPRESSION DURING ADOLESCENCE

1.1 Epidemiology of Depression in Adolescence

Adolescence, the transition period between childhood and adulthood (usually between 12-19 years), is one of the most dynamic stages of development, with rapid change and growth occurring across biological, psychological, cognitive, socio-emotional and interpersonal domains (Blakemore, 2008; Casey, Getz, & Galvan, 2008; Dick, Adkins, & Kuo, 2016; Guyer, Silk, & Nelson, 2016; Spear, 2000). The transformation that occurs during this developmental period presents both opportunity and challenge for the adolescent and whilst most individuals negotiate this period successfully, others may experience significant difficulty (Dahl, 2004). This phenomenon is reflected in a developmental health paradox for adolescents, who experience significant advancements in their physical and cognitive capabilities but also profound increases in overall morbidity and mortality in the same time interval (Dahl, 2004; Spear, 2000). Importantly, this rise in morbidity and mortality in this period has been found to be primarily related to difficulties in the control of emotions and behaviour, which contribute to intentional and unintentional injury, substance use, suicide, violence and risky behaviours, rather than being due to an upsurge in physical illnesses such as cardiovascular problems or cancer (Eaton et al., 2008; Masten, 1987; Steinberg, 2008). Indeed, national surveys indicate that the onset of many psychiatric illnesses increases substantially from childhood to adolescence (eg. Costello, Mustillo, Erkanli, Keeler, & Angold, 2003; Kessler, 2005).

A marked increase in the prevalence of depression over the period of adolescence has been documented. Prior to age 13, depressive disorder appears relatively uncommon,

with a point prevalence estimate of 2.8% (Costello, Erkanli, & Angold, 2006). However, the point prevalence increases twofold to 5.7% in adolescents aged 13-18 years, and by the age of 19 years, between a fifth and a quarter of individuals are estimated to have experienced a depressive disorder (Harrington & Dubicka, 2002; Lewinsohn, Rohde, & Seeley, 1998). Sub-clinical depressive symptomology may be even more common, with as many as 20-65% of adolescents reporting subclinical levels of symptoms at any one time (Kessler, 2001; Kessler, McGonagle, Swartz, Blazer, & Nelson, 1993). Delineating the relevant processes that contribute towards the emergence of this disorder is clearly a pressing goal given findings that depression is currently the most disabling disorder worldwide for individuals 15-44 years old (World Health Organization, 2008) and the leading cause of non-fatal disability in Australia (Begg et al., 2007).

The developmental processes that result in increased rates of depression onset in adolescence remain unclear. Suggestions implicate the onset of puberty with its multiple biological changes and emotional and psychosocial corollaries as well as neurological changes – specifically an imbalance in the rates of development of subcortical emotional structures and prefrontal cognitive control regions, which may make it challenging for young people to effectively regulate and understand their emotions (Casey et al., 2010; Essau & Chang, 2009; Kesek, Zelazo, & Lewis, 2008; Patton & Viner, 2007). Both variation in genetic background and environmental factors may influence the cascade of changes associated with puberty as well as neurodevelopmental trajectories and in turn lead to greater vulnerability in some individuals over others. In addition, the developmental period of adolescence involves a number of unique psychological and social challenges and stressors, such as developing a sense of identity, establishing both autonomy and

relatedness with parents, developing quality relationships with peers, navigating romantic relationships and making important educational and vocational decisions, which may also play a role in increasing vulnerability to depression in this age group (Shortt & Spence, 2006).

1.2 Onset, Clinical Features and Developmental Course

Adolescence appears to represent a critical period of vulnerability for depression. According to one longitudinal study, approximately three-quarters of individuals who receive a diagnosis of depression during their lifetime will experience onset of the disorder during childhood or adolescence (Kim-Cohen et al., 2003). The mean age of onset for depression is approximately 15 years (Lewinsohn, Clarke, Seeley, & Rohde, 1994) though girls report their first episode of depression to occur on average two years earlier than boys (Giaconia et al., 1994). Earlier age of onset has also been reported for children with a depressed parent (Weissman, 1984).

One of the most notable epidemiological features of depression is the significantly greater risk of this psychiatric disorder for girls. Whilst the ratio of female to male diagnoses of depression in childhood is approximately 1:1, this gender ratio increases to 2: 1 by adolescence (Essau, 2000; Hankin, 1998; Seeley, 2009), paralleling the adult ratio (Weissman et al., 1996). This differential risk begins to emerge in early adolescence (between 11-13 years) and is well-established by mid-adolescence (approximately 15 years) (Hankin, 1998; Lewinsohn et al., 1994; Lewinsohn et al., 1998).

On the whole, the phenomenology of adolescent depression is relatively similar to adult depression (Kovacs, 1996a; Lamers et al., 2012; Lewinsohn, Pettit, Joiner, & Seeley, 2003; Lewinsohn et al., 1998), though there is also recognition of some developmental

differences in symptom expression that potentially relate to cognitive, social, emotional, and biological changes experienced during this period (Harrington, 2001; Weiss & Garber, 2003). Most researchers and clinicians refer to the fifth edition of the Diagnostic and Statistical Manual of Mental Disorders – Text Revision (DSM-5; American Psychiatric Assocation, 2013) when making a diagnosis of depression. DSM-5 requires the presence of five of the following symptoms most of the time, on most days, for at least two weeks: depressed mood, anhedonia, disturbance in appetite and/or sleep, psychomotor agitation or retardation, fatigue or anergia, feelings of worthlessness or excessive guilt, reduced ability to concentrate or make decisions, and suicidal thoughts and/or behaviours.

In adults, there is a requirement that either depressed mood or anhedonia be present for a diagnosis, indicating that the DSM-5 regards mood disturbance as the integral feature of the disorder. DSM-5 allows a diagnosis of depression to be given to a child or adolescent if irritability rather than low mood or anhedonia is present. The remaining symptoms that contribute towards the clinical picture may differ markedly between individuals, making it a highly heterogeneous disorder. The difference in DSM-5 criteria for children/adolescents versus adults suggests that one potential developmental difference in the depression profile for children and adolescents compared to adults may be the presence of irritability as the defining symptom of the disorder rather than low mood or anhedonia, though at least one study has suggested that irritability without the co-occurrence of depressed mood is relatively rare (Stringaris, Maughan, Copeland, Costello, & Angold). Studies have suggested the possibility however that over the transition from childhood to adolescence, anhedonia, vegetative symptoms (e.g., eating and sleeping difficulties), hopelessness, psychomotor retardation and diurnal variation of mood may

increase, whilst somatic complaints become less frequent (Carlson & Kashani, 1988; Ryan et al., 1987).

Whilst longitudinal studies indicate that 60-90% of major depressive episodes experienced during adolescence will remit within a 12 month period (Dunn & Goodyer, 2006; Essau, 2007; Keller et al., 1988), adolescent-onset major depressive disorder caries a strong risk for recurrence. Individuals who experienced an onset of depression during adolescence are four times more likely to have an adult depressive disorder than adolescents who never experience a depressive episode (Harrington, Fudge, Rutter, Pickles, & Hill, 1990). Approximately 40% of adolescents with diagnosed depression will relapse within 2 years, and approximately 70% will relapse within 5 years (Kovacs, 1996b; Patton et al., 2014; Rao et al., 1995).

Moreover, whilst depression remains a leading cause of disability and morbidity for all ages, it may be particularly detrimental to experience an adolescent onset of the disorder. Depressed adolescents experience substantial concurrent impairment, including educational and relationship difficulties, social-cognitive distortions or biases, low self-esteem and high emotional reliance on others (Avenevoli, Swendsen, He, Burstein, & Merikangas, 2015; Lewinsohn et al., 1998; Rohde, Lewinsohn, & Seeley, 1994). Approximately two-thirds of clinically referred depressed adolescents experience suicidality (Kovacs, Goldston, & Gatsonis, 1993). Depression may also contribute towards teenage pregnancy, school drop out and obesity (Bardone, Moffitt, Caspi, Dickson, & Silva, 2009; Fergusson & Woodward, 2002; Fleming, Boyle, & Offord, 1993; Franko, Striegel-Moore, Thompson, Schreiber, & Daniels, 2005; Goodman & Whitaker, 2002). Furthermore, as depression can arrest normative developmental processes and cause

considerable impairment at a time when adolescents are often required to make many important long-term decisions, it may place the adolescent on a negative trajectory from which it may be difficult to diverge, even after recovery. In particular, adolescents may experience ongoing difficulties in social relationships and role functioning that continue into adulthood (e.g., Gotlib, Lewinsohn, & Seeley, 1998; Hammen, Brennan, Keenan-Miller, & Herr, 2008). A history of adolescent depression has also been associated with impaired occupational and educational functioning, poorer physical well-being, reduced reported quality of life and life satisfaction and greater substance use in later years (Fergusson & Woodward, 2002; Lewinsohn, Rohde, Seeley, Klein, & Gotlib, 2003; Rao et al., 1995). Subclinical levels of depressive symptoms also show similar concerning associations with negative outcomes (Gotlib, Lewinsohn, & Seeley, 1995; Seeley, 2009).

The age at which individuals first experience a depressive episode appears to have implications for prognosis. Within the adolescent period, an earlier onset of depression has been associated with longer duration of the disorder, greater risk of recurrence as well as a higher level of psychosocial impairment (Hammen et al., 2008; Kovacs et al., 1984; Lewinsohn et al., 1994). There is also greater risk of suicide amongst individuals with an earlier age of onset (Harrington et al., 1990; Lewinsohn et al., 1994; Zisook et al., 2007). Several explanations for the association between an earlier age of onset and poorer outcomes have been proposed. It has been argued that early onset may represent a more severe form of the disorder (Weissman, 1988), or indicate a biological vulnerability that is more easily triggered by environmental adversity (Kovacs et al., 1984). Alternatively, younger adolescents with depression may simply not yet developed the coping strategies to manage their depressive symptoms that older adolescents draw upon (Kovacs et al., 1984).

Depression in adolescence also shows considerable comorbidity with other psychiatric conditions, with one review suggesting that the presence of depression during this time increases the risk for another disorder at least twentyfold (Angold & Costello, 1993). According to both clinical and epidemiological studies, between 40-70% of children and adolescents with depression may have at least one comorbid psychiatric disorder (Essau, 2008; Kovacs, Obrosky, & Sherrill). Common comorbidities include anxiety disorders, followed by disruptive behaviour disorders, and substance use disorders (Seeley, 2009). Specifically, between 25-75% of individuals with depression may have a co-existing anxiety disorder, 21-50% may have conduct disorder and about 25% may have alcohol or drug abuse (Angold, Costello, & Erkanli, 1999; Axelson & Birmaher, 2001; Birmaher et al., 1996; Costello et al., 2003).

The observation of frequent comorbidity between anxiety and depression has led researchers to query a shared etiology for these disorders (e.g., Middeldorp, Cath, Van Dyck, & Boomsma, 2005). The experience of anxiety has been found to often precede the emergence of depression (Cohen et al., 1993; Cole, Peeke, Martin, Truglio, & Seroczynski, 1998; Pine, Cohen, Gurley, Brook, & Ma, 1998; Reinherz, Giaconia, Lefkowitz, Pakiz, & Frost, 1993), raising the possibility that these conditions may share common developmental pathways (Cummings, Caporino, & Kendall, 2014).

1.3 Theories regarding the Development of Depression

Numerous theories have been proposed to account for the experience of depression. The following sections review some of the key theories that suggest a role for biological processes in the disorder. These theories provide the foundation for much of the research into the influence of factors such as genetics, brain structure and function on the

development of depression but still also highlight the potential role of environmental risk factors.

1.3.1 **Diathesis stress theory**

Many of the dominant models of depression fall into the broad framework offered by the diathesis stress theory, which offers an account of psychiatric illness that considers the two obvious sources of risk – individual vulnerability and environmental adversity (Hankin & Abela, 2005; Monroe & Simons, 1991; Zuckerman, 1999). The specific relevance of this theory to the current thesis will be discussed in greater detail in CHAPTER 2 however the key proposition is that depression occurs only when there is both a diathesis (vulnerability) and the environmental stress – neither one on its own is sufficient. The model therefore offers one explanation for why some individuals but not others develop depression after certain occurrences. By allowing inclusion of a broad range of different vulnerabilities (e.g., genetic, temperamental, neurobiological, cognitive) and environmental stressors (e.g., abuse, chronic illness, adverse family environments), the diathesis stress theory also allows for equifinity - many different diathesis-stress pathways may confer risk for the same outcome of clinical depression.

The association between stress and depression is one of the most consistently documented findings (Hammen, 2005). According to one review, stressors are 2.5 times more prevalent in depressed patients compared to controls and major life events may precede as many as 80% of episodes of depression (Mazure, 1998). Furthermore, approximately 20-50% of people who experience a severe stressful event go on to develop depression. Adolescents experience more stressors than children, particularly interpersonal events (Ge, Lorenz, Conger, Elder, & Simons, 1994; Ge, Natsuaki, & Conger, 2006;

Rudolph & Hammen, 1999). Furthermore, whilst a variety of stressors may contribute to the subsequent development of depression in adolescence (Hankin, 2006), stress within relationships or stress associated with loss, including bereavement, separations, threats of separations or conflict, seems to carry particular risk (Eley & Stevenson, 2000; Paykel, 2003; Rudolph et al., 2000). Child abuse, maltreatment and neglect have particularly strong associations with depression, showing a two-to fourfold increase in risk for adolescents, who have experienced abuse in their earlier years (Brown, Cohen, Johnson, & Smailes, 1999; Norman et al., 2012).

1.3.2 **The Monoamine-deficiency hypothesis.**

The monoamine-deficiency hypothesis originated from the discovery that the first antidepressants, Iproniazid and Imipramine, boosted availability of serotonin and noradrenaline function (Andrews, Bharwani, Lee, Fox, & Thomson, 2015; Coppen, 1967). Since these monoamine enhancers were found to improve depressive symptoms, it was posited that depression must be caused by deficiencies or imbalances in monoamine neurotransmitters, particularly serotonin and/or noradrenaline (Belmaker & Agam, 2008). Indeed, investigations into the monoamine theory of depression yielded many of today's most commonly prescribed antidepressant agents, which act by either inhibiting monoamine reuptake (for example, selective serotonin reuptake inhibitors (SSRI's), such as fluoxetine) or by inhibiting degradation (for example, monoamine oxidase inhibitors such as tranylcypromine) (Wong & Licinio, 2004).

Whilst it has been arguably the most influential theory of depression for much of the last decade, several findings demonstrate the inadequacy of monoamine deficiency theory as a complete explanation of the psychophysiology of depression (Andrews, Bharwani, et

al., 2015). First, antidepressants' effects on monoamine function are immediate (Bymaster et al., 2002; Rutter & Auerbach, 1993) but mood-elevating effects are delayed, generally requiring several weeks of continuous treatment (Charney, Menkes, & Heninger, 1981; Oswald, Brezinova, & Dunleavy, 1972), indicating that additional mechanisms to the simple restoration of serotonin levels are likely involved. Second, therapeutic effects of antidepressants are only observed in between 50-70% of patients (Undurraga & Baldessarini, 2012) – a figure that seems less impressive when one also considers that as many as 30-50% of patients improve in the placebo-group of clinical studies (Bschor & Kilarski, 2016; Undurraga & Baldessarini, 2012). Third experimentally-induced monoamine depletion does not cause depressive symptoms in healthy individuals (though it does have mood lowering effects in individuals with a family history of Major Depressive Disorder (MDD) and in currently drug-free remitted patients who had previously been prescribed serotonergic antidepressants) (Ruhe, Mason, & Schene, 2007b). These findings suggest a simple deficiency of monoamines as the cause of depression to be highly improbable and it is now thought that downstream changes to alterations in monoamine function are likely to mediate the effects of antidepressants (Berton & Nestler, 2006; Jans, Riedel, Markus, & Blokland, 2007). Furthermore, the centrality of serotonin in the etiology of depression is being increasingly challenged by the development of other biological models, including those that suggest a role for the hypothalamic-pituitary adrenal axis or neurogenesis, though a clear role for serotonin neurotransmission remains evident in these theories.

1.3.3 **Hypothalamic-pituitary-adrenal (HPA) axis dysregulation**

The contribution of stress to the etiology of depression is well-recognised, though it is also acknowledged that stress is also not always necessary nor sufficient to precipitate a depressive episode (Hammen, 2005). Furthermore, an individual's pattern of physiological and psychological responses to stressors is now regarded as equally if not more important than the nature and intensity of the stressors themselves with regards to the development of depression (Guerry & Hastings, 2011). The current conceptualisation of the impact of adverse or stressful experiences in the etiology of depression has focused predominantly on pathways involving the HPA axis, one of the key neuroendocrine systems for regulating stress in the body (Juster et al., 2011; McEwen 1998; Sapolsky, 2000; Sapolsky, Krey, & McEwen, 1986). Briefly, activation of the HPA axis in response to physiological stress or perceived psychological stress is initiated by secretion of corticotropin-releasing factor (CRF) by the hypothalamus, which provokes release of corticotropin (ACTH) from the pituitary, which in turn results in secretion of glucocorticoids (cortisol in humans) from the adrenal cortex. The HPA axis is also regulated by a cortisol-mediated negative feedback mechanism, such that elevations in cortisol levels typically inhibit HPA axis activity via negative feedback from the hippocampus (Jacobson & Sapolsky, 1991).

Interestingly, there is evidence of complex interrelationships between serotonin system and the HPA axis (Porter, Gallagher, Watson, & Young, 2004; Tafet & Nemeroff, 2016). For example, studies have revealed that serotonin may activate the HPA axis and stimulate glucocorticosteroid secretion, as well as strengthen the negative feedback control of cortisol (Dinan, 1996; Fuller, 1992, 1996; van Praag, 2004). There is also some indication that increased cortisol levels may produce a higher central nervous system (CNS)

serotonin turnover initially but that over time, serotonin turnover and the mRNA production and sensitivity of particular serotonin receptors (e.g., 5-HT1A) may be reduced (McAllister-Williams, Anderson, & Young, 2001; van Praag, 2004).

Premorbid differences in HPA axis function may provide one explanation for why some but not others develop depression after a stressful event (Adam, Sutton, Doane, & Mineka, 2008). For example, impaired cortisol feedback due to reduced glucocorticoid receptor function (so-called glucocorticoid resistance) that results in higher levels of cortisol secretion may contribute to depression (Pariante & Miller, 2001). Changes in HPA axis function in response to stress may also represent a pathway to depression. The HPA axis is widely acknowledged to be plastic to the environment, and exposure to severe or chronic stress can produce alterations in HPA axis functioning that results in excessive or blunted glucocorticoid release. Alterations to glucocorticoid production in turn may cause further downstream structural changes in the brain (McEwen 1998; McEwen, 2012). In particular, glucocorticoids can result in structural and functional changes in brain regions with high densities of glucocorticoid receptors, including the hippocampus, prefrontal cortex (PFC) and amygdala, (McEwen, 2012; McEwen, Nasca, & Gray, 2016). In this case, the experience of stress has resulted in an accumulation of detrimental psychological and physical demands to the central nervous system (allostatic load) that may potentiate vulnerability to depression and other neuropsychiatric disorders. As discussed in detail in CHAPTER 5, variation in the volumes and activities of these regions have been implicated in depression.

A large body of research suggests developmental shaping of HPA axis function by childhood caregiving experiences (Gunnar & Hostinar, 2015; Hostinar & Gunnar, 2013),

and that exposure to adverse environments during early life can disrupt the developmental trajectory and activity of the HPA axis (Gunnar & Quevedo, 2007). This is the primary mechanism through which it is typically argued that adverse experiences may 'get under the skin' to shape brain structure and neural function and ultimately influence psychopathology (McEwen, 2012), though emerging research suggests a more wide-reaching impact of adversity on neurodevelopment that goes beyond the HPA axis, indicating a need to expand the focus to other potential mechanisms (McLaughlin, Sheridan, & Lambert, 2014).

Whilst research has consistently indicated the existence of HPA axis dysregulation in a significant proportion of depressed adults (Gillespie & Nemeroff, 2005), and dysregulation of the HPA axis has also been associated with early life adversities such as child maltreatment (Tarullo & Gunnar, 2006), there is debate about whether clear differences in HPA axis functioning exist between depressed and non-depressed adolescents. A recent review has argued that classic cortisol hypersecretion, common in depressed adult patients, is relatively rare in depressed adolescents relative to controls (Guerry & Hastings, 2011), though the authors did acknowledge that an overall non-significant trend is often observed. An earlier meta-analysis however found significantly higher basal cortisol levels in depressed children and adolescents compared to their non-depressed counterparts, which did not appear to be associated with dysregulation at any particular stage of the circadian rhythm (Lopez-Duran, Kovacs, & George, 2009). However, cortisol differences in depression during childhood and adolescence were smaller compared with cortisol differences during middle adulthood or during older adulthood. Another review noted that a more robust cross-sectional finding associated with adolescent

depression is of elevated cortisol levels in the evening, before sleep onset (Adam et al., 2008). Further research is required to clarify the extent to which HPA axis dysfunction plays a role in the emergence of depression in adolescence.

1.3.4 **Neurogenesis theory and the influence of neurotrophins**

It is now known that neurogenesis, the generation of new neurons, occurs throughout the lifetime, and is particularly prevalent in the subgranular zone of the dentate gyrus of the hippocampus and the subventricular zone (Bruel-Jungerman, Rampon, & Laroche, 2007; Eriksson et al., 1998; Gonçalves, Schafer, & Gage; Spalding et al., 2013). Brain imaging studies suggesting that depression is associated with reduced hippocampal volume in adults have supported a popular hypothesis implicating decreases in neurogenesis in the etiology of the disorder (Kempermann, 2002). Importantly, antidepressants have been shown to increase neurogenesis and support survival of new neurons, and neurogenesis appears to be required for the behavioural effects of antidepressants in animals (Santarelli et al., 2003).

Research suggests the system of trophic agents involved in stimulating neurogenesis may have an important role in the onset of psychiatric disorders and depressive disorders in particular. Brain-derived neurotrophic factor (BDNF) has been particularly implicated, with findings that serum concentrations of BDNF are lower in untreated patients with MDD but can be normalised by antidepressant treatment (Molendijk et al., 2014) giving the neurogenesis theory of depression further credence. Two imaging studies on hippocampal volume and BDNF in humans indicate the Met allele of BDNF is also associated with reduced volumes in the hippocampus in depressed patients and healthy volunteers (Frodl et al., 2007; Pezawas et al., 2004).

Chronic stress has also been shown to potently decrease adult hippocampal neurogenesis (Dranovsky & Hen, 2006). It has been speculated that reduced neurogenesis in hippocampal regions may occur as a result of stress-induced increases in glucocorticosteroid concentrations (Mirescu & Gould, 2006). Hippocampal atrophy in non-human primates and rodents following exposure to high doses of corticosteroids has been observed. This effect may be partially mediated by changes in BDNF. Corticosteroids are known to reduce BDNF, which in turn has been found to be associated with decreases in hippocampal neurogenesis. Conversely, hippocampal neurogenesis has also been shown to regulate HPA axis activity and the secretion of glucocorticoids in response to stress (Schloesser, Manji, & Martinowich, 2009; Snyder, Soumier, Brewer, Pickel, & Cameron, 2011). Interestingly, animal studies have suggested that adult hippocampal neurogenesis may be required for the ability of enriched environments to promote stress resilience and recovery from stress-induced depressive behaviours (Schloesser, Lehmann, Martinowich, Manji, & Herkenham, 2010).

Research also suggests strong interplay between the BDNF pathway and serotonergic system activity(Branchi, 2011; Martinowich & Lu, 2007). Antidepressants have been shown to increase BDNF concentrations and stimulate neurogenesis after 3-4 weeks administration, the time course for maturation of new neurons and also when the effects on mood are often observed (Castrén, 2004a, 2004b). BDNF mRNA levels in the rat brain have been shown to be modulated by serotonin (Zetterström et al., 1999). In turn, there are indications that BDNF levels affect the serotoninergic system; BDNF appears to stimulate growth and differentiation of serotonergic neurons (Mamounas, Blue, Siuciak, & Altar, 1995) and increased brain serotoninergic activity has been documented following

increases in BDNF levels (Mössner et al., 2000). Understanding of the contribution of neurogenesis and neurotrophins such as BDNF to the etiology of depression remains in its infancy however and the relevance of this theory to the emergence of this disorder in adolescence in particular is unclear.

1.4 Summary and Implications

Depression in adolescence is both a common and serious mental health problem that shows high rates of recurrence and impairment, at significant cost to both the individual and society. The criteria for major depressive disorder reveal a potentially highly heterogeneous presentation, which suggests the involvement of multiple etiological factors. An examination of the epidemiological evidence illustrates that the transition from childhood to adolescence and young adulthood marks a period of increased risk for onset of the disorder that is unique across the lifespan. Compared to adult-onset depression, adolescent-onset depression is associated with a high risk for recurrence and is associated with the presence of significant negative consequences well into adulthood. Adolescence is therefore a particularly important developmental period for investigating vulnerability to depression and research that attempts to situate depression etiology within the context of normative processes and changes that occur in adolescence is likely to be beneficial in elucidating the larger course of the disorder. Current frameworks and theories highlight the existence of a variety of factors (including genes, neural circuits and environmental experiences) and cross-level mechanisms, and thus emphasise the importance of a research lens that considers multiple levels of analysis that incorporates both individual difference as well as the critical role of environmental regulation of emotion and stress neurobiology when approaching questions regarding the emergence of psychopathology. Interestingly,

the direct or indirect influence of serotonin neurotransmission and alterations in brain structure, particularly in the hippocampus, but also in the amygdala and prefrontal regions, has been noted by the major theories for depression (though their applicability to depression in adolescence remains unclear). The role of one particular aspect of the serotonin system in the emergence of depression that has attracted particular research attention concerns individual differences in the serotonin transporter promoter region polymorphism (5-HTTLPR) and this will be discussed in greater detail in CHAPTER 2.

CHAPTER 2: THE ROLE OF THE SEROTONIN TRANSPORTER GENE X ENVIRONMENT INTERACTION IN DEPRESSION

2.1 The complex genetic basis of depression

Genetic epidemiological studies, including family and twin studies, have accrued convincing evidence that genetic factors contribute significantly to vulnerability to mood disorders, including depression, (Craddock & Forty, 2006; Kendler, Gatz, Gardner, & Pedersen, 2006; Rice, Harold, & Thapar, 2002; Sullivan, Neale, & Kendler, 2000). Depression is known to run in families; lifetime risk for the disorder is estimated to be between two to three times higher for individuals with a first-degree relative with depression (Sullivan et al., 2000). Twin studies, which allow for a simultaneous estimation of genetic and environmental influences, suggest that heritability for depression, namely the variation in the population risk of depression that is attributable to genetic variation, falls within the range of 31%-42% (Rice et al., 2002). Recurrent- early onset forms of the disorder, defined as the experience of two or more episodes before 25 years old, may carry a higher genetic loading, with heritability estimated to be approximately 70% (Zubenko, Zubenko, Spiker, Giles, & Kaplan, 2001).

Given the large body of epidemiological and behavioural genetic evidence supporting a strong genetic contribution to the disorder, it had been hoped that the search for genetic factors involved in depression would be relatively uncomplicated. Research that has aimed to identify the genetic architecture of depression however has encountered a puzzling incongruity between these high heritability estimates and a deficit of replicable gene-disorder associations – a phenomenon that has been termed the “missing heritability” problem (Lesch, 2011). Exploratory approaches entail searches of the entire genome

without *a priori* hypotheses about the contribution of specific genes or chromosomal areas and include linkage studies and genome-wide association studies. Both these approaches arguably have had limited success (Lohoff, 2010; Wray et al., 2012).

Linkage studies involve the systematic scanning of the genomes of large families of individuals with and without a particular disease to identify genetic regions "in linkage" with the disorder by showing that affected individuals have particular variants of genetic sequences (i.e., alleles) more frequently than would be expected by chance. Genome-wide association studies (GWAS) are based upon a similar premise, comparing genomes of affected and unaffected individuals to determine whether any allele of a genetic variant are more frequent in those with the phenotype of interest. Comparisons do not need to be between family members however and therefore can be completed in much larger sample groups. GWAS are usually performed at the level of single nucleotide polymorphisms (SNPs), and are typically executed in two stages; a discovery phase involving the screening of the entire genome, and a replication phase involving the testing of specific SNPS in an independent sample. Although linkage studies have suggested several regions in the genome that may contain risk alleles, they have not yet demonstrated any consistent findings (Lohoff, 2010).

Likewise, the majority of GWAS meta-analyses comparing MDD cases and controls have not identified any genetic variants that achieve genome-wide significance (Flint & Kendler, 2014; Major Depressive Disorder Working Group of the Psychiatric GWAS Consortium, 2013; Wray et al., 2012). More recently, a meta-analysis involving 84,847 cases and 241,266 controls (Hyde et al., 2016) identified 17 independent SNPs that showed a significant association with major depression diagnosis, with many of the top

associations appearing in or near genes encoding transcription factors with known neurodevelopmental functions though no previously identified role in depression. The magnitude of effects of the individual SNPs were very small however and the extent to which any of these findings can be replicated by other GWAS studies remains to be seen.

What these results demonstrate is that depression is a complex disorder with no single gene being necessary or sufficient for its development (Lohoff, 2010). Depression is now considered to have a complex mode of inheritance that most likely involves considerable genetic heterogeneity, incomplete penetrance, and significant interaction with the environment, resulting in each susceptibility gene contributing only a small amount to the overall risk for the condition. Furthermore, it is now acknowledged that some risk alleles may increase vulnerability to psychiatric disorders more broadly, rather than being specific to depression (Kendler, Neale, Kessler, Heath, & Eaves 1992).

This complex mode of inheritance of depression as well as the challenges associated with our current conceptualization of the disorder (which involves diverse phenomenology as well imprecisely defined traits) may make linkage and genome-wide association studies less appropriate for the identification of "causal depression genes." Linkage studies generally have low power to detect low-risk alleles, whilst association studies only possess sufficient power if the risk alleles are relatively common (minor allele frequency >.05) or if odds of detection are increased by focusing on a specific disease subtype or phenotype or by incorporating environmental factors into the study design (Flint & Kendler, 2014). In the absence of very large samples, currently a candidate gene approach may be more fruitful in the search for genetic factors involved in the etiology of depression. In contrast to linkage and genome-wide association studies, the candidate gene approach involves an assessment

of the validity of an "educated guess" regarding the association between the alleles of a specific (candidate) gene and a disease (Kwon & Goate, 2000). Selection of the candidate gene is based upon existing understanding of underlying disease mechanisms. Candidate gene approaches have been argued to be better suited to detecting genes associated with common and complex diseases where the contribution of any one gene may be relatively minor (Collins, Guyer, & Charkravarti, 1997; Risch & Merikangas, 1996).

2.2 The role of a serotonin transporter gene in depression

Search for candidate genes for depression has focused largely (although not exclusively) on the serotonin (5-hydroxytryptamine, or 5-HT) system given multiple lines of evidence that have implicated impaired serotonin neurotransmission in dysregulated emotional processing and in vulnerability to mood and anxiety disorders (Cowen, 2008; Lucki, 1998; Owens & Nemeroff, 1998; Sharp & Cowen, 2011). This evidence includes clinical, neuroimaging and neuroendocrine findings indicating that (i) many substances that increase serotonin neurotransmission by blocking 5-HT reuptake or metabolism (including selective serotonin reuptake inhibitor (SSRI) antidepressants) alleviate depressive symptoms (Blier & de Montigny, 1999; Nemeroff & Owens, 2002) whilst substances that deplete serotonin, such as reserpine can induce depressed mood (Freis, 1954; Goodwin & Bunney, 1971), (ii) plasma concentrations of tryptophan, a biochemical precursor of serotonin, may be lower in depressed patients (Cowen, Parry-Billings, & Newsholme, 1989; DeMyer, Shea, Hendrie, & Yoshimura, 1981; Maes, De Ruyter, Hobin, & Suy, 1987), (iii) acute tryptophan depletion induces a profound, temporary relapse of symptoms in recovered depressed patients who had responded to serotonergic antidepressant treatment (Booij et al., 2002; Delgado et al., 1990; Ruhe, Mason, & Schene, 2007a), (iv) blood

platelets of depressed patients show diminished uptake of serotonin (Sheline, Bardgett, Jackson, Newcomer, & Csernansky, 1995), and (v) brain tissue of depressed patients may contain fewer 5-hydroxytryptamine (serotonin) receptor 1A (5-$H1_A$) receptor binding sites (Drevets et al., 2007; Sargent et al., 2000) and potentially fewer serotonin transporter binding sites (Parsey et al., 2006; Selvaraj et al., 2011).

Serotonin is both a key neurotransmitter in the brain and a regulator of brain development. Serotonergic neurons originate primarily from the raphe nuclei in the brainstem, and innervate all areas of the human brain (Hensler, 2006; Jacobs & Azmitia, 1992). Serotonergic neurons with cell bodies in the dorsal and median raphe nuclei project to all regions of the forebrain, but particularly limbic structures, including prefrontal and cingulate cortices, the amygdala, hippocampus and the adjacent entorhinal cortex, ventral striatum, and hypothalamus (Hensler, 2006). A smaller group of serotonergic neurons with cell bodies in the caudal raphe nuclei project to the brainstem, cerebellum, and spinal cord. Serotonin thus unsurprisingly influences a wide range of functions, including sensorimotor activity, appetite, maintenance of circadian rhythm, sexual behaviour, mood cognition, learning and memory (Lucki, 1998), all of which may be disturbed in mood disorders, including depression. Similarly, the serotonin system appears to have a role in a number of other psychiatric or neurodevelopmental conditions, including anxiety disorders (Maron & Shlik, 2005; Ressler & Nemeroff, 2000), schizophrenia (Bleich, Brown, Kahn, & van Praag, 1988; Meltzer, 1999), bipolar disorder (Mahmood & Silverstone, 2001), addiction (Müller & Homberg, 2015), and autism spectrum disorder (Benza & Chugani, 2015).

Serotonin is also known to modulate neural cell proliferation, migration and differentiation and is also involved in synapse formation and neural network construction,

including the growth and guidance of axons and trans-synaptic signalling during brain development (Daubert & Condron, 2010; Gaspar, Cases, & Maroteaux, 2003). Serotonin may also have a critical role in the plasticity of both the developing and adult brain by regulating the action of synaptic cell adhesion molecules (Lesch & Waider, 2012), which form part of the system responsible for connecting pre- and postsynaptic neurons, facilitating neural transmission, controlling synaptic plasticity and refining neural circuits (Yamagata, Sanes, & Weiner, 2003). Thus, dysregulation of the serotonin system may not only contribute towards psychiatric disorders as a function of current levels of serotonin available to support neurotransmission, but also potentially as a result of its wide-ranging effects on earlier neurodevelopment.

Research investigating candidate genes for depression that belong to the serotonin system has centred on the serotonin transporter gene *(SLC6A4,* synonyms: 5-HTT, SERT*),* based upon findings that the gene is a key regulator of serotoninergic neurotransmission and a direct target for several antidepressants, including serotonin re-uptake inhibitors (Lotrich, Pollock, & Ferrell, 2001). The serotonin transporter gene is located on chromosome 17q11.1-q12 and comprises 14 exons spanning ~40kb (Aslund et al., 2009; Lesch et al., 1994; Murphy & Moya, 2011; Ramamoorthy et al., 1993). The serotonin transporter gene codes for serotonin transporter protein, which is responsible for removing serotonin from the neuronal synapse and returning it to the presynaptic neuron for degradation or rerelease (Blakely et al., 1991).

Numerous polymorphisms within the serotonin transporter gene exist, including an insertion/deletion polymorphism in the gene promoter (5-HTTLPR). Given that the 5-HTTLPR has been observed to be a functional polymorphism, it has been the focus of

intense study.[1] There are two common alleles within 5-HTTLPR – a short (*S*) variant and a long (*L*) variant, which contain 14 and 16 copies of a 43bp repeat cassette respectively (Heils et al., 1996). Functional studies of the activity of 5HTTLPR in transfected cell lines, post-mortem human brains and lymphoblasts have demonstrated that the S allele is associated with lower transcriptional efficiency and is associated with a nearly 50% reduction in serotonin availability (Heinz et al., 2000; Hu et al., 2006; Lesch et al., 1996). There is some evidence that the S allele is dominant over the L allele (Heils et al., 1996; Hranilovica et al., 2004), which has led many studies to analyse S/S and S/L individuals together as S-carriers (Caspi et al., 2003; Hariri et al., 2002; Klumpers, Heitland, Oosting, Kenemans, & Baas, 2012). A number of research findings however also support the possibility of an additive or intermediate dominance effect, where effects for the heterozygous S/L genotype are midway between participants with the homozygous genotypes (Chen, Joormann, Hallmayer, & Gotlib, 2009 ; Hankin et al., 2011; Pluess et al., 2011). A much smaller group of studies have identified a pattern that could be consistent with dominance of the L-allele over the S allele (e.g., Bakermans-Kranenburg & IJzendoorn, 2008; Williams et al., 2003).

There are striking differences in the frequencies of these 5-HTTLPR alleles in populations of different ethnic backgrounds. Amongst Caucasian samples, the frequency of

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[11] Other genetic variations within the serotonin transporter gene have been identified, including a 17bp variable-number-of tandem-repeats (VNTR) polymorphism in the second intron of the gene, which is currently presumed to have no functional consequences for serotonin production (MacKenzie & Quinn, 1999 but c.f. Lovejoy, Scott, Fiskerstrand, Bubb & Quinn, 2003), and hence has received little research attention. The serotonin transporter gene may also contain a number of SNPs, the majority of which have also been found to be non-functional and/or of extremely low prevalence and hence have usually not been considered when examining associations between different alleles and neuropsychiatric phenotypes (Kunugi et al., 1997; Nakamura, Ueno, Sano, & Tanabe, 2000).

S-allele is approximately 40% and the frequency of the L-allele is approximately 60% (Lesch et al., 1996). African/African American samples exhibit comparably lower frequencies of the S allele of approximately 27%, and higher frequencies of the L-allele of approximately 73% (Gelernter, Cubells, Kidd, Pakstis, & Kidd, 1999). Samples of participants of Asian background exhibit the reverse pattern, showing 74%/26% split in allele frequency for the S-allele and L-allele respectively (Gelernter et al., 1999; Goldman, Glei, Lin, & Weinstein, 2010).

More recently, An Adenine (A) → Guanine (G) SNP, rs25531, located within the L allele of 5-HTTLPR, has been identified. The L allele containing the rarer G substitution, which occurs at a frequency of 9-15% in Caucasians and 24% in African Americans, is thought to be functionally equivalent to the lower-expressing S allele. The 5-HTTLPR polymorphism is thus regarded as functionally biallelic (S/L_G and L_A) (Wendland, Martin, Kruse, Lesch, & Murphy, 2006). Genotyping of L_G and L_A is increasingly being incorporated into investigations, however many studies continue to focus on the effect of the S/L alleles.

Individual studies' findings of an overall relationship between the serotonin transporter gene promoter and depression have been inconsistent, but five meta-analyses to date provide evidence that S allele carriers, who possess either one or two copies of the S allele, have an elevated risk of depression compared to individuals homozygous for the L allele (Clarke, Flint, Attwood, & Munafò, 2010; Furlong et al., 1998; Kiyohara & Yoshimasu, 2010; López-León et al., 2008 ; Lotrich, 2004) whilst another four meta-analyses obtained negative results (Anguelova, Benkelfat, & Turecki, 2003; Culverhouse et al., 2017; Lasky-Su, Faraone, Glatt, & Tsuang, 2005; Risch et al., 2009). The conflicting

findings of individual studies and meta-analyses suggest that if 5-HTTLPR genotype on its own does incur direct risk for depression, large samples may be critical for the detection of this likely very small contribution of this genetic polymorphism against the background of the action of many genes in combination with numerous other biological and psychosocial risks. Interestingly, some support for a direct role of the action of the serotonin transporter gene but in the opposite direction may come from results of a meta-analysis which indicated a more favourable response to SSRI antidepressants amongst individuals with an L-allele than those homozygous for the S allele (Serretti, Kato, De, & Kinoshita, 2007).

2.3 Gene-environment Interactions involving the Serotonin Transporter Gene

Alternatively, the somewhat inconsistent findings of an overall association between 5-HTTLPR and depression may point to stronger effects of this polymorphism in different environments. A seminal study by Caspi and colleagues (2003) found that individuals carrying the S-allele of the serotonin transporter gene were more vulnerable to the depressogenic effects of childhood maltreatment or multiple negative stressful life events in the preceding 5 years than individuals homozygous for the L allele. This study is considered to be of one of "extraordinary quality," (Wankerl, Wust, & Otte, 2010, p. 584), not only for its prospective longitudinal design with an epidemiologically representative cohort but also for its use of gold-standard measures. The study used repeated interview-based assessments of stressful life events that had been conducted prior to depression onset, thus reducing the well-known risk of biases that may occur through mood-congruent memory revision, in the form of overestimation of the frequency or severity of stressful events (Joormann, Hertel, LeMoult, & Gotlib, 2009). The presence of 14 stressful life events, including employment, financial, housing, health and relationship stressors, were

assessed for with the aid of a life history calendar (Caspi et al., 1996) which has high reliability (Belli, Shay, & Stafford, 2001). In addition, an objective measure of stress, childhood maltreatment that could be verified by external sources, was also included in analyses. Likewise, four depression phenotypes (1) major depression diagnosis, (2) depressive symptoms and (3) probability of suicidal ideation/attempt in the context of a depressive episode, assessed according the Diagnostic Interview Schedule, and (4) depressive symptoms per informant report were included as outcomes. Participants were 849 individuals of Caucasian background from the longitudinal Dunedin Multidisciplinary Health and Development Study, stratified into three groups according to their 5-HTTLPR genotype. The proportions of S/S homozygotes (possessing two copies of the S allele), S/L heterozygotes (possessing one copy of the S allele) and L/L homozygotes (possessing two copies of the L allele) were 17%, 51% and 31% respectively, and were in Hardy-Weinberg equilibrium, with no gender differences in genotype frequencies. Caspi and colleagues' (2003) findings indicated that in the absence of stressful life events there was no difference in depression risk between the 5-HTTLPR genotype groups. Increased exposure to stressful life events however was associated with greater risk for depression in a dose-dependent manner, but only for individuals carrying one or more copies of the S allele. Amongst individuals homozygous for the L allele, increasing stressful life events did not incur a significantly increased risk for depression. These findings were reproduced across all four depression phenotypes and were also evident when child maltreatment was examined as a measure of stress.

This demonstration of a gene-environment interaction involving the serotonin transporter gene introduced a new paradigm for genetic investigations into the etiology of

depression, which resulted in a rapid proliferation of research attempting to extend or replicate these results (Uher & McGuffin, 2010). Findings of these studies have been somewhat conflicting and this has led some researchers to query the validity of the interaction (e.g., Risch et al., Culverhouse et al., 2017; Dick et al., 2015; Duncan & Keller, 2011; 2009). In particular there are concerns about the possibility that published positive results reflect chance findings that have become overrepresented in the literature due to publication bias (Dick et al., 2015; Duncan & Keller, 2011).

Caspi and colleagues (2010) have argued however that positive results have been replicated both cross-sectionally and longitudinally, with diverse samples, and across a variety of observational research designs. They have maintained that this makes it increasingly unlikely that this finding has occurred simply by chance. Early on, a potential relationship between the type of assessment procedures employed and the study outcome was also noted (Uher & McGuffin, 2008), such that positive results appeared to be more consistently detected by studies that investigated effects of specific stressors (e.g., child maltreatment or medical illness), whilst findings of studies that used a compilation measure of a diverse range of adverse events seemed somewhat variable.

In addition, positive results appeared to be more commonly obtained by studies that utilised semi-structured interviews with contextual ratings or objective measures of stress that are able to verified externally. Negative findings seemed to be predominantly found by studies that examined a simple count of stressful life events according to a self-report questionnaire. Caspi and colleagues (2010) have contended that this pattern of results is because self-report questionnaire measures, which are usually in the form of checklists, fail to consider variation in the impact of particular events on the individual, having a tendency

to both over-report and under-report severe events by including trivial incidents whilst failing to capture events shown by semi-structured interviews to be associated with depression onset.

Notably, many of the larger studies that have conducted investigations of an interaction involving the serotonin transporter gene and stressful life events have had to rely on brief, self-report checklist questionnaires due to both budgetary and time constraints (Caspi et al., 2010; Uher & McGuffin, 2010). Studies that have investigated this GxE interaction with larger samples may therefore have done so at the cost of weaker measurements, compromising their power to detect effects. This is potentially a significant concern given simulation studies have demonstrated that even moderate declines in the measurement accuracy of the environmental variable of interest may weaken statistical power to detect a GxE interaction effect by as much as twenty-fold (Luan, Wong, Day, & Wareham, 2001; Wong, Day, Luan, & Wareham, 2003).

2.3.1 **Findings from meta-analytic studies**

Given the inconsistency of individual study findings, meta-analytic strategies may have had the potential to clarify these relationships, however these efforts have also produced varying outcomes. The first meta-analysis, which was conducted by Munafò and colleagues (2009), of 5 studies (N=2 999) provided no evidence of a significant GxE interaction effect between 5-HTTLPR genotype, stressful life events and depression (OR 1.16, 95% CI .89-1.49, $Z = 1.11$, $p = .27$). Risch et al., (2009) then conducted a meta-analysis of 14 studies (14 250), which also failed to support a gene-environment interaction involving 5-HTTLPR (OR, 1.01; CI, 0.94-1.10).

However, a number of researchers (Caspi et al., 2010; Rutter, 2009, 2010; Uher & McGuffin, 2010; Wankerl et al., 2010) have noted several possible limitations to these analyses. Perhaps of most concern are the selection criteria of the meta-analyses by Munafo and colleagues (2009) and Risch and colleagues (2009), which have been suggested to favour studies were more likely to obtain negative findings (Rutter, 2010; Uher & McGuffin, 2010; Wankerl et al., 2010). Critically, these studies tended to involve larger samples and examined the number of stressful life events according to self-report questionnaires. In fact, two negative studies that used self-report comprised 63% of the total sample in Munafo et al. (2009), and 37% of the sample in Risch et al. (2009)'s meta-analyses. It has been suggested that the overall null findings of these meta-analyses therefore may well be due to their preferential inclusion of larger non-replications using poorer methodologies that outweighed the contribution of smaller positive studies with better methodologies (Caspi et al., 2010; Wankerl et al., 2010).

Both meta-analyses also included only studies that considered depression as a dichotomous outcome (presence/absence of diagnosis according to DSM-IV or ICD-10 or established cut-off scores to define depression from standardized ratings scales), disregarding the findings of studies that had used continuous measures of depression. Given that dichotomization often leads to a decrease in the measured strength of an association between variables (Cohen, 1983) and may increase statistical error due to incorrect classification of "subthreshold" cases (Plomin & Davis, 2009), it is possible that this inclusion criterion resulted in a further underestimation of the interaction. Furthermore, these meta-analyses did not incorporate the large body of evidence for the interaction from studies examining single stressors, such as childhood abuse, loss of employment or medical

illness. This has been argued to represent a serious omission given studies of the effect of a single stressor have arguably decreased between-subject heterogeneity in their exposure and therefore superior internal validity in their study design as a result of their focus on a specific, homogeneous and more clearly operationalized experience (Caspi et al., 2010). It has been contended that these single stressor studies have more consistently yielded positive findings (Caspi et al., 2010).

Interestingly, the results of these two studies contrast with a more inclusive meta-analysis conducted by Karg and colleagues (2011), which attempted to address some of the criticisms made of the previous meta-analyses. This meta-analysis found strong support for the hypothesis that 5-HTTLPR moderates the relationship between stress and depression. It was performed on the entire body of studies up until November 2009 (54 studies, N=40 749) that have tested for the presence of this interaction. Instead of performing a traditional meta-analysis involving combining raw data from the primary studies, the authors utilised the Liptak-Stouffer z-score method to combine the studies at the level of significance tests (*P* values). This allows the inclusion of different classes of studies that might employ different designs and measures, though it also incorporates any errors or bias present in the original studies into the meta-analysis. Results demonstrated strong evidence that 5-HTTLPR moderates the relationship between stress and depression, with the 5-HTTLPR S allele associated with an increased risk for the development of depression under stress (P = .00002).

Stratification of the studies by the type of stressor examined showed strong evidence for an association between the *s* allele and increased stress sensitivity following childhood maltreatment (P = .00007) and the experience of a specific medical condition (*P*

= .0004). There was also only marginal evidence for an association in the subset of studies that simply measured the number stressful life events experienced in a particular period (*P* = .03) and findings of a significant association disappeared completely when analyses were restricted to only the studies examined in the previous meta-analyses (Munafò and colleagues (2009), *P* = .16; Risch and colleagues (2009), *P* = .11). This indicates that differing results between meta-analyses are due to differences in their inclusion criteria rather than their choice of meta-analytic statistics. To address the question of publication bias, the authors calculated that more than 729 unpublished or undiscovered studies with an average sample of 755 participants and a non-significant result would be needed to render the results of the meta-analysis non-significant. This represents a fail-safe ratio of 14 non-included studies for every included study in the meta-analysis. Neither of the previous meta-analyses by Risch et al. (2009) or Munafò et al. (2009) provided these statistics.

Sharpley, Palanisamy, Glyde, Dillingham and Agnew's (2014) update to the meta-analysis by Karg et al (2011) based on 81 studies (N=54 996) identified to June 2013 found even stronger evidence of a significant interaction between 5-HTTLPR s-allele and adverse environments and depression (p = .0000009). The interaction remained significant across separate meta-analyses of studies stratified according to research design (exposed only, case control, longitudinal and cross-sectional), type of stressor (medical illness, childhood adversity, stressful life events) and method of stress assessment (self-report questionnaires, interview and objectively verified measures), though the effect again was smaller when self-report stress assessment questionnaires were used. The authors calculated that more than 1808 studies of an average sample size of 682 participants and with nonsignificant outcomes would be required before the results of the overall analysis became nonsignificant

at the .05 level. This corresponds to a fail-safe ratio of 45 studies not included for every study included in this meta-analysis.

Sharpley and colleagues (2014) also noted that whilst the majority of studies (65%) supported an association between the s-allele, adversity and depression, nearly 26% of the included studies failed to show a significant interaction, and approximately 10% found opposite results to those expected, implicating the L-allele as vulnerable to depression in the presence of adversity. Further analyses were conducted to explore possible differences between the studies that supported the interaction between the s allele and stress predicting depression versus those which did not. Gender composition of the sample, average age of the participants, research design, type of stressor examined, method of stressor assessment, or method of depression assessment (self-report depression scale or clinical interview) did not predict which studies obtained s-allele supportive or non-supportive findings (ie. that obtained null findings or findings of an association between the L allele and depression following stress). Studies with a greater sample size were however more likely to identify null findings, suggesting that these results were not simply due to insufficient statistical power. A significant interaction between the methods of assessment for stress and depression was also identified, such that the most significant S-allele findings (lowest one-tailed *p* values) were observed when both variables of interest were assessed according to 'gold-standard' measures (i.e., depression was measured by clinical interview and stress was measured according to objectively verified methods or clinical interview, rather than self-report), whilst the least significant S-allele finding was recorded when depression was measured by clinical interview and stress was measured by self-report questionnaires. A question that does not appear to have been addressed was whether the studies that

employed gold-standard measures for both depression and stress were based on smaller samples. The extent to which the significant finding is driven by smaller studies of higher methods specifically has not therefore been clearly determined.

Sharpley and colleagues (2014) concluded that the current available literature included in their meta-analysis provides compelling support for an association between the short form of the 5-HTTLPR, adversity and depression, but that it *also* provides some support for the opposite finding (i.e. the L-allele association with adversity and depression), as well as highlighting that that a fairly substantial proportion of studies find no significant links between the serotonin transporter linked polymorphic region and depression following adversity. They contended that these non-conforming findings do not necessarily weaken the hypothesis of association between this polymorphism, adversity and depression but that they rather indicate that the interactive effect may be more complex than originally conceptualised, with potential exceptions to the typically found 5-HTTLPR S-allele, stress and depression. The authors suggested that greater exploration of the reasons why those non-conforming findings might occur would promote a more nuanced understanding of these relationships.

More recently, Culverhouse and colleagues (2017) performed a collaborative meta-analysis on 31 datasets containing participants genotyped for 5-HTTLPR that had completed assessments of stress and depression. Only studies with a minimum of 300 participants were eligible to contribute to the meta-analysis. To minimise heterogeneity of statistical models between studies, each contributing research group performed identical de novo statistical analyses that had been determined a priori (with the pre-registered protocol and analysis script published prior Culverhouse et al. (2013), which were then meta-

analysed by the coordinating research centre. Only individuals of European ancestry were included. After data harmonisation, 38 802 participants contributed to at least one of the analyses. The meta-analysis provided no clear evidence to support the presence of an interaction between 5-HTTLPR and adverse environmental experiences predicting depression.

In contrast to the analyses of Karg and colleagues (2011) and Sharpley and colleagues (2014), there was no evidence to suggest that findings in the Culverhouse (2017) meta-analysis might vary depending on whether a broad measure of stress or a more specific measure of childhood maltreatment was considered. Findings also remained non-significant regardless of whether an outcome of lifetime depression or current depression was examined, or whether analyses were based on a particular genetic model (additive, dominant or recessive). There was also no indication of any difference in the findings for males or females, or for two separate age-ranges (all ages, and young adults between 21-30 years). Moreover, to address concerns that significant findings might be obscured by studies failing to consider the timing of stressors and depression (and in particular lifetime measures precluding the establishment of a temporal order between the hypothesised cause and outcome), a sub-analysis was conducted that was limited to longitudinal studies that had queried the specific timing of these variables. These analyses did not show any significant interactive effects. Analyses that included only participants for whom life stress was documented to have occurred within the five years prior to the point when depression was measured were also non-significant.

The authors concluded that if an interaction between the S-allele of the serotonin transporter gene and stress does exist, it is likely to be of modest effect, observable in more

limited situations and not necessarily broadly generalisable. The meta-analysis did not however consider the role that the type of measurement might have played on whether a significant interaction was detected. This meta-analysis has also been criticised by some investigators for excluding studies with less than 300 participants, as these smaller studies are more likely to have been prospective-longitudinal, to have use gold-standard measures such as face-to-face interviews, and to have considered the influence of specific stressors such as medical illness, hence reducing the problem of homogeneity in the environment between subjects (Moffitt & Caspi, 2014).

It thus appears that meta-analyses that have taken a broader approach to study inclusion have tended to find evidence of a significant interaction, whilst meta-analyses that have taken a more exclusive or purely statistical approach have not identified a significant interaction. Given the inconsistencies between meta-analyses, future research arguably needs to aim to identify systematic differences between studies, including potentially more fine-grained distinctions between the populations of individuals or the environmental situations where the interaction appears to be present and where it is not.

2.4 The Diathesis Stress Hypothesis and the Differential Susceptibility Hypothesis: Frameworks for understanding serotonin transporter gene x environment interactions?

In order to more accurately identify how variations in the 5-HTTLPR genotype could interact with the environment, it is important to understand the frameworks that studies of gene-environment studies currently assume. Until recently, the majority of gene-environment interaction research has been conducted within a diathesis-stress or vulnerability paradigm (Belsky et al., 2009; Belsky & Pluess, 2009; Belsky & Pluess,

2013), as illustrated in Figure 2-1A. This framework suggests that when exposed to *adverse* environments or *negative* experiences, some individuals are more or even uniquely susceptible to *maladaptive* outcomes than others due to their possession of some endogenous "vulnerability" characteristic (e.g., a temperamental characteristic or particular allele of a gene) (Belsky et al., 2009; Pluess & Belsky, 2013). Findings pertaining to the serotonin transporter gene have most often been interpreted within this framework, which has focused on the S-allele as a "risk" allele that confers greater sensitivity to stress and hence increases susceptibility to psychiatric disorders such as depression in contexts of adversity (Caspi et al., 2010). As pointed out by Reiss and colleagues (2013), this same form of interaction, as shown in Figure 2-1A, might also be attributable to an inherited resilience variant, associated with a particular trait that confers a capacity to offset environmental challenges. When applied to gene-environment interactions involving the serotonin transporter gene, this interpretation would suggest that characteristics associated with an L-allele might offer some protection in adverse environments that may otherwise not seem particularly beneficial in more neutral or supportive environments. This interpretation is perhaps implied in allusions to the L-allele as a "resilience" allele (Markus & De Raedt, 2011; Murrough & Charney, 2011) but systematic discussions of the extent to which the L-allele's "resilience" could account for serotonin transporter gene x environmental adversity interactions do not appear within the literature, which centres rather on the S-allele's "risk."

One limitation with the diathesis-stress theory is it cannot account for why the frequency of the S-allele has been preserved in the population if it is a risk allele, given that evolution selects for favourable traits that increase the likelihood of reproduction. It seems

improbable that the S-allele would have survived unless it conferred some reproductive advantage in at least some circumstances. It has been suggested that the diathesis-stress theory's failure to consider the potential impact of serotonin transporter genotypic variation in response to positive environments may have meant that this reproductive advantage was not easily apparent (Belsky et al., 2009). In fact, as shown in Figure 2-1A, the diathesis-stress theory arguably assumes no difference in S-carriers and L-homozygotes' outcomes in non-adverse or enriched environments.

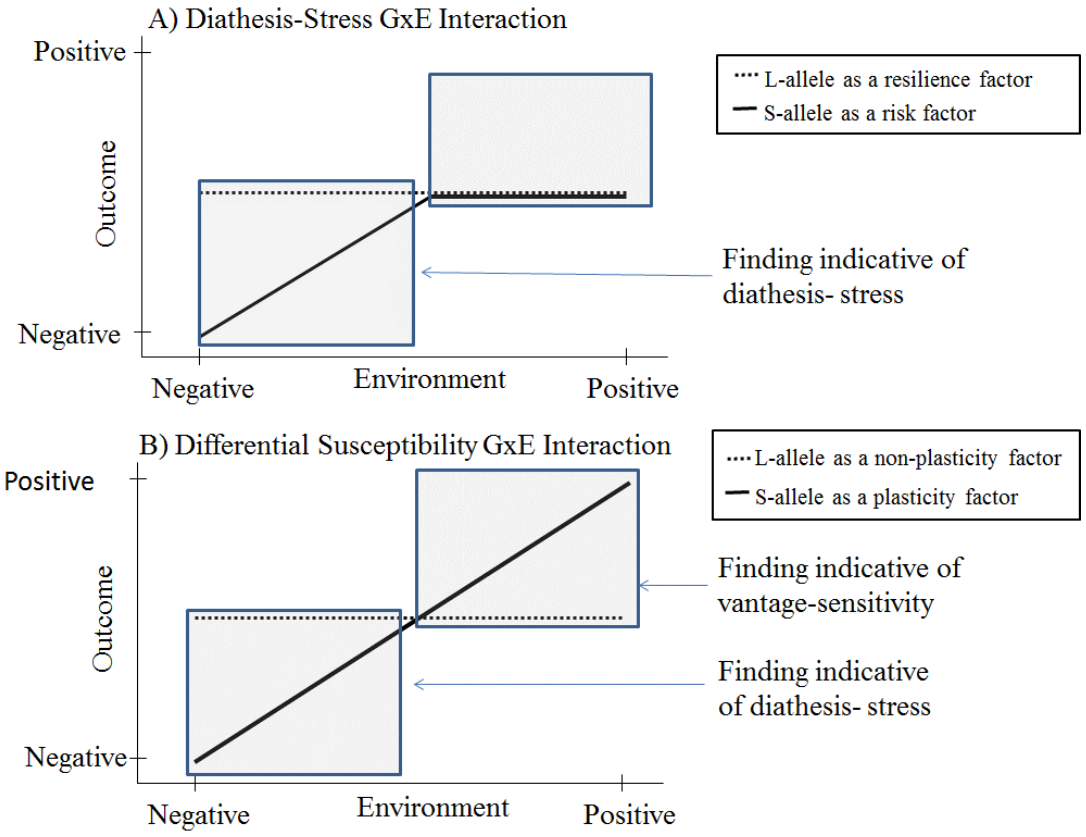

Figure 2-1: *Diathesis-stress and Differential Susceptibility Frameworks.*
A) Diathesis-stress describes individual differences in response to the presence of negative influences only. An "at risk" group experiences negative outcomes when exposed to a negative environment, whilst a "resilient group" remains unaffected. There is no difference in the groups' outcomes in a positive environment. B) Differential susceptibility refers to variability in responses to the presence of negative and positive influences and therefore represents the combination of diathesis-stress and vantage sensitivity as a function of the same sensitivity factor (e.g., the serotonin transporter gene). Adapted from Bakermans-Kranenburg and Van Ijzendoorn (2007).

This shortcoming led to an alternate explanation for the occurrence of gene-environment interactions, referred to the differential susceptibility hypothesis (DSH), which goes beyond consideration of maladaptive outcomes of negative environments. The DSH proposes that the S-allele as a "plasticity" allele, that exhibits differing levels of adaptive fitness depending on the environmental context (Belsky et al., 2009; Belsky & Pluess, 2009). In this model, S-carrier status is not a risk factor for sensitivity to adversity and psychiatric disorder *per se*, but is rather associated with a broader sensitivity to environmental influences. In propitious or enriched environments, this sensitivity may be beneficial, resulting in positive outcomes ('the bright side'), whilst in adverse, challenging environments it may increase risk for negative outcomes, such as stress-related psychiatric disorder ('the dark side'). Differential susceptibility can thus be said to be present when a cross-over interaction is evident, with some individuals affected to a significantly greater extent by both *positive* and *negative* experiences in a 'for better or worse' manner than other individuals, whose functioning remains relatively unchanged by their environmental conditions, as illustrated in Figure *2-1*B.

The DSH thus encompasses the notion of *diathesis stress*, as well as *vantage sensitivity*, the term that has been used to describe the potential for some individuals to derive more benefit from positive environmental experiences than others (Pluess & Belsky, 2013). The differential susceptibility hypothesis has resulted in a shift in thinking about the serotonin transporter gene by reframing S-carriers as not just especially "vulnerable" to adversity, but more generally "developmentally plastic or malleable" to environmental experiences (Belsky et al., 2009; Boyce & Ellis, 2005; Ellis, Boyce, Belsky, Bakermans-Kranenburg, & van Ijzendoorn, 2011).

Proponents of the differential susceptibility theory have argued that it is based in evolutionary reasoning; that because the future is inherently uncertain, natural selection should have shaped individuals to differ in their plasticity or degree of susceptibility to environmental conditions, whatever these conditions might involve (Belsky & Hartman, 2014; Belsky & Pluess, 2013; Ellis et al., 2011). Greater plasticity is thus a selectable trait in and of itself (Sinn, Gosling, & Moltschaniwskyj, 2008). Whilst more plastic individuals (i.e. S-carriers) may receive greater benefit from propitious environments and therefore increased opportunities to pass on their genes to future generations compared to less plastic or environmentally sensitive individuals (i.e. L-homozygous individuals), their reproductive fitness may suffer disproportionately in adverse environments. In contrast, less plastic individuals do not incur the reproductive advantages of favourable environments but also do not bear the costs of adverse environments.

The argument that evolution would have shaped a population of individuals to vary in their malleability or plasticity to the environment is somewhat problematic however. Natural selection is an unconscious, automatic and iterative process by which alleles from one generation have increased probability of being present in future generations if they confer a reproductive advantage in the current environment. Given natural selection has no foresight or fortune telling capacity, it cannot "hedge bets" by retaining genetic variation in the population, with an expectation that more plastic or less plastic alleles might become adaptive in the future. Indeed, as argued by Manuck and McCaffery (2014), attributing purpose to genetic variants, whose activities that are biological and distant from evolutionary outcomes, is at odds with a natural selection framework.

Moreover, differential susceptibility reasoning arguably suggests – at least implicitly – that carriage of a 5-HTTLPR S-allele may confer a *non-specific, global plasticity* to environmental influence, whilst L-homozygosity may bestow relative immunity from all environmental influence, *irrespective of the type of exposure under consideration*. Whilst this hypothesis appears to have been embraced with great enthusiasm and has generated a significant body of research, current evidence for serotonin transporter gene moderation of environmental effects per the differential susceptibility hypothesis is arguably somewhat equivocal according to a meta-analysis of 30 studies with children and adolescents (N=9,361) (van Ijzendoorn, Belsky, & Bakermans-Kranenburg, 2012). Rather than testing for the specific crossover interaction pattern indicative of differential susceptibility, two sets of analyses testing for the presence of diathesis-stress and vantage sensitivity separately were conducted. Studies with data that would have allowed testing of the full model of differential susceptibility, were only included in one of the two analyses (diathesis-stress or vantage sensitivity). Effect sizes drawn from 16 studies were concerned with diathesis stress ('the dark side' shown on the left side of Figure *2-1*B) – that is, how 5-HTTLPR genotype moderated associations between an adverse environment and negative developmental outcome, which were categorised as *negative*. Effect sizes drawn from 14 studies were concerned with vantage sensitivity (the 'bright side' shown on the right side of Figure *2-1*B) – that is, how 5-HTTLPR genotype moderated relationships between supportive environments and positive developmental outcomes, which were categorised as *positive* associations. Here, vantage sensitivity appeared to encompass both the *presence* of arguably desirable outcomes (e.g., higher levels of positive emotionality; Pauli-Pott,

Friedel, Hinney, & Hebebrand, 2009) and the *absence* of arguably undesirable outcomes (e.g., lower levels of conduct problems; Sonuga-Barke et al., 2009).

In the overall sample drawn from all the available studies, participants with S-alleles in this meta-analysis experienced more deleterious effects from adverse contexts than LL homozygous participants, but they did not appear to capitalise more from supportive environments to achieve greater positive outcomes. However, when the analysis was limited to studies with mostly Caucasian participants (52 effect sizes; N=6626), both the associations between positive environments and positive developmental outcomes and adverse environments and negative developmental outcomes were evident for SS/SL participants but not LL carriers. This finding suggests that the two components of differential susceptibility – diathesis-stress and vantage sensitivity – may occur for Caucasian S-carriers, but not L-homozygous individuals. However, there were not enough studies with participants from other ethnic backgrounds to perform analyses on these groups, making it difficult to know whether ethnicity or some other factor is a moderator of this interaction.

A closer examination of 13[2] of the 14 studies included in the meta-analysis testing the vantage sensitivity aspect of differential susceptibility (i.e., the association between the presence of supportive environments and putatively positive outcomes) suggests significant variation in the findings of these studies. Seven of the 13 studies (54% - Brody, Chen, Beach, Philibert, & Kogan, 2009; Drury et al., 2012; Eley et al., 2012; Fox et al., 2005;

[2] I could not locate any study by Cicchetti et al. (2011) with a sample of 92 participants which involved the serotonin transporter gene, therefore this paper could not be included in my analysis of the studies classified as containing positive effects according to the meta-analysis by van Ijzendoorn et al. (2012).

Kochanska, Kim, Barry, & Philibert, 2011; Pauli-Pott et al., 2009; Sonuga-Barke et al., 2009) documented at least one association that implicated the S-allele as having enhanced sensitivity to supportive environments. For example, Drury et al. (2012) reported a GxE interaction reflective of the full differential susceptibility model (i.e. both vantage sensitivity and diathesis-stress components), documenting that outcomes of children with an SS or SL genotype appeared to vary significantly in a 'for better or for worse' fashion depending on the type of care they were allocated to receive following their removal from a Romanian orphanage; compared to their LL-homozygous counterparts, they demonstrated much lower levels of indiscriminate behaviour when placed in high quality foster care and the highest levels of indiscriminate behaviour when provided with care as usual. In contrast, children with the LL genotype showed little difference in indiscriminate behaviours over time as a function of the type of care they received.

It is also worth noting that several of these studies classified as providing support for vantage sensitivity did not identify significant gene-environment interactions consistent with this phenomenon across all their outcomes of interest (e.g. Sonuga-Barke et al. 2009, Kochanska et al., 2011, Pauli-Pott et al., 2009). For example, Sonuga-Barke and colleagues (2009) found support for vantage sensitivity in a sample of children with ADHD when considering the outcome of comorbid conduct problems but not the outcome of emotional problems. They identified children with an S allele (SS/SL) displayed lower levels of comorbid conduct problems than LL homozygous children when they experienced high levels of positive maternal expressed emotion there were no apparent differences in conduct problems between S-carriers and L-homozygous children in environments of low positive

maternal expressed emotion, and no significant GxE interaction predicting emotional problems.

Importantly, results from three of the 13 studies (23% - Gilissen, Bakermans-Kranenburg, van Ijzendoorn, & Linting, 2008; Sadeh et al., 2010; Sulik et al., 2012) were consistent with the L-allele displaying differential susceptibility to the environment. For example, amongst children with an LL genotype but not SS/SL genotype, supportive parenting was negatively related to non-compliance, such that LL children who received high quality parenting showed the lowest levels of non-compliance whilst those that received low quality parenting showed the highest levels of non-compliance (Sulik et al., 2012). The pattern of results was similar when aggression was the outcome, though these results were only marginally significant.

Another study by Paaver, Kurrikoff, Nordquist, Oreland, and Harro (2008) did not provide evidence of vantage sensitivity indicated by the presence of more optimal outcomes in the *presence* of supportive positive environments; rather their results suggested that carriers of an S-allele experienced less optimal outcomes in the *absence* of supportive environments. Paaver and colleagues (2008) found specifically that low warmth in the families was associated with higher thoughtlessness, disinhibition and impulsivity in S-allele carrying girls relative to their LL-counterparts, but there were no apparent differences in outcomes between LL and S-carriers from high warmth families. Interestingly, lower family warmth showed a strong negative correlation with higher maltreatment (e.g., physical discipline, family violence), though maltreatment did not appear to be included as a covariate in the analyses. Maltreatment did not interact with the serotonin transporter gene to predict the outcomes of interest.

Only one study of the 13 studies identified only non-significant interaction findings (Luijk et al., 2011). The remaining study suggested that individuals with a heterozygous LS 5-HTTLPR genotype who belonged to families with either neutral or adverse family relationships may be at elevated risk of higher alcohol consumption compared to their LS counterparts living in families with good family relations, or those of either SS or LL 5-HTTLPR genotypes (Nilsson et al., 2005).

The findings of this meta-analysis thus suggest that whilst there is a large body of research that appears to support the proposition that the S-allele may confer increased sensitivity to positive environments as well as negative environments, there is a sizeable group of studies with "non-conforming" results, including those that implicate the L-allele as susceptible to a variety of outcomes following exposure to particular environments - as previously noted by previous meta-analyses examining the interactions involving the serotonin transporter gene with adversity (e.g., Sharpley et al., 2014). Whilst the overall findings of meta-analysis appear to provide some support for the presence of vantage sensitivity and diathesis stress, two components of differential susceptibility, these results also perhaps suggest the need for caution in the use of broad-sweeping conclusions that carriers of a 5-HTTLPR S-allele experience greater susceptibility to *all* environmental influence, whilst L-homozygotes bestows relative immunity from *all* environmental contexts.

2.5 Making Sense of Inconsistent findings of the Serotonin Transporter Gene-Environment Interaction

2.5.1 **A brief note on the putative roles of gender and ethnicity**

Gender is one individual or within-person factor that has been proposed to potentially distinguish individuals for whom the serotonin transporter gene x environment interaction appears more relevant, and this has been the focus of some attention. Studies testing interactions between 5-HTTLPR and stress have occasionally demonstrated differential effects for males and females (e.g.,Aslund et al., 2009; Grabe et al., 2005; Priess-Groben & Hyde, 2013; Surtees et al., 2006). Higher serotonin transporter (5-HTT) availability has been detected among females relative to males (Staley et al., 2001). A recent systematic review noted the possibility of an increased risk of depression as well as other internalising phenotypes amongst women carrying an S-allele (Gressier, Calati, & Serretti, 2016). In contrast, amongst men, carriage of an S-allele seemed to be associated with an increased risk of aggressiveness, conduct disorder and symptom counts of externalizing behaviour among men. Moreover, this association appeared to be reinforced by the presence of adversity or stressful life events and appeared to be particularly pronounced during adolescence, becoming less consistent with age, suggesting a plausible role for hormonal and neurobiological changes associated with the puberty. Importantly however, the authors noted that it was not yet possible to draw definite conclusions about the effects of gender with respect to a 5-HTTLPR-depression association given limitations of the review, including the small number of included papers, conflicting findings between studies, that the samples of a number of studies were predominantly or exclusively male or female, and that the consideration of the role of gender varied substantially across studies,

with many failing to explicitly consider statistical differences in gender effects. Importantly, meta-analyses have not identified moderating effects of gender on the interaction (Culverhouse et al., 2017; Risch et al., 2009; Sharpley et al., 2014).

It has also been argued that the effect of this polymorphism gene may vary according to race/ethnicity – for example, some researchers have suggested that the L-allele may act as the risk allele for depression in adverse environments more commonly in African American samples (Anderson & Mayes, 2010; Davies & Cicchetti, 2014; Williams et al., 2003; Williams et al., 2008). As noted above, the meta-analysis of differential sensitivity found that vantage sensitivity associated with carriage of an S-allele was only evident when samples were limited to those composed primarily (>80%) of White children (van Ijzendoorn et al., 2012). There has been no systematic exploration of the role of ethnicity/race in the interaction however, nor have any theories been proposed as to why race/ethnicity might have a moderating effect. A role for both gender and ethnicity therefore remains highly speculative.

2.5.2 **Considering the Influence of Potential Underlying Traits Associated with 5-HTTLPR Variation on the Interaction**

Characterising the S-allele

The underlying traits associated with the serotonin transporter gene polymorphism have not been conclusively identified, however a number of reviews have suggested that the S-allele may be associated with increased emotional reactivity and heightened psychological sensitivity to stress (Caspi et al., 2010; Homberg & Lesch, 2011). Some meta-analyses suggest that S-carriers display higher levels of negative affect/neuroticism, a personality marker of stress sensitivity and the tendency to experience negative emotional

states (Schinka, Busch, & Robichaux-Keene, 2004; Sen, Burmeister, & Ghosh, 2004 but c.f. Munafò, Clark & Flint, 2005). A meta-analysis has also suggested that carriers of a 5-HTTLPR S-allele show a significant attention bias for towards negative or threat-related stimuli, including angry or fearful facial expressions, interpreted as a greater tendency to allocate processing resources towards threat-relevant stimuli (Pergamin-Hight, Bakermans-Kranenburg, van Ijzendoorn, & Bar-Haim, 2012). Moreover, this attentional or emotion perception bias has been shown to be enhanced in S-carriers with a history of more threatening experiences, such as more critical parenting (expressed emotion criticism) within the normative range (Gibb et al., 2011) and abuse (Antypa, Cerit, Kruijt, Verhoeven, & Van der Does, 2011).

Numerous studies have documented enhanced threat-related amygdala activity in S-carriers compared to L-homozygous individuals across a range of aversive stimuli and neuroimaging techniques (e.g., Hariri et al., 2002; Munafò, Brown, & Hariri, 2008; Murphy et al., 2013 but see Bastiaansen, 2014). Further studies have suggested there may be decreased functional coupling between the amygdala and the ventral and perigenual anterior cingulate cortex in S-carriers (Pezawas et al., 2005). Structural abnormalities in the amygdala and anterior cingulate cortex, as well as in the hippocampus and orbitofrontal cortex have been observed (Scharinger et al., 2010). These neuroimaging findings will be discussed in more detail in CHAPTER 6, and are noteworthy because they indicate the presence of variations in circuitry critical to emotion processing and regulation and the stress response (Phillips, Drevets, Rauch, & Lane, 2003; Whalen, Shin, Somerville, McLean, & Kim, 2002).

Studies have additionally linked the presence of one or two S-alleles to stronger HPA axis activity, particularly higher waking cortisol levels (Chen et al., 2009) and greater cortisol reactivity in response to aversive or stressful stimuli (Miller, Wankerl, Stalder, Kirschbaum, & Alexander, 2012). Newborns (aged 3 days old) with an S/S genotype have also been observed to exhibit a significantly greater cortisol stress response to a painful heel prick than their S/L or L/L counterparts that remained when the potential role of other pre- or perinatal environmental factors were taken into account (Mueller, Brocke, Fries, Lesch, & Kirschbaum, 2010), indicating that this genetic contribution to stress responsivity is present from birth. S-carriers have also been found to show enhanced startle responses (Brocke et al., 2006; Klumpers et al., 2012; Lonsdorf et al., 2009) and greater skin conductance reactivity following vicarious conditioning to threat cues (Crişan et al., 2009).

There is also some indication that, relative to their LL-homozygous counterparts, S-carriers may show impairments in inhibitory control - the capacity to suppress a strong automatic responses and instead perform a more appropriate action (Holmes, Bogdan, & Pizzagalli, 2010; Jasinska et al., 2012; Landrø et al., 2015; Walderhaug, Herman, Magnusson, Morgan, & Landro, 2010). Similarly, individuals homozygous for the functional S-allele have difficulties with down-regulating negative emotion relative to L-carriers (Gilman et al., 2015). Links between the S-allele and aggression and impulsivity, including violence and suicide (Liao, Hong, Shih, & Tsai, 2004; Retz, Retz-Junginger, Supprian, Thome, & Rösler, 2004) particularly in the context of environmental adversity (Carver, Johnson, Joormann, Kim, & Nam, 2011; Reif et al., 2007), provide further support for a role of the S-allele conferring difficulties with emotional regulation and stress.

One interpretation of these findings is that the S-allele is associated with enhanced processing of salient environmental, particularly emotional, cues, which could amplify the risk for affective disorders in the presence of distressing or stressful experiences (Hariri & Holmes, Dannlowski et al., 2012; 2006). Critically, carriage of an S-allele genotype has been associated with a number of psychiatric phenotypes, many of which are characterised by stress-reactivity and emotional dysregulation, including borderline personality disorder (Lyons-Ruth et al., 2007), post-traumatic stress disorder (Xie et al., 2009), anxiety sensitivity/reactivity (Gunthert et al., 2007; Stein, Schork, & Gelernter, 2007), bipolar disorder (Cho et al., 2005) and stress-related alcohol and substance problems (Brody, Beach, et al., 2009; Covault et al., 2007). It seems plausible that variation in the serotonin transporter gene may therefore represent a broad risk factor for psychiatric disorder rather than a specific risk for depression, as a function of its impact on stress sensitivity and emotion processing.

There is increasing discussion however about whether enhanced emotional processing or sensitivity to environmental stimuli could potentially confer superior social cognition relative to L-homozygous individuals (Glenn, 2011; Homberg & Lesch, 2011). Support for this notion comes from studies suggesting that individuals carrying an S-allele show greater atunement to the emotions expressed by others. For example, S-carriers show more sensitive responding to both their romantic partner's affect (Schoebi, Way, Karney, & Bradbury, 2011) and to their infants' cues (Mileva-Seitz et al., 2011). There is also some indication (albeit at trend) that S-carriers with depression show an enhanced ability relative to L-homozygous individuals to decode mental states of a negative (though not positive) valence on a task assessing theory of mind (Zahavi et al., 2016). Toddlers carrying the S-

allele also display increased social mimicry and imitation of an adult's manipulations of a set of toys (Schroeder, Asherson, Blake, Fenstermacher, & Saudino, 2016). Adult S-allele carriers have also been found to demonstrate greater social learning on an observational fear conditioning task and greater susceptibility to environmental framing cues in a decision making task (Crişan et al., 2009). It seems plausible that in the absence of adversity and particularly in the presence of more enriched environments, enhanced social cognition conferred by more sensitive emotional processing and stress responsivity might be advantageous to S-allele carriers, consistent with the vantage sensitivity component of differential susceptibility.

Characterising the L-allele

Importantly, research to date has almost exclusively been concerned with characterisation of the S-allele in comparison to the L-allele, and with how traits associated with S-allele carriage might confer risk for psychological disorder. These same studies however may also offer insights into the traits that may be associated with an L-allele, and any risks that these too might pose for the development of psychopathology. Two reviews that have considered this body of research from this viewpoint have contended that possession of two L-alleles may be associated with reduced emotionality (including shallow affect, reduced empathy and lower levels of fearfulness) and lower stress sensitivity, which may potentially increase risk for psychopathy in the context of additional genetic and environmental factors (Glenn, 2011; Yildirim & Derksen, 2013). For example, compared to those with the LS or SS genotype, women with an LL genotype self-reported significantly greater difficulties with identifying feelings on a subscale measuring Alexithymia, a personality construct that captures problems with recognising, expressing

emotions and understanding others' emotions (Kano et al., 2012). High expressing 5-HTT individuals have also been found to show poorer emotion recognition accuracy compared to low expressing 5-HTT (Boll & Gamer, 2014). Two large laboratory studies based on independent samples have also suggested relationships between L-homozygosity and reduced emotional reactivity of two different types (Gyurak et al., 2013). In the first study, L-homozygous individuals were found to display less emotionally expressive behaviours and reported less amusement, shame and anger when viewing film clips of themselves in embarrassing situations. In the second study, L-homozygous individuals demonstrated reduced levels of prosocial emotional empathy and exhibited lower cardiovascular and electrodermal activity when watching films of others in serious distress. Spouses carrying two L-alleles have also been identified to show less sensitivity to their partner's positive affect and anxiety/nervousness during marital interactions compared to spouses carrying an S-allele (Schoebi et al., 2011). Individuals homozygous for the L-allele have been found to display higher levels of callous-unemotional traits compared to S-carriers (Brammer, Jezior, & Lee, 2016), though one study found this effect to be limited to the group of individuals brought up in socioeconomically disadvantaged environments (Sadeh et al., 2010).

The L-allele may also be associated with a bias towards positive emotional stimuli and/or a bias away from negative stimuli (Fox, Ridgewell, & Ashwin, 2009; Kwang, Wells, McGeary, Swann, & Beevers, 2010; Pérez-Edgar et al., 2011), a pattern of attention that may be consistent with the reward-dominant response style that is seen in individuals with psychopathy or who are high in callous-unemotional traits (Dadds & Salmon, 2003). Psychiatrically healthy 5-HTTLPR LL-homozygous women showed greater accuracy in the

recognition of happy faces than their s-allele carrier counterparts (Defrancesco et al., 2011). Superior accuracy in decoding mental states of a positive valence has also been observed in individuals with an LL-homozygous genotype (Zahavi et al., 2016).

Neural or biological processes to underlie the reduced emotional processing in individuals with an L-allele are hypothesised to involve reduced activation of limbic regions, particularly the amygdala, and potentially increased activation of frontal regions, which likely dampen the strength and duration of internal physiological responses to relevant emotional events (Glenn, 2011). L-homozygous individuals show minimal change in amygdala response to viewing aversive stimuli (e.g. only 3% increase in activity in response to fearful faces in the original study by Hariri and colleagues (2002) compared to neutral stimuli). Moreover, LL-homozygous women have been found to show greater neural activation in face processing regions, namely the left fusiform gyrus, in response to positive emotional stimuli than s-allele carriers (Demaree et al., 2009).

Furthermore, genetic association studies have suggested a possible link between the 5HTTLPR L-allele and reduced cognitive flexibility (Borg et al., 2009; den Ouden et al., 2013; Finger et al., 2007; Tukel et al., 2016; Wilkosc et al., 2010), an important executive skill involving the capacity to adjust thinking or attention in response to changing goals and/or environmental stimuli (Banich, 2009; Miyake & Friedman, 2012). Individuals with the LL genotype have also been found to perform poorly relative to their S-carrier counterparts on several other aspects of executive functioning, including sustained attention (Strobel et al., 2007) and visual planning (Roiser, Rogers, Cook, & Sahakian, 2006). In a sample of children from low SES backgrounds, LL-homozygotes have also been identified to display attenuated responses (event-related brain potentials) on a neural index of

selective attention compared to S-carriers when asked to focus on only one of two simultaneously aurally presented stories (Isbell, Stevens, Hampton Wray, Bell, & Neville, 2016). Findings are somewhat more mixed for working memory, where impaired performance has been associated with both an L-allele (Roiser, Müller, Clark, & Sahakian, 2007) and S-allele (Havranek et al., 2015; Weiss et al., 2014). Interestingly, two studies have suggested a role for genetic sensitivity to the family context on executive function that implicates the L-allele. The first study found that L/L homozygous children with mothers who had endorsed high levels of depressive symptoms demonstrated impaired performance on executive function tasks assessing cognitive flexibility, working memory and inhibition compared to children with one or two S-alleles, although they also performed better than S-carrier children on these tasks when their mothers endorsed few depression symptoms (Weikum et al., 2013). The second study found that youth with the L/L genotype who experienced very low levels of parental supervision performed worse on cognitive flexibility compared to youth with S/S or S/L genotypes (Li et al., 2015). Moreover, it appears that L-homozygosity is a significant though modest, risk factor for ADHD (Gizer, Ficks, & Waldman, 2009). Interestingly, whilst weakness in executive function is not ubiquitous in ADHD, executive function deficits are thought to be one component of the complex neuropsychology of ADHD (Willcutt, Doyle, Nigg, Faraone, & Pennington, 2005). In addition, poorer social cognition, particularly associated with the recognition of emotion, has been found to be compromised in individuals with ADHD (Bora & Pantelis, 2016; Graziano & Garcia, 2016). Elevated callous-unemotional traits have also been noted as a potential feature of ADHD (Graziano & Garcia, 2016) and callous-unemotional traits

have been found to mediate the association between L-homozygosity and ADHD (Brammer et al., 2016).

Critically, Yildrim and colleagues (2013) have highlighted the difference between a constitutional *deficit* in emotional processing and a *disturbance* in emotional processing. They argue specifically that carriage of two L-alleles may be associated with a more inherent emotional processing *deficiency,* or *emotional hyporesponsivity*. This emotional deficiency, associated with unusual fearlessness, affect restriction, dampened emotional empathy and an underdeveloped moral conscience, could, in a more extreme form, represent a risk factor for a primary or inherent psychopathy. In contrast, an interactive effect between emotional reactivity/stress responsivity associated with S-allele carriage and destructive social experiences, such as abuse and maltreatment in childhood, may result in a *disturbance* in emotional processing that rather involves a more maladaptive level of *emotional hyperresponsivity*. This emotional disturbance, in the form of impaired appraisal, regulation and control of emotions may be associated with internalising psychopathology but also violence, aggressive behaviour and impulsivity, consistent with a secondary form of psychopathy that has often been referred to as sociopathy. Whilst the authors suggest that environmental socialisation processes may have a larger effect on an emotional hyperresponsivity disturbance than on an inherent emotional deficit or hyporesponsivity associated with primary psychopathy, they emphasise findings of a recent study of incarcerated boys which indicated that parental neglect (i.e., deprivation) more often characterised youth with emotional deficits consistent with psychopathy, whilst sexual abuse (i.e., threat), more often characterised youth showing emotional dysregulation,

including impulsive aggression, associated with sociopathy (Kimonis, Fanti, Isoma, & Donoghue, 2013).

It is interesting to note some similarities between this finding and some findings in the GxE literature that possibly suggest an association between the S-allele and externalizing behaviours in contexts involving high adversity, such as childhood abuse, but an association between the L-allele and externalizing behaviours in contexts involving low support or parental responsivity. For example, Reif and colleagues (2007) obtained an interaction effect between childhood environment and serotonin transporter genotype on violent behaviour in an incarcerated sample, whereby the experience of an adverse childhood environment was associated with later-life violence for S-carriers only. In contrast, Sulik and colleagues (2012) reported a negative relationship between supportive parenting and noncompliance in early childhood that was evident only in the group of children with an L homozygous genotype and not for S-carriers. Similarly, Davis and Cicchetti (2014) found that maternal unresponsiveness predicted greater externalizing problems, such as aggression and defiance for children with the homozygous L genotype but not for their counterparts with a functional S-carrier genotype.

Taken together, these findings suggest that the S-allele confers increased emotional reactivity and greater sensitivity to stress, traits which, in the absence of adversity, may not be problematic in and of themselves, and in fact, may also be associated with better social cognition. However, exposure to harsh, traumatic or negative environments may result in disturbances in S-carriers' emotional processing that may increase vulnerability to broad range of psychiatric difficulties involving emotional dysregulation, such as depression, but also aggression and impulsivity. In contrast, the L-allele has been associated with relatively

dampened emotional reactivity, reduced sensitivity to stress and more limited cognitive flexibility. The possibility has therefore been raised as to whether L homozygous individuals may be more likely to develop traits associated with psychopathy and more readily engage in antisocial behaviour when exposed to "cold," less engaged or neglectful parental care (Yildirim & Derksen, 2013). An important question is whether emotional hyporesponsivity conferred by an L-allele could also represent a risk factor for depression in certain environments. Importantly, deficient emotional experiences, in the form of increased negative and reduced positive emotions but also reduced emotional reactivity or low emotional responsiveness to changing contexts, have also been associated with depressive disorders (Bylsma, Morris, & Rottenberg, 2008; Kuppens, Allen, & Sheeber, 2010). Deficits in executive function more broadly, and in cognitive flexibility specifically, have also been linked with depression (Lee, Hermens, Porter, & Redoblado-Hodge, 2012; Snyder, 2013; Wagner, Muller, Helmreich, Huss, & Tadic, 2015).

2.5.3 **Considering the role of the environment**

As reviewed in the previous section, the S-allele has been associated with emotional *hyper*reactivity as well as heightened stress responsivity - traits that would plausibly increase vulnerability to a range of psychological disorders in the presence of adversity. However, as noted in the previous section, there is emerging evidence that emotional *hypo*reactivity, decreased stress responsivity and impaired executive function (particularly cognitive flexibility) associated with L-homozygosity may too represent risk factors for various psychological conditions such as psychopathy, when combined with other genetic and environmental risk factors, including, potentially, parental neglect. The prospect that L-homozygotes carry their own vulnerability to psychological conditions that may be

heightened in certain environments conflicts somewhat with the proposal that only S-carriers show sensitivity to their environment. Instead it may be that *both* S-allele and L-allele individuals possess specific characteristics that may be advantageous or detrimental, depending on the *type* of environmental experiences they encounter.

Relevant to this idea is a novel framework recently proposed by McLaughlin and Sheridan (2014), which recognizes two dimensions that may cut across various forms of adversity to differing degrees; threat and deprivation (Figure 2-2). Critically, the authors propose that whilst exposure to deprivation and threat experiences frequently co-occur, they can be measured separately and may influence emotional and behavioural outcomes, including onset of various psychopathologies via different neurobiological mechanisms. McLaughlin and Sheridan (2014) discuss threat as the *presence* of experiences involving actual or perceived harm to one's physical integrity, consistent with the definition of trauma according to the Diagnostic and Statistical Manual of Mental Disorders (5th ed.; American Psychiatric Association (2013). The dimension of threat is therefore central to experiences such as physical abuse, sexual abuse, emotional abuse involving actual or perceived threats of physical violence or coercion, witnessing of domestic violence, and exposure to other forms of violent victimization in the home, school or community (Sheridan & McLaughlin, 2014). In contrast, deprivation, involves the *absence* of expected social or cognitive inputs and species- and age-typical complexity in environmental stimuli. McLaughlin, Sheridan, and Nelson (2017) have suggested that at the most fundamental level, deprivation involves the absence of a stable, sensitive, and responsive caregiver, as perhaps the most important species-expectant experience. Emotional and physical neglect and institutional rearing may

constitute forms of adversity that are underpinned by the dimension of deprivation (McLaughlin et al., 2014; McLaughlin et al., 2017).

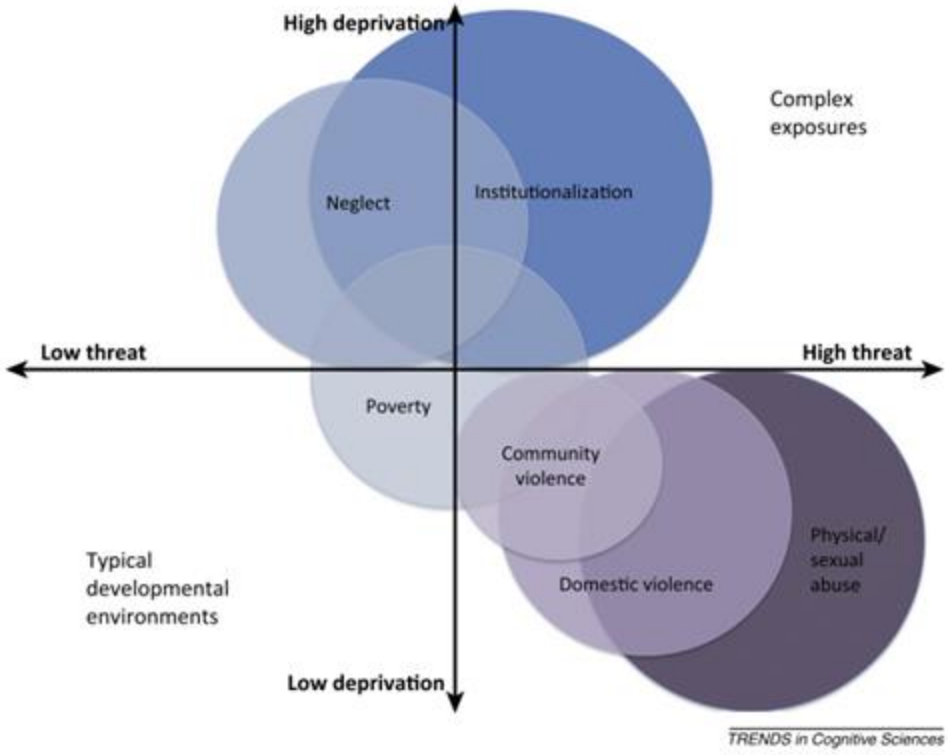

Figure 2-2. *Dimensions of threat and deprivation associated with commonly occurring adverse childhood experiences (ACEs).*
This figure illustrates the argument by Sheridan and McLaughlin (2014) that threat and deprivation are measurable dimensions of experience that underpin a range of adverse childhood experiences, including those that occur in isolation (e.g., a single incident of community violence exposure) and those that are co-occurring (e.g., physical abuse and physical neglect). The term 'complex exposures' refers to experiences that involve aspects of both threat and deprivation. Reprinted from (Sheridan & McLaughlin, 2014).

There may also be environmental experiences that are *markers* of exposure to either deprivation or threat, such that they are associated with increased risk of threatening

experiences or reduced exposure to cognitive, social and environmental complexity but not necessarily characterised by these experiences (McLaughlin et al., 2014; Sheridan & McLaughlin, 2014). For example, the experience of poverty does not inherently involve subjection to threat or deprivation (i.e., individuals may be poor and have no exposure to threatening experiences and typical exposure to cognitive, social, and environmental complexity) but can often bring with it some enhanced risk of maladaptive experiences, particularly deprivation in the form of more limited access to enriching and cognitively complex environments. The degree of threat and deprivation associated with these marker variables may therefore be heterogeneous.

Critically, McLaughlin and colleagues (2016; 2014; 2017) suggest that the type of adversity experienced by a child may have specific effects on a child's ability to process emotions. Children from environments characterized by high or uncontrollable *threat* appear to exhibit patterns of information processing that promote a rapid identification of salient emotional cues in the environment (e.g. biased attention to threat). For example, children with abuse histories display an attentional bias towards angry facial expressions (an important signal of potential threat), accurately identify angry facial expressions based on less perceptual information, require greater resources to disengage from angry faces, and display anticipatory monitoring of the environment following interpersonal displays of anger compared to typically developing children (Pollak, Cicchetti, Hornung, & Reed, 2000; Pollak & Sinha, 2002; Pollak & Tolley-Schell, 2003; Pollak, Vardi, Putzer Bechner, & Curtin, 2005; Shackman, Shackman, & Pollak, 2007).

A review by Teicher and Samson (2016) of studies assessing the relationship between amygdala volume and early adversity noted a possible link between reductions in

amygdala volume and maltreatment histories involving threatening experiences and abuse. The authors also noted that reduced hippocampal volume was a highly consistent finding in adults with threat histories of abuse or maltreatment. Children who have been exposed to a high level of threat in the form of interpersonal violence or physical or sexual abuse also experience greater activation in the amygdala in response to a variety of different negative emotional stimuli (Garrett et al., 2012; Grant, Cannistraci, Hollon, Gore, & Shelton, 2011; McCrory et al., 2013; McCrory et al.; McLaughlin, Peverill, Gold, Alves, & Sheridan, 2015). Increased amygdala activation has also been reported in maltreated adults with no history of psychopathology (Dannlowski et al., 2012).

Information processing biases that facilitate both identification of threat and maintenance of attention to threat cues seem to be specific to children who have experienced violence, as they do not appear to have been identified in children with a deprivation history involving neglect (Pollak et al., 2000). Instead, there is evidence suggesting that neglected children have difficulty identifying facial expressions of affect (Pollak et al., 2000; Vorria et al., 2006; Wismer Fries & Pollak, 2004). It is thought that this difficulty with discriminating facial emotions is related to their experience of impoverished expressive environments that has hampered the development of emotion recognition (Camras, Grow, & Ribordy, 1983; During & McMahon, 1991).

A growing body of evidence also points to a link between environmental deprivation and enduring difficulties in executive function, such as problems with cognitive flexibility, working memory, planning ability and inhibitory control (McLaughlin, 2016; McLaughlin et al., 2017). Relative to both children who have abused and children raised in typical environments, children exposed to a range of deprivation experiences, including

institutional settings, neglectful home environments and poverty have been found to be at greater risk for a variety of cognitive difficulties, including reduced general intellectual ability, expressive and receptive language problems, and attentional and executive function difficulties (Hildyard & Wolfe, 2002; Spratt et al., 2012).Whilst difficulties in other cognitive domains tend to abate somewhat following placements into stable family environments, deficits in executive functioning and higher rates of attention-deficit/hyperactivity disorder (which is associated with executive function problems) persist over time (Bos, Fox, Zeanah, & Nelson, 2009; Humphreys et al., 2015; McDermott et al., 2013; Tibu et al., 2016a; Tibu et al., 2016b; Zeanah et al., 2009).

Children with deprived backgrounds may also show signs of atypical development of neural systems relevant to emotion processing and executive function. Teicher and Samson's (2008) review identified that studies of individuals experiencing forms of deprivation, such as caregiver neglect, those belonging to chronically depressed mothers, or with a history of disrupted attachments, tended to document amygdala volume increases. There is also some limited evidence to suggest that that a pattern of increased amygdala activation to negative emotional expression levels of activation might be present in children with deprivation histories (Goff et al., 2013; Maheu et al., 2010; Tottenham et al., 2011). However, institutionally reared children show no significant differences in their amygdala responses to viewing caregiver and stranger faces, compared to family-reared children who show significantly greater relative amygdala activation to their caregiver's face versus that of a stranger (Olsavsky et al., 2013). Parental presence and caregiving behaviour has been suggested to have a key role in entraining or scaffolding development of the amygdala-medial prefrontal cortex (mPFC) circuit, laying out the blueprint by which stable patterns of

connectivity between these brain regions are achieved (Callaghan & Tottenham, 2016). Children with a history of deprivation (institutionalism) show evidence of developmental *acceleration* in amygdala-medial prefrontal cortex connectivity in response to emotional stimuli. Whilst typically raised children who showed the immature pattern of positive coupling between the amygdala and mPFC, children who had experienced institutionalism rather demonstrated negatively correlated amygdala-mPFC connectivity that is more typical of the pattern seen in adults (Gee et al., 2013). Whilst the group of post-institutionalised children displayed higher levels of anxiety on average relative to typically raised children, adult-like amygdala-mPFC phenotypes were associated with lower levels of anxiety within the group of children with a history of institution. It was suggested that this acceleration of connectivity may serve as an ontogenetic adaptation to allay heightened amygdala reactivity. Children who spent their early lives in institutional settings have also been found to show increased recruitment of the dorsal anterior cingulate gyrus, inferior prefrontal cortex and striatum during an executive function task measuring inhibitory control and reduced performance on this task (Mueller, Maheu, et al., 2010).

The framework by McLaughlin and colleagues that differentiates effects of threat and deprivation thus potentially provides an important step forward in delineating the mechanisms by which brain and biological systems might impact psychopathology. Teicher and colleagues (2016) have proposed that rather than adversity producing alterations that constitute damage to the brain that predisposes to psychopathology, these experiences may encourage the brain to progress along alternative developmental pathways that might enhance the likelihood of reproduction and survival in what, based on experience, appears to be a world with specific challenges. It seems plausible that there may be specific

polymorphisms, of which the serotonin transporter gene might be one, that render some individuals more susceptible to certain experience-dependent neurodevelopmental changes. In particular, the serotonin transporter gene might be a marker of characteristics such as emotion processing and executive functioning that create propensities to interact with these two forms of environmental experience in different ways. Parallels between the findings of studies of the influence of threat versus neglect and 5-HTTLPR S-carrier versus L-homozygous on emotional processing and executive functioning suggests the possibility that S-allele carriers may be more vulnerable to threat-induced neurodevelopmental changes whilst L-homozygous individuals may be more susceptible to deprivation-induced neurodevelopmental changes.

2.5.4 **The Differential Capability Hypothesis: Considering the match/mismatch between different dimensions of environment and the traits associated with 5-HTTLPR genotype**

The differential susceptibility hypothesis specifies that more plastic individuals (e.g. S-carriers) are broadly susceptible to all environments. To date, as illustrated in Figure 2-3, frameworks and resulting research on the serotonin transporter gene have tended to classify interactions as involving the presence of "negative" (i.e. adverse) environments and negative or maladaptive outcomes (quadrant 1) or the presence of "positive" (i.e., propitious, supportive) environments and positive outcomes (vantage sensitivity quadrant 2) (Sharpley et al., 2014; van Ijzendoorn et al., 2012). A broad set of exposures commonly analysed in studies of 5-HTTLPR x environment interaction such as child maltreatment, institutional rearing, natural disasters, marital conflict, divorce, chronic poverty, bullying victimization experiences and unresponsive or punitive parenting have fallen under the

umbrella of "negative" environments. Similarly, a "positive" environment encompasses a range of distinct experiences such as responsive parenting, supportive educational environments, high quality foster care after early institutional deprivation, and formal psychological interventions for those experiencing mental health difficulties. This approach implicitly assumes that very different kinds of experiences influence outcomes through similar mechanisms. Indeed, a basic tenet of the differential susceptibility model is that virtually any adversity or stress may result in the experience of illness whilst any positive environment would promote positive developmental outcomes for a "sensitive" individual, such as an S-allele carrier. Inspection of findings included in meta-analyses (Sharpley et al., 2014; van Ijzendoorn et al., 2012) however point to the heterogeneity in patterns of results between individual studies.

Although some overlap in the mechanisms linking various forms of adversity to psychopathology (or different forms of positive experiences to beneficial outcomes) is likely, it is possible that the lack of specificity associated with this dichotomous categorisation approach may obscure the distinct ways that the serotonin transporter gene interacts with particular environmental experiences to influence development. It is noteworthy that the most consistent support for an interaction between the serotonin transporter gene and negative environments appears to come from studies that have considered a single, homogenous exposure, such as childhood abuse or medical illness. Whilst these events might be different, they both load highly on the dimension of threat involving either the experience or anticipation of significant harm. (Caspi et al., 2010). In contrast, the group of studies that have employed composite or count measures of adverse

experiences, particularly in the form of checklists, which often incorporate experiences of both threat and deprivation, have obtained more heterogeneous findings.

Moreover, many studies that have investigated GxE interactions according to a differential susceptibility hypothesis have tended to equate the lack of negative outcomes (e.g., no depression) and the presence of a positive outcome. Attention to the distinction between the absence of mental ill-health and the existence of positive functioning and wellbeing is being increasingly highlighted by research perspectives such as the positive psychology/positive development field (Tolan, Ross, Arkin, Godine, & Clark, 2016). Thus, truly including the vantage sensitivity component of differential susceptibility phenomenon involves a consideration of the adaptive spectrum rather than simply the maladaptive spectrum, as the absence of negative outcomes (i.e. no psychopathology) may not the same as the presence of positive outcomes that would characterize thriving or optimal functioning.

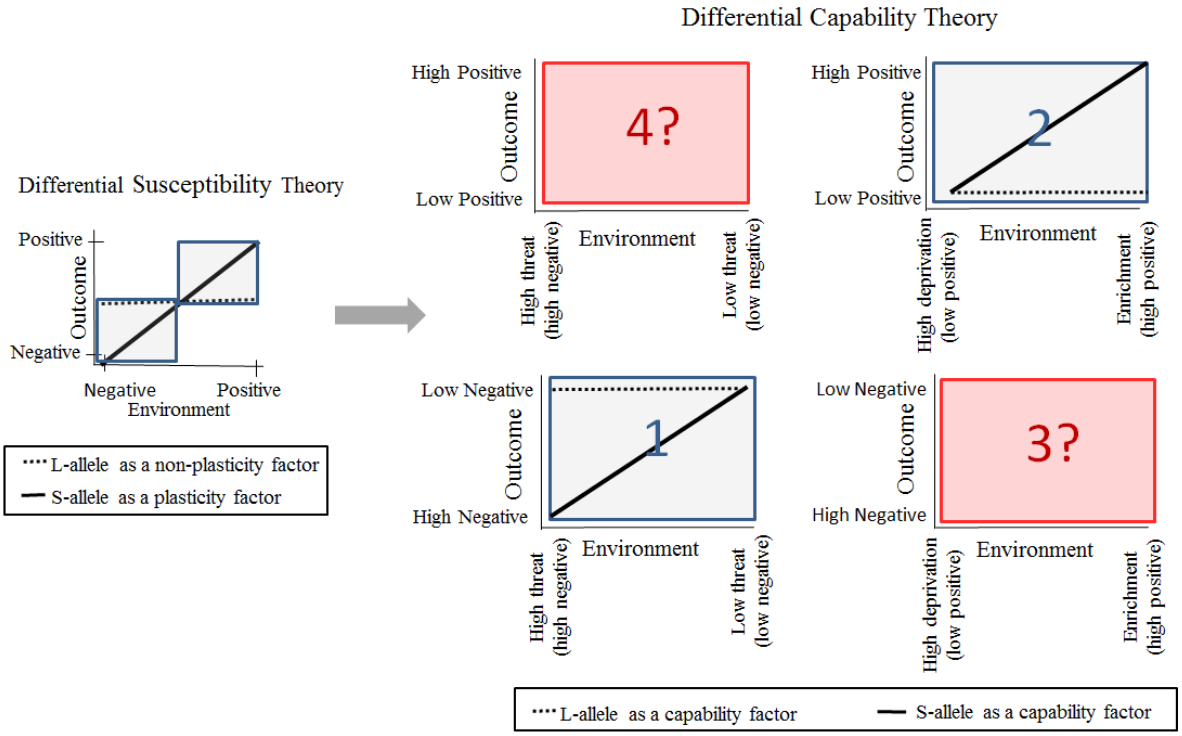

Figure 2-3: *Progression from the Differential Susceptibility Hypothesis to the Differential Capability Hypothesis.*
As shown, both the environment and outcomes are typically depicted by the DSH as falling on continua that extend from negative to positive. The Differential Capability Hypothesis rather depicts positive and negative continua on separate but adjacent axes to differentiate between high deprivation and high threat environments/outcomes and high positive and low negative outcomes. Interactions that fall in quadrant 1 reflect those interactions between threatening-non-threatening environments and the likelihood of maladaptive outcomes. According to the differential capability theory, the more emotionally reactive, stress-sensitive S-carriers who experience higher levels of threat will show greater vulnerability to particular maladaptive (stress-related) outcomes. Interactions that fall in quadrant 2 focus on interactions between deprived-enriched environments and the likelihood of positive outcomes. The Differential Capability Hypothesis suggests that in nurturing, supportive (enriched) environments, S-carriers are likely to achieve particular positive outcomes as a function of their putatively enhanced emotional reactivity, and cognitive flexibility. The differential capability hypothesis equally allows for the possibility that characteristics associated with an L-allele, namely reduced emotional reactivity and stress responsivity, and greater inhibition, could place L-homozygous individuals at a particular advantage in certain environments. Interactions in quadrant 3 represent the effects of deprived environments, where the Differential Capability Hypothesis would suggest that in these less nurturant, environments, LL-homozygous individuals could be vulnerable to maladaptive outcomes. Interactions in quadrant 4 represent those involving the continuum of threatening to non-threatening (but not enriched) environments and the likelihood of positive outcomes. It is unclear which genotype might be at greater advantage in this environment and consideration of this interaction is beyond the scope of the current thesis. Areas of the graph highlighted in blue thus represent predictions of the differential capability theory that are consistent with the DSH, and also represent findings where there is at least some research shown by at least one meta-analysis that supports this pattern of interaction. Areas of the graph highlighted in red represent predictions of the differential capability theory that have not yet been systematically investigated. Breakpoints in the axes of the graph and the lines representing the two different genotypes denote that positive and negative environments as well as positive and negative outcomes may not exist on the same continuum.

As shown in Figure 2-3, it may therefore be important to distinguish additional components of the relationship between the serotonin transporter gene and environments, and in particular to consider whether 5-HTTLPR genotypes might influence associations between environments involving deprivation that *lack* positive, supportive, or nurturant features and subsequent negative, undesirable outcomes (quadrant 3).[3] Questions of this nature are important because, as pointed out by McLaughlin and colleagues (2014), deprived environments or the absence of a favourable environment may not necessarily contribute to the presence of psychopathology in the same way as the presence of a threatening environment, just as the absence of a harsh or threatening environment may not confer the same benefits as the presence of a favourable, nurturing environment. Arguably, individuals developing in deprived environments that *lack* supportive and nurturing features may face very different adaptive challenges to those who are developing in environments that are stressful because of the *presence* of threat involving harsh or conflictual elements from which they need to protect themselves.

[3] A further potential interaction shown in quadrant 4 of Figure *2-3* that could be examined is whether the serotonin transporter gene moderates the relationship between threatening environments and likelihood of positive outcomes. Given its focus on the prediction of the negative outcome of depression, consideration of this interaction is beyond the scope of the current thesis, however, investigations of this quadrant could include study of variability in the experience of post-traumatic growth (such as enhanced interpersonal relationships, appreciation for life, personal strength, and positive changes in life priorities) after aversive events (Cordova & Andrykowski, 2003; Tedeschi & Calhoun, 1996). It is interesting to posit whether the putative characteristics that might allow S-carriers to incur greater benefit from positive environments (such as greater social cognition, emotional reactivity) might also allow them to achieve positive outcomes in negative environments, whilst simultaneously being vulnerable to negative outcomes. Alternatively, L-allele homozygous individuals, who are thought to be less likely to experience depression in threatening environments, might also be more likely to achieve positive outcomes than their S-carrier counterparts in these circumstances.

The Differential Capability Hypothesis

One relatively simple way of conceptualizing environments lacking positive and nurturing features is that they provoke emotional distress, in which case S-carriers might be expected to be more likely to experience negative outcomes. In harsh, threatening environments S-allele carriers, who are thought to be more emotionally reactive and especially sensitive to their context (Homberg & Lesch, 2011), may have a disadvantage over less emotionally responsive L-homozygotes. By contrast, in environments that *lack* important nurturing features, the primary affective task may be to engage and extract nurturance and support from surroundings, a task for which S-carriers might be better suited than L-homozygous individuals due to their greater capacity for affective engagement and social cognition. In interpersonal environments where the primary challenge is to elicit care and support that is lacking, greater capacity for emotional responding and engagement with others may be beneficial. Thus, in these contexts it may be the emotionally hyporesponsive, less cognitively flexible L-homozygous individuals who are less adaptive, placing them at greater risk of psychopathology. Rather than a differential *susceptibility* model, the possibility that the serotonin transporter gene might interact with different environmental experiences in different ways would be consistent with a differential *capability* model whereby the *fit* (or lack thereof) between genetic or biological predispositions and environmental challenges determines functioning and wellbeing. This paradigm has some parallels with Thomas, Chess and Birch's (1968) 'goodness-of-fit' theory, which suggests that the degree of match or mismatch between a child's characteristics (temperament, capacities and motivations) and the demands and

expectations of the caregiving environment in which he or she functions is an important determinant of behavioral adjustment.

The differential capability framework is consistent with evolutionary thinking that where a continua of a particular trait of polygenic inheritance exists, natural selection will preserve innate differences that comprise the continua as long as the different levels of the trait are advantageous in different circumstances or tasks even though they may prove disadvantageous in others (Belsky, 1997; Nettle, 2006). If there is no universal optimum of this context-sensitive fitness cost-benefit ratio, it would be expected that the allelic variation in genes such as the serotonin transporter gene that might contribute towards variation in the associated phenotype will be maintained in the population. In threatening environments, where there is risk of harm, particularly of a social nature, it may be risky to experience and display strong emotions. Emotional hyporesponsivity associated with L-homozygosity may therefore be more advantageous in these environments. In safe, hospitable and nurturing environments, greater emotional reactivity and expressiveness may promote connection and intimacy with others. In these environments, it may therefore be beneficial to be an S-carrier rather than an L-homozygous individual. Thus, findings to date of S-carriers being susceptible to positive and negative environments in a 'for better or for worse manner' which have commonly been cited in support of the DSH would therefore also fit within a differential capability theory. According to a differential capability theory however, selection thus occurs at the level of the specific traits conferred by serotonin transporter genotype, and the degree to which they match the individual's current environment, rather than at the level of plasticity more broadly as in the DSH. Predictions about reproductive fitness would also differ according to the differential capability

Hypothesis, with both S-carriers and L-homozygous individuals showing fluctuations in reproductive fitness that depended on the current match or mismatch between the individual traits and the environment.

By emphasising the importance of the match or mismatch between environments and genetic dispositions, the differential capability model encourages researchers to carefully consider the theoretical implications of particular environmental influences on certain outcomes. As already discussed in the current chapter, there is endorsement for this theory from studies indicating that L-homozygous children and adolescents are more susceptible than their S-carrier counterparts to externalizing symptoms such as defiance and aggression in environments in environments involving low parental support or positivity (Davies & Cicchetti, 2014; Sulik et al., 2012), but it is less clear how this interaction might extend to internalizing conditions, such as depression. The following chapter will consider the relationship between family environment and depression as well as how the serotonin transporter gene may interact with specific exposures, such as parenting behaviour, to predict depression.

2.6 Summary and Implications

This chapter reviewed research suggesting that variation in serotonin transporter genotype might interact with the environment in predicting depression, whilst noting inconsistencies within the literature regarding the direction of this potential effect. It was hypothesised that inconsistent results across studies might reflect that particular types of environmental conditions differentially affect the relationship between 5-HTTLPR genotype and depression outcomes; more specifically that possession of an S-allele might

increase vulnerability to outcomes associated with environmental experiences higher on the dimension of threat, whilst possession of an L-allele might increase susceptibility to outcomes associated with experiences higher on the dimension of deprivation. This chapter also noted the preponderance of studies examining interactions involving the serotonin transporter gene on the contribution of experiences involving the dimension of threat, particularly extreme, traumatic or stressful events, such as childhood maltreatment but also other experiences such as medical illness, natural disaster and war. The generalisability of these findings may be somewhat limited however as many individuals with depression may not have experienced adversity of this extreme nature. Moreover, there is a paucity of research examining the contribution of experiences involving the dimension of deprivation – those interpersonal or physical environments lacking socioemotional or cognitive features important for optimal developmental and wellbeing. Investigation of different environments that occur on a continuum and may be more applicable to the general population may have greater success in explaining the etiology of depression. CHAPTER 3 therefore provides a review of the literature that suggests how the family environment, a developmentally-relevant exposure that can be examined as a continuous measure, might interact with 5-HTTLPR genotype to predict the emergence of depression.

CHAPTER 3: THE INTERACTION BETWEEN THE SEROTONIN TRANSPORTER GENE X FAMILY ENVIRIONMENT IN PREDICTING DEPRESSION DURING ADOLESCENCE

Gene-environment interactions involving more normative environmental influences, such as less optimal parenting or family environments (compared with, for example, extreme abuse or neglect) have received little attention to date, despite the possibility that they may have greater success in explaining the etiology of depression. This chapter will consider various aspects of the family environment, and in particular, more aversive, harsh parenting as well as less warm, less responsive parenting, as developmentally-relevant risk factors for depression during the adolescent period. It will also examine how individuals might be differentially affected by known family risk factors for depression, with a particular focus on reviewing the emerging body of evidence that supports an interaction between 5-HTTLPR genotype and maladaptive parenting in predicting depression.

3.1 Parenting and broader family processes as a candidate environmental exposure for gene-environment research

A number of researchers have expressed concern that the investigations that followed the original GxE results of Caspi and colleagues (2003) involving the serotonin transporter gene have considered a wide variety of ad hoc alternative indices of environmental stress, involving almost any form of adversity or hardship at any time in a person's life and over any range of time (Dick et al., 2015; Monroe & Reid, 2008). In the same way that genes are assessed as potential candidates in gene-environment research, there should be candidate "environments," that are suggested based on prior research and a theoretical understanding of the plausible underlying pathways or mechanisms linking them

to the outcome of interest (Dick et al., 2015). Just as no one would expect to uncover a 'true' GxE interaction for a specific condition if the incorrect gene is considered, a potentially valid GxE interaction is unlikely to be identified if the incorrect form of environment is assessed, or if the correct form of the environment is assessed improperly (Monroe & Reid, 2008).

Theoretical reviews have argued there are several key principles that should be observed by researchers when selecting environmental risks in order to conduct hypothesis-driven studies of GxE predicting psychological disorders (Moffitt, Caspi, & Rutter, 2005). First, there should be evidence that the putative candidate environment has causal pathogenic effects on the disorder of interest. Second, there should be plausible effects of the environmental risk on biological systems involved in the disorder. Third, there should be evidence of variability in response to the selected environmental exposure. The following sections will consider how parenting and the broader family environment might represent promising candidate environment exposures for gene-environment research.

3.2 The family environment is a significant risk factor for depression

The influence of families on child and adolescent development and on risk for psychopathology has been the focus of a large body of research, reflecting the widely-held view that the caregiving that children receive or the home environment they grow up in has significant implications for their adjustment (Rapee, 1997; Repetti, Taylor, & Seeman, 2002; Stocker, Richmond, Rhoades, & Kiang, 2007). Whilst it has been suggested that parents' influence diminishes during adolescence as adolescents negotiate increasing autonomy and independence from the family, and peers become increasingly salient (Turner, Irwin, Tschann, & Millstein, 1993), parents have been found to remain as

particularly important influences during this developmental period (Stocker et al., 2007). Moreover, there is marked continuity in the affective quality of parent-child relationships and interactions across childhood and adolescence (Collins & Laursen, 2004; Michalik et al., 2007).

3.2.1 **The influence of parental behaviours**

It is widely accepted that the ways in which parents behave or interact with their children have an impact on young people's risk of developing depressive disorders during adolescence, consistent with an understanding of depression as a disorder that exists in an interpersonal context. Parental behaviours refer to both the specific, goal-related behaviours or parent-child relationship factors that impact directly on the child (referred to as parenting practices), and non-goal-related behaviours that are engaged in by parents, such as facial expressions of emotion gestures, and changes in tone of voice (Cowan & Cowan, 2002; Darling & Steinberg, 1993; Prevatt, 2003).

The effects of family violence, physical, emotional and sexual abuse, as well as severe neglect on risk for psychopathology throughout childhood and into adulthood are well-documented (Mandelli, Petrelli, & Serretti, 2015). However, adverse effects may also occur as a result of less severe and arguably more "normative" family dysfunction. A recent meta-analysis reveals a large and compelling literature implicating adverse parent-child interactions characterized by elevated conflict, high levels of parental hostility, rejection and control, and low levels of parental affective warmth, support and approval in the occurrence of child and adolescent depression (Yap, Pilkington, Ryan, & Jorm, 2014). These associations have been documented in both community samples (e.g., Hopkins, Lavigne, Gouze, Lebailly, & Bryant, 2013; Sijtsema, Oldehinkel, Veenstra, Verhulst, &

Ormel, 2014; Vazsonyi & Belliston, 2006) and clinical samples (e.g., Guberman & Manassis, 2011) and according to observational assessment of parenting behaviour (Schwartz et al., 2012; Sheeber, Davis, Leve, Hops, & Tildesley, 2007) as well as parent and child/adolescent reports (Cole & McPherson, 1993; Hops, Lewinsohn, Andrews, & Roberts, 1990; Sijtsema et al., 2014; Stark, Humphrey, Crook, & Lewis, 1990).

Whilst negative, harsh or aggressive parenting behaviour and positive, warm, nurturing behaviour could be conceived as falling on opposite ends of a single spectrum, research suggests that they rather represent distinct, albeit correlated, dimensions that make opposite and independent contributions to depression (Barrera, Chassin, & Rogosch, 1993; Dallaire et al., 2006). Conceptualizing warmth and hostility as separate dimensions also allows a more nuanced examination of parenting. It allows, for example, a consideration of the presence of warmth and the absence of warmth (which may not necessarily have the same effect as the presence of hostile parenting), as well as the presence and absence of hostile parenting (which may not necessarily have the same effect as the presence of parental warmth).

Indeed, individuals developing in environments that *lack* supportive and nurturing features may face very different adaptive challenges to those who are developing in environments involving the *presence* of harsh, conflictual or threatening behaviours. Hostile, irritable behaviour from parents may represent a source of ongoing stress for children that increases psychological distress, feelings of hopelessness and worthlessness, and diminishes a sense of control, all of which are symptoms of depression (Burge & Hammen, 1991; Downey & Coyne, 1990; Ge, Best, Conger, & Simons, 1996). A lack of parental warmth, sensitivity and responsiveness might promote an increased reliance on an

inward-focused coping response of withdrawal or disengagement which, over time, may also place the child at greater risk of depressive symptomatology (Field, 1992; Tronick & Gianino, 1986). The distinctiveness of positive and negative parenting dimensions may be further supported by findings of reviews and recent meta-analyses that conclude the influence of parental warmth on depression is well established but that the link between parental warmth and anxiety remains more equivocal, (Rapee, 1997; Wood, McLeod, Sigman, Hwang, & Chu, 2003; Yap et al., 2014).

Critically, a number of prospective longitudinal studies have indicated that adverse family interactions or parenting behaviours are present prior to depression, and hence may potentially elicit depressive symptomatology. For example, a study by Schwartz, Byrne, Simmons, Whittle, Dudgeon, Yap, Sheeber and Allen (2013) revealed that higher rates of maternal aggressive (hostile, critical) behaviour and lower rates of maternal positive (warm, supportive) behaviour observed during mother-adolescent interactions at age 12 prospectively predicted MDD onset across the entire course of adolescence to age 18-19. Sheeber, Hops, Alpert, Davis, and Andrews (1997) found that family support and conflict predicted adolescent depression one year later, controlling for initial levels of depression at time 1. Rueter, Scaramella, Wallace, and Conger (1999) observed that increased frequency in parent-adolescent disagreements, according to parent-report, from age 12-13 years to age 14-15 years predicted adolescent-reported internalising symptoms which in turn were predictive of a first onset of case-level depression at age 19 years. Stice, Ragan, and Randall (2004) found in a sample of 11-15 year old girls that perceived deficits in parental support by adolescents were associated with increases in their depressive symptoms as well as first onset of major depression two years later.

However, the association between parenting and child or adolescent depression may not be entirely unidirectional. It is widely accepted that children and adolescents actively influence their environment, including the behaviour of family members (Pardini, 2008; Pettit & Arsiwalla, 2008), and it is conceivable that child depression in particular could evoke, reinforce and/or shape particular parenting behaviours. Indeed, whilst the reverse pathway from depressive disorder or symptoms to parenting was not evident in the aforementioned studies, evidence for bidirectional or reciprocal effects between parenting and child or adolescent depression has been detected by a number of other longitudinal studies (Branje, Hale, Frijins, & Meeus, 2010; Hale, Vander Valk, Akse, & Meeus, 2008; Hipwell et al., 2008).

Further evidence supporting a potentially causal relationship between parenting and depression comes from a body of research that has indicated that the quality of parent-child interactions or family climate predicts changes in depressive symptoms over time (Garrison, Jackson, Marsteller, McKeown, & Addy, 1990; Schwartz et al., 2012) and response to treatment, including relapse and recurrence of MDD (Asarnow, Goldstein, Tompson, & Guthrie, 1993; Birmaher et al., 2000; Brent et al., 1998; Feeny et al., 2009; Kennard et al., 2008; McCleary & Sanford, 2002). There is also emerging evidence that targeting the specific parenting processes in family-based interventions implicated in the emergence of depression can reduce symptoms (Sandler, Schoenfelder, Wolchik, & MacKinnon, 2011). Changes in parenting factors, particularly increases in parental responsiveness/warmth and decreases in parental criticism/guilt induction, have been found to mediate intervention effects on youth mental health outcomes and coping (Compas et al., 2010; Zhou, Sandler, Millsap, Wolchik, & Dawson-McClure, 2008). Overall this literature

suggests a clear association between particular patterns of parenting behaviours, parent-adolescent relationships and adolescent depressive disorders.

3.2.2 **The influence of contextual family factors**

A number of factors that relate to the broad family context have also been found to increase risk for depression during the adolescent period, including the presence of parental psychopathology, high interparental conflict and low socioeconomic status (SES).

With regard to parental psychopathology, depression in parents has been consistently identified as a particularly potent predictor of adolescent depression, possibly because causal relationships between parental and child depression can occur through both environmental transmission (i.e. impaired parenting, observational learning resulting from exposure to depressed cognitions and affect) and genetic transmission (Beardslee, Gladstone, & O'Connor, 2011; Birmaher et al., 1996; Goodman & Gotlib, 2002). Research suggests that risk for depression during childhood and adolescence is three-to-four times higher amongst children with a parent that has experienced depression compared to children without a parental history of the disorder (Beardselee, Versage, & Giadstone, 1998; Weissman, Warner, Wickramaratne, Moreau, & Olfson, 1997). Parental depression has also been found to predict greater risk of relapse and poorer recovery from depression experienced by those same parents' offspring (Brent et al., 1998; Essau, 2004).

Previous studies on the family context of depressed parents have identified difficulties in parenting and the parent-child relationship, particularly in the quality of parent–child interaction as relevant factors (for reviews, see Dix & Meunier, 2009; England & Sim, 2009; Lovejoy, Graczyk, O'Hare, & Neuman, 2000). This work, which has tended to comprise studies of mother (rather than father)-child interactions, has revealed that,

compared to both psychiatrically healthy and non-depressed psychiatric controls, depressed mothers are less warm and responsive to their children, exhibit more punitive responses, and engage in less effective problem-solving techniques and inconsistent discipline strategies to resolve conflict or difficulties. Findings that depressed mothers tend to display increased rates of withdrawn, disengaged behaviours have led some researchers to regard maternal depression as a potential marker of deprivation (Lupien et al., 2011).

Moreover, a number of studies have suggested that maladaptive parenting may have a critical mediation role in the association between parental psychopathology and child psychopathology (e.g., Burt et al., 2005; Elgar, Mills, McGrath, Waschbusch, & Brownridge, 2007; Johnson, Cohen, Kasen, Smailes, & Brook, 2001). For example, one large prospective, community-based longitudinal study revealed that parents with psychiatric conditions showed higher levels of harsh punishment, more inconsistent enforcement of rules, and low warmth, and that maladaptive parenting behaviours in turn predicted the occurrence of offspring psychiatric conditions during late adolescence and early adulthood (Johnson et al., 2001). Critically, the relationship between parental psychopathology and child psychiatric condition was no longer significant when parenting was included in the model, indicative of its mediating role. The majority of youths who experienced high levels of maladaptive parenting experienced onset of a psychiatric illness, independent of whether their parents had a diagnosis, whilst children of parents with a psychiatric condition were only at greater risk when they experienced maladaptive parenting. These associations were present for parent and child psychiatric conditions more broadly, and for parent and child depression specifically.

Interparental conflict is another broad family context factor that has been linked to poorer psychological adjustment in children, including increased risk for depression (Cummings & Davies, 2002; Grych & Fincham, 1990). Similar to parental psychopathology, a significant body of research indicates that interparental relationships characterized by conflict, anger, and aggression may have an indirect impact on offspring via disruptions to caregiving behaviours and the parent-child relationship (e.g., Cui & Conger, 2008; Stroud, Meyers, Wilson, & Durbin, 2015; Sturge-Apple, Davies, Cicchetti, & Cummings, 2009) It has also been argued that exposure to interparental discord may threaten feelings of safety and emotional security and promote feelings of guilt in children, which may increase vulnerability to depression and other disorders (Cummings, Schermerhorn, Davies, Goeke-Morey, & Cummings, 2006; Davies & Cummings, 1994; Du Rocher Schudlich & Cummings, 2007; Fosco & Grych, 2008).

Studies have reliably identified a family's socioeconomic status (SES) as a risk factor for internalizing problems such as depression in individuals of all ages, including adolescence (e.g., Reiss, 2013; Slopen, Fitzmaurice, Williams, & Gilman, 2010). Research suggests that a disadvantaged socio-economic background adversely affects children's socioemotional development through not only an accumulation of risk experiences such as poor living conditions, exposure to more chronic and uncontrollable life events, and reduced access to resources (Evans & English, 2002; Schoon, Sacker, & Bartley, 2003; Wadsworth et al., 2008), but also by influencing the psychological wellbeing of parents, and thereby their parenting practices and the broader parent-child relationship (Bøe et al., 2014; Conger & Donnellan, 2007; Grant et al., 2003; Reising et al., 2013).

To summarise, several potential mechanisms may account for the associations between these broad contextual family factors and depression. One possibility is that they are inherently stressful or distressing and thus directly affect child psychological adjustment. A common theme however that emerges in this brief review of these factors is that their effects on psychological disorder, such as depression, appear to be at least partly mediated by their effects on parenting behaviours. Financial strain, interparental discord or the experience of a psychological disorder may leave parents overwhelmed, anxious, irritable, angry and helpless, with fewer emotional or practical resources to direct towards their children. This in turn may decrease a parent's ability to parent effectively and reduce the quality of parenting that a child receives. Thus, children and adolescents growing up in more challenging or problematic family environments may be exposed to increased levels of potentially maladaptive parenting practices, in addition to the direct impact of exposure to broader family adversity, which, in turn, may augment the risk of depression and other disorders (Cummings & Davies, 2002; England & Sim, 2009; Grant et al., 2003).

3.2.3 **The family environment influences biological systems involved in depression**

There are a number of reviews of the body of evidence suggesting that parenting and broader family experiences, particularly those involving a high level of adversity, may shape brain structure and function (e.g.,Andersen & Teicher, 2008; Belsky & de Haan, 2011; Lupien, McEwen, Gunnar, & Heim, 2009). Particularly relevant to the current thesis is an emerging body of research that indicates that variation in more normative parental care can influence an individual's affective neural circuitry, namely the amygdala, hippocampus, orbitofrontal cortex (OFC), and anterior cingulate cortex (ACC) morphology. For example, one study identified associations between increased family poverty

experienced at preschool age (i.e., 3-6 years old) and smaller hippocampal and amygdala volumes, with the effects of poverty on hippocampal volume mediated by less optimal parenting involving lower support/higher hostility (Luby et al., 2013). Supportive parenting in early childhood has also been shown to predict larger hippocampal volumes at school-age, particularly for non-depressed (versus depressed) children (Luby et al., 2012). In contrast to these findings, enlarged amygdala volumes but comparable hippocampal volumes have been found in ten-year old children of chronically depressed versus non-depressed mothers (Lupien et al., 2011). A longitudinal neuroimaging study identified that maternal support during the preschool (but not primary school) years influenced the trajectory of hippocampal volume growth into later school age and early adolescence, with reduced levels of maternal support associated with a shallower slope of hippocampal volume enlargement over time (Luby, Belden, Harms, Tillman, & Barch, 2016). Another recent longitudinal study by Whittle, Simmons, et al. (2014) found that early adolescents (aged approximately 12 years) whose mothers showed more warm, positive behaviours during laboratory-based parent-child interaction tasks displayed attenuated volumetric growth in the right amygdala, and accelerated cortical thinning in the right anterior cingulate (males only) and left and right orbitofrontal cortices between baseline and follow up four years later. Whilst not necessarily consistent with one another, together the findings from these studies indicate that family processes may contribute to the developmental trajectories of brain structures thought to have a role in affective processing. This is important because morphometric differences in these structures have been associated with depression, as will be discussed in CHAPTER 6.

3.3 Individuals may be differentially affected by their family environment

Evidence discussed so far in this chapter suggests that adverse family environments represents an important risk factor for developing depression, however it is also generally well accepted that the effect of the family environment can vary significantly between individuals (e.g., Cowan & Cowan, 2002). Certainly, as shown in the meta-analysis by Yap and colleagues (2014), effect sizes of various parenting variables on depression were generally small to medium, indicating that these factors did not perfectly predict depression outcomes. Research on gene-environment interactions, including that reviewed in the previous chapter, highlights the very real possibility that part of the heterogeneity in response to maladaptive parenting or adverse family environment might be related to inherited genotype, including allelic variation in the serotonin transporter gene. However, the majority of the gene-environment interaction research that has considered the influence of family experiences, has typically focused on highly adverse, distressing childhood circumstances such as those involving abuse or maltreatment (e.g., Caspi et al., 2003; Ressler et al., 2010), which the majority of people have not experienced. Less is known about the impact of relatively less severe environments or more normative experiences, including the range of exposures pertaining to the family environment and parenting, which have been empirically shown to be associated with depression and other forms of psychopathology. The following sections thus critically review the current body of studies in humans that consider how the serotonin transporter gene and family environments might interact to influence depression.

3.3.1 **Evidence for a 5-HTTLPR X family environment interaction predicting depression**

Objective

A broader narrative review was conducted, which drew on systematic review guidelines (Moher et al., 2015) to identify the studies that have examined association between the 5-HTTLPR, family environment and depression, and guide critical appraisals of these studies, particularly around issues relating to methodology. The aim was to explore aspects of study designs that might contribute to variation in findings. A systematic search procedure was employed to identify relevant articles that specifically included an assessment of how the serotonin transporter gene might interact with various aspects of the family environment to affect risk for depression.

Method

Studies

A literature search of Medline and PsychInfo was conducted in April 2016 using a combination of database-specific index terms (e.g. 'Serotonin Plasma Membrane Transport Proteins', 'Family Relations', 'Parent-Child Relations', 'Child Rearing,' 'Parenting,' 'Family Characteristics,' 'Parents,' 'Caregivers,' 'Family Structure,' 'Attachment Behaviour' and 'Depression') and individual terms located in the title or abstract (e.g. 'serotonin transporter', '5-HTTLPR,', 'family environment,' 'attachment'). A complete list of search terms for each database is provided in

Table 3-1. There are slight variations in the search terms used in PsychInfo and Medline due to differences in the databases' specific index terms. Studies were included in this review if: (1) they were conducted with human participants; (2) they were written in English and published in peer reviewed journals; (3) they specifically tested a two-way interaction between variation in the serotonin transporter gene and a measure of family environment that predicted either case level depression, depressive symptoms or a change in depression or symptoms in either males and females together, or in males and females separately, and conducted sufficient post-hoc analyses to identify the direction of the interaction; (4) the specific measure of family environment examined in the 2-way interaction did not focus on trauma and child maltreatment (sexual, physical or emotional abuse or severe neglect, such as institutionalism), though other interactions involving these phenomena may also have been tested separately in the study and (5) the time period associated with the family environment measure was childhood or adolescence. The time period associated with the outcome of depression was variously defined.

Table 3-1. *Search parameter specification for review based on systematic review guidelines.*

	PsychInfo	**Medline (Ovid)**
Gene		
Index term	-	Serotonin Plasma Membrane Transport Proteins
Free-text keyword	serotonin transporter OR 5-HTTLPR OR 5HTTLPR	serotonin transporter OR 5-HTTLPR or 5HTTLPR
Family Environment		
Index term	Family Relations OR Child Discipline OR Childrearing Practices OR Family Conflict OR Marital Relations OR Parent-Child Relations OR Parental Role OR Parenting or Authoritarian Parenting OR Parent Child Communication OR Parental Involvement OR Parenting Style OR Permissive Parenting OR Parental Characteristics Parent Educational Background OR Parental Attitudes OR Parental Occupation OR Parenting Skills OR Parents OR Fathers OR Mothers or Single Parents OR Stepparents OR Caregivers OR Family Structure OR Attachment Behaviour	Family Relations OR Parent-Child Relations OR Child Rearing OR Parenting OR Family Characteristics OR Parents OR Caregivers OR Family OR Family Health
Free-text keyword	family health OR family environment OR family adversity or family stress	family structure OR attachment OR family environment OR family adversity or family stress
Depression		
Index term	Depression (Emotion) OR Major Depression	Depression OR Depressive Disorder
Free-text keyword	-	-

Figure 3-1 shows the PRISMA flow diagram describing the selection process for studies examining interactions between variation in the promoter of the serotonin

transporter gene and aspects of the family environment. After removal of duplicates, the search generated 101 potential articles.

The ancestry approach, which involved searching the reference lists of review articles or articles dealing broadly with relevant subject matter, was also used to uncover 2 additional potential papers. In total, 103 papers were examined for potential inclusion based on abstracts. Two reviewers (K.L. & A.P.) assessed the titles and abstracts of the papers to determine whether the study was appropriate to include in the current review. Where a title or abstract suggested a study might be eligible, the full article was obtained and assessed based on the inclusion criteria. If it was not clear that a study met the inclusion criteria, a third reviewer (N.B.A.) was enlisted to reach consensus. Fifty-two full text articles were retrieved and assessed for eligibility, of which 22 articles were finally included.

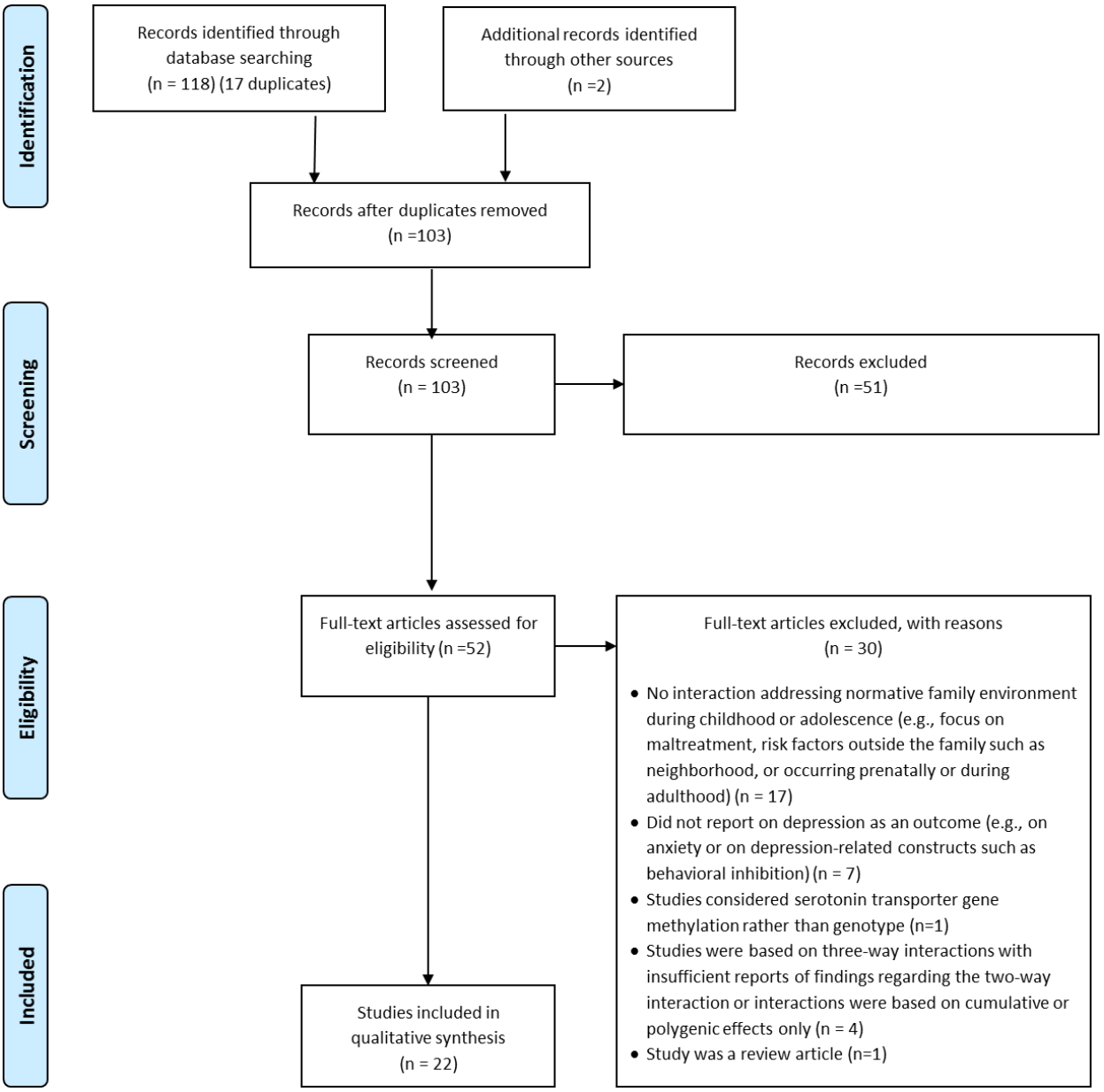

Figure 3-1*: PRISMA flow diagram of the study inclusion/exclusion process.*

Results

Table 3-2 presents details regarding the complete set of 22 studies that examined an interaction of interest between the serotonin transporter gene and a measure of the family environment predicting depression as an outcome. There is a total of 13, 740 participants in the studies[4], and the number of participants per study ranges from 118 to 4,334 (M =723.16, SD=961.60).

[4] . The analyses from the studies by Conway, Hammen, Brennan, Lind, and Najman (2010) and Hammen, Brennan, Keenan-Miller, Hazel, and Najman (2010) both draw on participants from the same longitudinal study (the Mater-University Study of Pregnancy), with slightly different inclusion criteria. Similarly, the analyses contained in the articles by Jenness, Hankin, Abela, Young, and Smolen (2011) and Oppenheimer, Hankin, Young, and Smolen (2013) draw on participants from the same study (the GEM study), with slightly different inclusion criteria. The analyses by Nobile et al. (2014) are based on the participants from Nobile et al. (2009), who were available for longitudinal follow up. The figure on which the total number of participants is calculated uses the largest number of participants, which is from Conway et al., 2010, Oppenheimer et al., 2013 and Nobile et al., 2009.

Table 3-2. *Summary data for the 22 studies included in the systematic review*

Author, Year	Sample gender (M;F) ethnicity	Study design	Serotonin Transporter Genotype	Developmental period/age when Family Environment Measured	Aspect of Family Environment	Family Environment Assessment	Developmental Period/age at outcome	Outcome	Outcome assessment	Ratio of Significant Interactions: Tested Interactions	Finding
1. Araya et al. (2009)	4,334 M: 53% 94% C	Longitudinal	**rs25531 SNP:** SS, SL_G, L_GL_G vs SL_A, L_GL_A vs L_AL_A	Infancy	Maternal post-natal depression	Parent (i.e. self) report	Early childhood	Emotional (internalising) symptoms	SDQ	0:9	Null
2.Brummett et al. (2008)	142 M: 54.9% 47.2% C 52.8% AA	Cross-sectional	**VNTR:** SS vs SL vs LL	Childhood (measured retrospectively)	Family SES (father's level of education)	Participant report	Adulthood mean (SD) age in years =34.0 (8.8)	Depressive symptoms	OBD Scale from MMPI (self-report questionnaire)	2:3	Positive /Opposite
3. Chipman et al. (2007)	544 M: 269 (49%) >99% C	Longitudinal	**VNTR:** SS vs SL vs LL	Late childhood and adolescence	Family stress in the previous 12 months Persistent family adversity across 6 years	Study-derived parent report questionnaire	Adolescence (15-16 years; 17-18 years)	Depression	SMFQ (self-report questionnaire)	1:4	Partially opposite
4. Conway et al. (2010)	381 M: 39% 92% C 8% Other	Longitudinal	**rs25531 SNP:** SS, SL_G, L_GL_G, SL_A, L_GL_A vs L_AL_A Exploratory analyses: SS, SL_G, L_GL_G vs SL_A, L_GL_A vs L_AL_A	Adolescence (age 15)	Chronic family stress	Multi-informant: composite of parent, and child reports on questionnaires and interview	Early adulthood (20 years)	Depressive symptoms Depression	BDI-II (self-report questionnaire) SCID (diagnostic interview)	0:6	Null
5. Eley et al. (2004)	377 M:42% Ethnicity not reported	Cross-sectional	**VNTR:** SS vs SL vs LL	Adolescence (age 12-19)	Broad family stress	Parent report questionnaires	Adolescence (12-19 years)	High or low depression group based on cut-off scores	SMFQ (self-report questionnaire)	1:3	Partially positive

6. Fandino-Losada et al. (2013)	1758 M: 40.3% 89 % C in larger study 11% Other	Cross-sectional, case-control design	**rs25531 SNP:** S_AS_A, S_AL_G, L_GL_G, S_GS_A vs S_AL_A, L_GL_A, L_AL_A	Childhood and adolescence	Parental separation/loss	Study-derived questions	Adulthood (20-64 years)	Case-level depression (Major Depressive Disorder, mixed Anxiety/Depression or Dysthymia)	MDI; Sheehan Patient-Rated (Panic) Anxiety Scale (both self-report questionnaires)	0:8	Null
7. Fergusson, Horwood, Miller, and Kennedy (2011)	893 Sex not reported 85% C 15 % Maori/PI	Longitudinal	**VNTR:** SS vs SL vs LL; LL, SL vs SS LL vs SL, SS	Early childhood (averaged over 3, 4 & 5 years)	Punitive parenting behaviour Changes of parents	Interviewer observation of mother-child interaction Study-derived questions on changes in family situation	Adulthood (18, 21, 25 and 30 years)	Depressive symptoms Major depression diagnosis	CIDI based on self report / 'significant other' report (diagnostic interview)	0:240	Null
8. Hammen et al. (2010)	346 M: 38% 93% C 7% other	Longitudinal	**rs25531 SNP:** SS, SL_G, L_GL_G vs SL_A, L_GL_A vs L_AL_A	Adolescence (15 years)	Chronic family stress	Multi-informant: composite of parent, and child reports on questionnaires and interview	Adulthood (20 years)	Depressive symptoms	BDI-II (self-report questionnaire)	2:3	Partially positive
9. Jenness et al. (2011)	200 M: 43% 67% C 7% AA 7% Latino 4%A/PI 14% Other	Prospective longitudinal	**rs25531 SNP** SS, L_GL_G, SL_G vs SL_A, L_GL_A vs L_AL_A **VNTR:** SS vs SL vs LL	Childhood & adolescence (7-16 years)	Chronic family stress	Semi-structured interview with child	Childhood & adolescence (7-16 years)	Change in depressive symptoms over six months	CDI (self-report questionnaire)	3:3	Positive
10. Laucht et al. (2009)	309 M: 65% >99.0% European descent.	Prospective longitudinal	**VNTR:** SS; SL; LL **rs25531 SNP:** L_GS, L_GL_G, SS vs L_AS; L_AL_G vs L_AL_A	Infancy (3 months old)	Family adversity	Structured interview with parent	Adolescence (15-19 years)	Diagnosis of depression/anxiety Depressive symptoms	SCID (semi-structured clinical interview) BDI (self-report questionnaire)	3:4	Partially opposite

11. Lavigne et al. (2013)	175 M: 55.4% 100% C	Cross-sectional	**VNTR:** SS, SL vs. LL	Childhood (4 years old)	Family conflict; parental stress; SES; caretaker depression; parental support; parental scaffolding; parental hostility	Parent report questionnaires, interviewer observation of caretaker-child interaction	Childhood (4 years old)	Depressive symptoms	Composite of DISC-YC major depression scale (parent interview), CSI major depression scale, and the CSI dysthymia scale (parent-report questionnaire)	4:14	Partially opposite
12. Li, Berk, and Lee (2013)	1 030 M: 56% 100% C	Longitudinal	**VNTR:** SS, SL vs. LL SS vs SL, LL LL vs SL vs SS	Adolescence and early adulthood (12-27 years old)	Perceived family support	Structured interview with adolescent	Adolescence and early adulthood (12-27 years old)	Depressive symptoms	Abbreviated CES-D (self-report questionnaire)	2:6	Partially positive/
13. Nobile et al. (2009)	607 M: 51.9% 99% C 1% other	Cross-sectional	**VNTR:** SS, SL vs. LL **rs25531 SNP:** L_GS, L_GL_G, SS, L_AS; L_AL_G vs L_AL_A	Childhood & Early adolescence (10-14 years)	Family structure SES (parental employment)	Parent report Parent report	Childhood & early adolescence (10-14 years)	Affective (mainly depressive) problems Broader internalising (anxiety, depressive and somatic) problems	CBCL/6-18 (parent report questionnaire)	1:5	Partially positive
14. Nobile et al. (2014)	287 M: 59% Ethnicity not reported	Longitudinal	**VNTR:** SS, SL vs. LL **rs25531 SNP:** L_GS, L_GL_G, SS, L_AS; L_AL_G vs L_AL_A	Childhood & adolescence (10-19 years)	Family structure SES (parental employment)	Parent report Parent report	Childhood & adolescence (10-19 years)	Anxious/ depressed symptoms Withdrawn/ depressed symptoms Internalising problems	CBCL/6-18 (parent report questionnaire)	2:30	Partially opposite
15. Oppenheimer et al. (2013)	241 43% M 66 C 7 % AA 8% Latino 4%A/PI 15% Other	Prospective longitudinal	**VNTR:** LL vs SL vs SS	Childhood & adolescence (9-16 years)	Ideographic changes in maternal depressive symptoms Nomothetic changes in maternal depressive symptoms	Self-report questionnaire (i.e., mother reporting on own symptoms)	Childhood & adolescence (9-16 years)	Depressive symptoms	CDI (self-report questionnaire)	2:3	Partially positive

16. Petersen et al. (2012)	574 M: 52% ~81% C 17% AA	Prospective longitudinal	**VNTR:** LL vs SL vs SS **rs25531 SNP:** (L_GS; L_GL_G; SS) vs (L_AS; L_AL_G) vs (L_AL_A)	Adolescence (12-17 years)	Stressful life events (changes and adjustments) within the family	Parent-report questionnaire	Adolescence (12-17 years)	Anxious/ depressed symptoms	CBCL/6-18 (parent-report questionnaire); YSR (self-report questionnaire)	**5:20**	**Partially positive**
17. Ritchie et al. (2009)	942 M: 41.9% Ethnicity not reported	Cross-sectional	**VNTR:** SS, SL vs. LL LL vs SL vs SS	Childhood/adolescence (retrospective measurement in adulthood)	Paternal mental health problems; Maternal mental health problems; Poverty/financial difficulties; Parental oversharing of problems; Maternal affection; Happy childhood; Parents did their best	Self-report questionnaire	Adulthood	Depression	MINI, CES-D anti-depressant treatment	4:9	Partially opposite
18. Sen et al. (2010)	409* M: 45.7% 65.5% C 20.5% A 13.9% Other	Prospective longitudinal	**rs25531 SNP:** L_GS, L_GL_G, SS, L_AS, L_AL_G vs L_AL_A	Childhood and adolescence measured retrospectively in adulthood	Early family environment	Self-report questionnaire	Adulthood (first year of medical internship)	Change in depressive symptoms over one year	PHQ-9 depression module (self-report questionnaire)	0:3	Null
19. Sjoberg et al. (2006)	200 M: 40.5% Ethnicity not reported	Longitudinal	**VNTR:** LL vs SL vs SS	Childhood and early adolescence (retrospective in late adolescence/early adulthood)	Family conflicts, type of family residence, family constellation	Semi-structured interview	Adulthood (19-22 years)	Depressive symptoms	DSRS of the DSM-IV (A-criterion) for major depression	8:12	Positive/ opposite
20. Taylor et al. (2006)	118 M: 43.2% 33.9% C 38.1% A 27.9% Other	Cross-sectional	**VNTR:** LL vs SL vs SS	Childhood/adolescence measured retrospectively in adulthood	Early family environment	Self-report questionnaire	Adulthood (18-29 years)	Depressive symptoms	BDI (self-report questionnaire)	2:3	Partially positive

21. Van Roekel, Engels, Verhagen, Goossens, and Scholte (2011)	306 M: 46.7% Ethnicity not reported	Prospective-longitudinal	**VNTR:** LL vs SL, SS	Early adolescence	Maternal depressive symptoms, paternal depressive symptoms, perceived maternal support, perceived paternal support	Self-report questionnaire for parental depression (i.e., parent reporting on own symptoms), child report for perceived support	Adolescence (13-18 years)	Depressive symptoms	DSS (self-report questionnaire)	0:12	Null
22. Vrshek-Schallhorn et al. (2014)	400 M: 30.7% 48.25% C 13.50%AA 14.25% Hispanic/Latino 4.5%A .75 PI 5.50 Other 13.25% Multiple	Longitudinal	**VNTR:** LL vs SL, SS	Adolescence	Chronic family stress	Semi-structured interview	Adolescence	Major Depressive Disorder	(SCID-I/NP (Semi-structured interview)	:2	Positive

M=Male; F= Female; C= Caucasian; AA = African American; A=Asian; PI=Pacific Islander *= note that GxE was conducted in the separate ethnicity groups only, not in the overall sample; SDQ=Strengths and Difficulties Questionnaire; OBD Scale from MMPI=Obvious Depression Scale from the Minnesota Multiphasic Personality Inventory; SMFQ = Short Mood and Feelings Questionnaire; BDI-II = Beck Depression Inventory-II; MDI = Major Depression Inventory; CIDI=Composite International Diagnostic Interview; CDI=Children's Depression Inventory; SCID=Structured Clinical Interview for DSM-IV; DISC-YC= The Diagnostic Interview Schedule for Children–Parent Scale—Young Child; CSI= The Child Symptom Inventory; CBCL/ 6–18= Child Behaviour Checklist for Ages 6-18 (Achenbach & Rescorla, 2001); PHQ-9= Patient Health Questionnaire; SCID-I/NP= Structured Clinical Interview for DSM- IV Axis I Disorders (First, Spitzer, Gibbon, & Williams, 2001) ; DSR= Depression Self-Rating Scale of the DSM-IV (A-criterion) for major depression

Table *3-3* summarises the total range of categories of outcomes, for the interactions of interest (but not for other interactions, such as those involving environmental exposures unrelated to the family environment, or involving abuse/neglect, or predicting other outcomes than depression).

Table 3-3*: Study outcomes.*

Outcome	**N (%)**
Positive/partially positive (supports S-allele in all analyses or in part of the analyses – e.g., for a subsample, particular environment factor or particular outcome)	9 (40.91)
Opposite/partially opposite (supports L allele in all analyses or part of the analyses (e.g., for a subsample, or for a family environment factor or particular outcome)	5 (22.73)
Null (no significant GxE interaction)	6 (27.27)
Partially positive/opposite (mixed support - supports both the S allele and L allele as risk factors for a particular subsample, family environment factor or particular outcome)	2 (9.09)

This table indicates that findings from 9 of the 22 studies (41%) provided some support for increased risk of depression in participants with the low-expression short alleles who had experienced an adverse family environment in childhood and/or adolescence (i.e. a *positive or partially positive* finding). A number of studies however did not conform to this result; six of the 22 studies (27%) obtained only null findings, failing to show any interaction between 5-HTTLPR and the family environment predicting depression, whilst 5 studies (23%) found that, for at least one factor of the family environment, it was the LL homozygous participants who were more likely than their S-carrying counterparts to experience depression in the context of an unfavourable family environment (i.e. an *opposite* or *partially opposite* result). Two studies (9%) provided *mixed support*, indicating that both the L-allele and the S-allele were associated with risk for internalising difficulties

in particular subgroups (for example, in boys versus girls) or under particular environmental circumstances. This analysis suggests that the most common finding for studies in terms of whether 5-HTTLPR interacts with the family environment to influence risk for internalising difficulties is at least one positive result, implicating the S-allele as a risk allele in adverse family environments. However, this finding was identified by less than half of studies.

It is interesting to note how this analysis compares to the largest meta-analysis/systematic review of 81 studies by Sharpley et al. (2014), which considered how depression might be predicted by an interaction between the serotonin transporter gene and more broad measures of stress (i.e. not confirmed to an adverse family environment). This review identified 53 studies (65.4%) that could be classified as finding some support for a positive interaction, 21 studies (25.9%) that obtained a null finding, and 6 studies (7.4%) that provided some support for an opposite finding. Whilst it is not possible to make any conclusive comparisons given the smaller number of studies relating to the family environment, positive findings appear somewhat less frequent and opposite findings somewhat more frequent when a measure of family environment is considered rather than a broader measure of stress.

An alternate method of quantifying the results of the studies is to identify the numbers of significant positive and opposite *interactions* that considered an aspect of the family environment. Many of the studies included in this analysis conducted multiple tests for the presence of such an interaction by varying their genetic models (e.g., Fandino-Losada et al., 2013; Laucht et al., 2009), environmental exposures (e.g., Lavigne et al., 2013; Nobile et al., 2014) and outcome of interest (e.g., Chipman et al., 2007; Conway et

al., 2010), altering the covariates (e.g., Nobile et al., 2009) or testing the interaction in different groups based on gender (e.g., Brummett et al., 2008; Taylor et al., 2006) or ethnic background (e.g., Oppenheimer et al., 2013; Petersen et al., 2012). A total of 402 interactions were tested across the 22 studies (additional analyses involving interactions between 5-HTTLPR and an aspect of the environment that did not pertain to family circumstances may also have been conducted but these are not included in the following analyses). Only 44 (10.95%) of these interactions identified a significant result at the $p<.05$ level. Overall, 23 of these interactions (5.72% of the total number of interactions) provided support for the S-allele as a risk allele and 17 interactions (4.23% of the total number of interactions) provided support for the L-allele as the risk allele in unfavourable family environments that was significant at the $p<.05$ level.[5] When the study by Fergusson and colleagues (2011)[6] — which tested 240 interactions (59.70% of the total number of interactions under consideration, all null results) and hence was considered to be an extreme outlier — was removed from analyses, significant interactions comprised 27.16%

[5] Several studies reported a significant two-way interaction involving the serotonin transporter gene and an aspect of the family environment but did not complete sufficient post-hoc analyses to clearly identify the nature of the interaction (Petersen et al., 2012) (Sjoberg et al., 2006) or they failed to report the findings of two-way interactions when testing three-way interactions between the serotonin transporter gene, an environmental factor and a third variable (Brummett et al., 2008; Hammen et al., 2010). There were a total of 15 interactions of this nature.

[6] Fergusson et al., (2011) tested a number of permutations of interactions involving two environmental variables (punitive parenting and change/loss of parents) and the serotonin transporter gene predicting depressive symptoms and case level depression at ages 18, 21, 25 and 30. The possibility of an additive or multiplicative interaction was tested for, and genotype was analysed as three groups (SS versus SL versus LL), as well as in an S-allele dominant form (SS and SL versus LL) and in an S-allele recessive form (SS versus SL and LL). Additional analyses involved including or excluding respondents of New Zealand Maori/Pacific Island ethnicity, and assessing the effects of additional covariate factors, including gender and measures of prior mental health problems on findings. Analyses were also conducted using a 'significant other' report of depressive symptoms instead of self-report. Finally the cohort was stratified by gender to examine gene-environment interactions separately for males and females.

of the remaining 162 interactions tested. The positive and opposite interactions formed 14.20% and 10.49% of this total. In contrast to the previous analysis which record findings summarised at the level of the study, these figures recorded at the level of the interaction arguably do not implicate the S-allele as a risk allele at a greater frequency than the L-allele. In fact, this method of analysis particularly highlights the large number of null findings obtained by studies.

An exploration of variation in interaction findings

A key aim of this review is to explore possible differences between the studies that identified the hypothesised interaction between the S-allele and family environment predicting depression versus those studies which did not identify this association. Given the number of included studies is relatively small, descriptive statistics have been used to quantitively summarise particular features of these studies in order to identify any potentially fruitful avenues that appear to warrant further exploration. Traditional statistical procedures that are typically used to inform comparisons have not been conducted given their reliance on larger sample sizes. Any interpretations of potential trends that might be visible in comparisons need to be interpreted with significant caution.

Factors Related to Study Design

Number of analyses

As highlighted in the previous section, there are a large number of ways in which analyses might be conducted; alternative outcomes, environmental variables and genetic models can be tested, different covariates can be included and the presence of the original finding can be investigated in various subsamples. Certainly, concern has been raised about the issue of multiple testing combined with bias towards presenting only significant results

that conform to the paradigm of the S-allele acting as a risk factor in adverse environments in manuscripts as well as publishing only these findings. These issues have led some to deem this interaction as "unrealistically positive" (Duncan & Keller, 2011, p. 1047).

The median number of interactions related to the family environment that were tested by studies was 5.50, whilst the mean number of interactions tested was 18.27 interactions (range 2-240). When the study by Fergusson and colleagues (2011) was removed from analyses, the median and mean reduced to 5.00 and 8.28 interactions respectively (range 2-30).

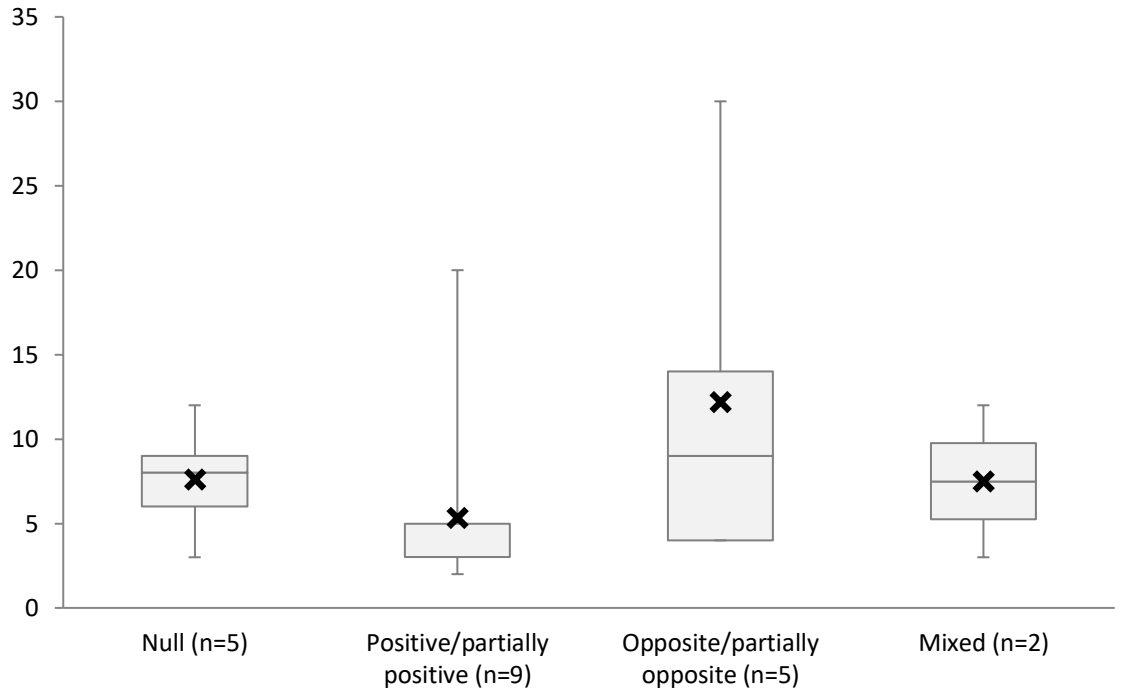

Figure 3-2. *Box plots of the number of tested interactions for four classifications of studies examining serotonin transporter gene x family environment interaction research.*
Null findings did not identify any significant interactions. Positive/partially positive studies identified at least one significant finding ($p<0.05$) that implicated the S-allele as the risk allele in adverse family environments. Opposite/partially opposite studies identified at least one significant finding ($p<0.05$) that implicated the L-allele as the risk allele in adverse family environments. Mixed studies implicated both alleles as the risk allele in adverse family environments. Boxes are first and third quartiles separated by the median value; black lines represent whiskers (maximum and minimum values); black crosses represent the mean values.

Figure 3-2 displays a box plot that shows the minimum, first quartile, median, third quartile and maximum, as well as the mean of the number of interactions tested by the groups of studies with different findings. The study by Fergusson et al. (2011) was excluded from this analysis given its outlier status. This graph shows that the median number of interactions tested by studies with at least one positive result was 3.00 (M=5.33, range 2-20), whilst the median number of interactions tested by studies with at least one opposite result was 9.00 (M= 12.20, range 4-30). The median number of interactions tested by studies that obtained null findings or mixed findings was 8 (M=7.26, range 3-12) and 7.50 (M= 7.50, range=3-12) respectively. A noted previously, the number of studies under consideration is small, and it is therefore not possible to statistically determine whether there are significant differences in these statistics, however inspection of the graph does suggest that studies reporting positive findings identifying the S-allele as the risk allele may be conducting fewer tests of interactions compared to studies that do not provide clear support for this hypothesis. There are a number of potential interpretations of this analysis. One possibility is that a confirmation bias for positive findings identifying the S-allele as a risk allele may exist; researchers may have "cherry picked" their findings somewhat, such that they are reporting fewer analyses in their papers than were conducted overall, with a tendency to include analyses that produced outcomes aligning with the broader literature suggesting an interaction between the 5-HTTLPR S-allele and stress. Alternatively, it is possible that the higher number of analyses conducted by studies identifying the L-allele as a risk allele reflects a publication bias, such that reviewers are more sceptical of opposite findings and are therefore more reluctant to publish them. They may therefore request a

greater number of extra analyses to demonstrate the robustness of these results before agreeing to accept the manuscript.

Sample size

It has also been argued that in the absence of publication bias, and when hypotheses being tested are true, studies with positive findings might be expected to have larger sample sizes than studies with null or opposite findings, because (holding effect size constant) larger sample sizes should afford greater statistical power (Duncan & Keller, 2011). Conversely, in the presence of publication bias, one might expect studies with null or opposite findings to be based upon larger sample sizes; much larger sample sizes are needed for such findings to be deemed publishable. There is likely to be less concern about sample size amongst positive studies, hence smaller studies are likely to be preferentially published when they yield positive results. Indeed, the phenomenon of smaller samples of studies with positive results relative to those with null or opposite results was noted in the meta-analysis by Sharpley and colleagues (Sharpley et al., 2014). In the current set of studies under consideration, the average sample size of those that obtained only null results was 1346.83 participants (*SD*= 1561.65), which was approximately three times the average sample size of studies that identified an S-allele association (M=478.14, *SD*=297.94). The average sample size of studies that identified an L-allele association (M=492.5, *SD*= 336.244) appeared similar to the sample size of studies that identified an S-allele association. Given the small number of studies under consideration, it was not possible to determine whether any differences in sample size were significantly different.

Measurement of primary variables of interest

Family environment

Importantly, as noted by Moffitt and Caspi (2014), the finding that positive findings appear to be identified more commonly by smaller studies does not by itself constitute evidence of this publication bias. They have rather suggested that systematic differences in quality between smaller and larger studies may also account for differences in findings. For example, Caspi et al. (2010) and Lotrich and Lenze (2009) argued that smaller studies tend to use prospective measures of higher quality that result in increased power to detect significant findings, whereas larger studies tend to use retrospective reports of lower quality that compromises power. This issue has been discussed with particular reference to studies that have looked at interactions between 5-HTTLPR x adverse/stressful life events, where the influence of increasing *numbers* of stressful events are considered. Here, larger studies have been particularly criticized for their use of brief self-report questionnaires, which are cost-effective but more vulnerable to recall bias and interpretation problems, and associated with both over-reporting adverse events by including relatively inconsequential occurrences, and under-reporting more critical or serious events (Monroe, 2008). Smaller studies are more likely to employ face-to-face interviews, which have superior reliability and validity but are more expensive, which has often required them to limit their sample size (Dohrenwend, 2006; Uher & McGuffin, 2010). Caspi and colleagues (2010) have argued that the vast majority of non-replications have utilized brief self-report measures of stress, whereas studies that have relied on objective indicators or face-to-face interviews to assess stressful events have obtained positive findings. Interestingly, method of stress assessment (objective, self-report questionnaire, interviewer), on its own was not found to be associated with the nature of study finding (i.e., positive or non-conforming) in Sharpley

and colleagues' (2014) meta-analysis, but it did interact with method of depression assessment (self-report depression scale, clinical interview) to predict whether a study obtained a positive or non-conforming finding. Specifically, when stress was assessed via objective or interview methods, greater support for the S-allele was obtained by studies that assessed depression according to structured clinical interview. In contrast, when stress was measured via self-report questionnaire, there was greater support for the S-allele by studies that also measured depression via self-report. This interaction arguably points not only to the more reliable detection of findings amongst studies assessing the environment and the outcome of depression according to 'gold-standard' methods, but also the contribution of shared method variance to the potential significance of findings.

The extent to which such measurement issues may affect studies looking at *adverse family environments* rather than counts of past stressful life events in predicting depression is unclear. Certainly, some aspects of family environment may be better assessed using more objective measures. Parenting behaviours, for example, are thought to be more optimally captured by observational paradigms than by questionnaire measures (Sheeber, Hops, & Davis, 2001).

In the current analysis, fifteen of the studies assessed aspects of adverse family environments solely by questionnaire, whilst five studies relied solely on interview methods. Two of studies used a composite measure of family relationship stress that drew on both questionnaires and interview, and two studies used observational methods to measure parenting and questionnaire measures to capture other aspects of family environment. Studies that relied solely on questionnaire measures contained approximately double the number of participants (M=818.38, SD=1141.13) than studies that drew on

interview or observational methods (*M*=437.11, *SD*=310.19). Fergusson and colleagues (2011), which used an observational measure and a questionnaire measure, was excluded from the following analyses because it contained a much larger number of permutations of the interactions under consideration than were completed by other studies, and hence could obscure relationships between the method of stress measure and study outcome. Of the 124 interactions that were based on questionnaire, 93 (75.00%) obtained null findings, and 24 (19.35%) obtained significant findings (12 positive and 12 opposite). Findings for the remaining seven interactions (5.65%) were not reported. Of the 36 interactions that drew on interview measures, 12 (33.33%) obtained null findings, and 20 (55.56%) obtained significant findings (11 positive, 5 opposite, direction of 4 not reported). Findings of four interactions (11.11%) were not reported. The two interactions that were based on observational measures both identified null findings (100%). These figures do suggest the possibility that significant findings could be identified more readily by interview approaches compared to questionnaire measures. Given the limited number of studies that drew on observational measures, it is not possible to reach any conclusions about their capacity to detect significant results.

Measurement of Depression

Seventeen studies examined depression as an outcome according to questionnaire measure, and four studies examined depression as an outcome according to diagnostic interview (with two studies looking at multiple depressive outcomes using both questionnaire and diagnostic interviews). One study based their outcome measure of case-level depression on either a current diagnosis of MDD based on clinical interview, or

endorsement of symptoms on a questionnaire measure above the clinical cut off, or prescription of antidepressants (Ritchie et al., 2009), and was therefore excluded from the following analyses. The study by Fergusson and colleagues (2011) was also excluded. Of the 146 interactions that were based on questionnaire, 101 (69.18%) obtained null findings, and 36 (24.66%) obtained significant findings (21 positive, 11 opposite, direction of 4 not reported). The significance values of nine interactions were not reported. Of the 7 interactions that drew on interview measures, 1 (14.29%) obtained a null finding, and 4 (57.14%) obtained significant findings (2 positive, 2 opposite). The significance values for two interactions were not reported. This analysis suggests the possibility that that interview approaches may perform better than questionnaire measures in terms of the percentage of significant interactions.

Content of environment measures

Previous reviews have noted that studies of specific stressors, such as child maltreatment or medical illness, yield positive findings more consistently than studies of counts of stressful life events (Caspi et al., 2010; Uher & McGuffin, 2010). Caspi et al. (2010) have suggested that considering specific, homogeneous, developmentally relevant, and clearly operationalized depression-inducing events improves the validity of the study design by reducing between-subject heterogeneity in the exposure. In the current analysis, it therefore seemed important to consider homogeneity amongst the measures to identify whether capacity to detect significant interactions was improved amongst studies with a narrower environmental focus or whether significant interactions implicating a particular allele could be more common for a specific aspect of the family environment. Inspection of the different measures of environment suggested possible grouping of these measures into

six different categories: (1) broad family stress or adversity, (2) family relationships and emotional climate of the home environment, (3) socioeconomic status, (4) changes to family structure relating to parental loss or separation, (5) parental psychopathology and (6) parenting behaviours. Categorisation of these studies in these groups also allows an examination of the issues related to methodology, and in particular, the methods used to assess the family environment, which, as discussed in the previous section, has been highlighted by a number of researchers as particularly important to the detection of the interaction. Once again however, given the small numbers of studies in each category, conclusions are speculative.

Interactions with broad family stress or adversity

As shown in Table 3-4, five studies considered a total of six measures of "family stress or adversity," indexed by either the report of stressors, changes or adjustments for the family or a composite scale that was designed to capture a broad range of experiences constituting a more challenging or adverse family environment, including stressful events, problems relating to housing, finance or work (including unemployment), single-parenthood and the experience of parental psychopathology. In each of these studies, a score indicative of high stress or adversity could therefore capture a variety of different experiences, and between-subject heterogeneity was presumably quite high. Significant interactions were documented by four of the studies, with the S-allele was identified as the risk allele in (Eley et al., 2004; but only in girls) and (Petersen et al., 2012) and the L-allele as the risk allele in Chipman et al. (2007) and Laucht et al. (2009). Thus, whilst the majority of studies examining interactions involving family adversity or stress identified at

least one significant result, there was not any clear indication to support one allele as associated with greater vulnerability to depression in more adverse family circumstances. There were also no clearly discernible differences in the content of these measures that appeared related to a positive, opposite or null result. Interestingly however, the only study that failed to identify an interactive effect involving broad family stress was based on a sample of much younger children, aged four years (Lavigne et al., 2013). All of the studies that reported a significant finding (positive or opposite) were conducted with adolescents.

Table 3-4. *Interactions testing between 5-HTTLPR and family stress or adversity*

Study	Measure	Findings
Chipman et al. (2007)	*Family stress:* 2 or more events with negative impact on the family (study devised item asking participants to list changes, losses or problems in last 12 months, without prompts, and rate their effect).	Null for depression at 15-16 years and 17-18 years
	Composite measure of persistent family adversity: Study devised index consisting of 6 items (unemployed father, father in unskilled occupation, many family moves, large family size, non-intact family and high levels of family stress in last 12 months). Required two or more items to be endorsed at 2 or more timepoints.	Null for depression at 15-16 years, opposite for depression at 17-18 years
Eley et al. (2004)	*Composite measure of family adversity:* Social problems/pressures encountered by family (Social Problems Questionnaire (Corney, 1988) which covers housing, occupation, finance, social and leisure activities, child/parent and martial relationships, relationships with relatives, friends, neighbours and workmates, and legal problems), low parental education (study devised item), adverse family events (List of Threatening Events (Brugha & Cragg, 1990) which covers events related to the family as a whole, including serious illness, bereavement, relationship breakdown, unemployment and financial crisis) .	Null in overall sample and in boys, positive for girls
Laucht et al. (2009)	*Composite measure of family adversity*: Standardised interview based on enriched index proposed by Rutter and Quinton (1977) probing presence or absence of 11 adverse family factors (low educational level of a parent, overcrowding in the home, parental psychiatric disorder, history of parental broken home or delinquency, marital discord, early parenthood, one parent family, unwanted pregnancy, poor social integration and support of parent, severe chronic difficulties, poor coping skills of a parent)	Opposite for diagnosis of depression or anxiety, and for depressive symptoms[a]
Lavigne et al. (2013)	*Composite measure of parent and family stress:* Perceived Stress Scale (Cohen, Kamarck, & Mermelstein, 1983), the total stress score of the Parenting Stress Index Short Form (Abidin, 1995), the McCubbin Family Changes & Strains Scale (McCubbin, Thompson, & McCubbin, 1996)	Null for single-interaction analysis and multiple-interaction analysis which controlled for six other interaction terms

Petersen et al. (2012)	*Family stress:* Changes and Adjustments Questionnaire (Dodge, Pettit, & Bates, 1994) - 18 stressful life events over the last 12 months that might have described experiences of the family, such as death of a close relative, divorce of the child's parents, or financial problems	Positive for outcome of accelerated growth of anxious/depressed symptoms at age 16-17, null for outcomes of initial symptoms at age 12, initial symptom growth at age 13, and accelerated growth of symptoms at age 14-15[b]
Sjoberg et al. (2006)	*Family stress:* Derived from dichotomous psychosocial variables (fathers' and mothers' education, parental occupation, family economy, quality of family relationships and conflicts within the family) based on interviewer rating according to study-derived unstructured interview, which were then merged into an index and dichotomized again into psychosocial risk (no and one risk/ two or more risks)	Interaction significant in overall sample but findings of post-hoc analyses not reported. Positive in girls, opposite in boys.

[a] interactions predicting both outcomes were significant when 5-HTTLPR was analysed according to the traditional LS classification but only the interaction predicting diagnosis of depression or anxiety was significant when 5-HTTLPR was analysed as follows : LGS, LGLG, and SS were designated as S'S', LAS and LALG as L'S', and LALA as L'L'; [b] comparable findings when analyses repeated for males and females, European Americans, and traditional VNTR coding of 5-HTTLPR.

Interactions with family emotional climate

As can be seen from Table 3-5, ten studies considered a total of 12 measures of family emotional climate, indexed by either the report of family relationship stress, family support, family conflict or the broader family milieu including the level of positive and negative emotion expressed by parents, care directed to the child and the level of organisation at home. Overall, eight studies identified at least one significant interaction based on six different indices. Findings implicating the S-allele as the risk allele for increased depression were perhaps particularly prevalent, identified in six studies (Hammen et al., 2010; Jenness et al., 2011; Li et al., 2013; Sjoberg et al., 2006; Taylor et al., 2006; Vrshek-Schallhorn et al., 2014), whilst the L-allele was clearly implicated as the risk allele in two studies (Lavigne et al., 2013; Ritchie et al., 2009). Two studies failed to identify any significant findings pertaining to the serotonin transporter gene, family emotional climate and depression (Conway et al., 2010; Sen et al., 2010).

Positive interactions implicating the S-allele were also perhaps more consistently identified by studies with a longitudinal design that utilised interview measures examining family relationship stress (the GEM study in Jenness et al. (2011), the Youth Emotion Project in Vrshek-Schallhorn et al. (2014) and the Mater-University Study of Pregnancy in Hammen et al., (2010) in girls but not in boys; see also the null finding in the overall sample by Conway et al. (2010) based on the same group of participants). Interestingly, method descriptions indicate the youth version of the UCLA Chronic Stress Interview was used with all three of these independent samples. Taken together, these studies suggest that this measure can detect interactions implicating the 5-HTTLPR S-allele and family relationship stress with some reliability.

There was also some direct overlap in a broader measure of the family milieu; early adverse family environment was assessed retrospectively in two independent samples by The Risky Family Questionnaire (Taylor, Lerner, Sage, Lehman, & Seeman, 2004). The cross-sectional study by Taylor et al. (2006), which was based on participants who were affiliated with UCLA as either employees or students, identified a significant interaction implicating the S-allele as increasing vulnerability to depressive symptoms in adulthood in the context of a more adverse family environment. Whilst this particular measure does not directly measure trauma or neglect, it does assess aspects of family environment involving a higher level of threat and deprivation than perhaps the other measures considered in this review. However, the authors identified the significant finding as particularly noteworthy given that the degree of adversity in this particular sample was fairly mild, consisting of some conflict, moderate household chaos, and/or cold, unaffectionate and distant parental behaviours (and no physical or sexual abuse). This finding was in contrast to the null finding of the longitudinal prospective study by Sen et al. (2010) based on a sample of medical interns. Though no information was provided about the level of early family adversity experienced by this sample, given that participants were also university educated, a relatively similar low level of family adversity to the sample based on UCLA students and employees seems plausible. Whilst the UCLA-based sample in the study by Taylor and colleagues (2006) comprised participants from a range of degrees and positions, the sample in the study by Sen and colleagues (2010) was composed entirely of participants in the medical field, a profession associated with particularly high rates of depression (Schneider & Phillips, 1993). Given that the outcome measure was a change in depressive symptoms following commencement of medical internship, an interaction between early family

environment and 5-HTTLPR may not have been a particularly salient predictor of this particular depression outcome in this sample, which was likely to be more strongly accounted for by the more proximal stressors encountered during the medical training year. These two studies perhaps provide a concrete example of the limited conclusions that may be drawn about the reliability of the finding, or the reliability of the measure in detecting the interaction, when comparing findings across different studies with different depression outcomes, methodologies and samples.

Findings were also somewhat mixed when the level of family conflict was directly assessed, rather than family relationships or the broader milieu; one community-based study of adolescents obtained a seemingly positive interaction (based on a graph of the interaction) when family conflict was assessed as present or absent according to responses on an unstructured, study-derived interview (Sjoberg et al., 2006). The findings of this particular study are somewhat challenging to evaluate however because only the overall significance value was provided, and more detailed post-hoc analyses regarding the significance of the individual relationships between the environment and depression for the different genotypes do not appear to have been conducted. An opposite finding implicating the L-allele was noted by a community-based study of young children (Lavigne et al., 2013), which assessed family conflict on a continuum according to a composite of questionnaire measures. The finding by Lavigne et al. (2013) however became non-significant following the inclusion of additional aspects of the family environment (socioeconomic status, life stress, caretaker depression, parental support, hostility, and scaffolding skills) and their interactions with 5-HTTLPR, raising the possibility that the significant family conflict interaction in fact reflected an association that this variable has

with another variable. Alternatively, the study may not have had appropriate power to detect a significant interaction whilst controlling for the main effects of six other environmental factors and each of their interactions with the serotonin transporter gene in a relatively small sample of 175 participants.

An opposite finding was also recorded by the study by Ritchie et al. (2009) that assessed excessive sharing of parental problems according to a single item with a 'yes/no' response. No information about the type of information that was shared by the parent or its effect on the participant was available, and no other studies appear to have measured an interaction with a similar construct. Excessive sharing of information might however point to an expectation within the family for the child to provide care and nurturance to the parent, rather than the parent assuming this role for the child; this item may therefore indicate a level of deprivation within the family environment. Two null findings were also identified by this study, relating to participants' perceptions that they 'had a happy childhood,' and their impression that their 'parents did their best.' These constructs were also assessed by single items with a 'yes/no' response, and lacked specificity about the type of family environment experienced that might typically lead a participant to endorse these items. The study provided no information about the validity and reliability of these items, including the extent to which these single items can adequately discriminate between individuals with different family experiences according to more objective measures. Limited conclusions about the nature of these findings are therefore possible.

Table 3-5. *Studies testing interactions between 5-HTTLPR and family emotional climate*

Study	Measure	Findings
Conway et al. (2010)	Composite measure of family relationship stress based on 11 self-report and interview measures: parent and youth versions of UCLA Chronic Stress Interview (Hammen et al., 1987), covering quality of mother's marital/romantic relationship, parent (mother) relationship with the youth, and the youth's relationship with immediate family members; the Satisfaction subscale of the Dyadic Adjustment Scale (Spanier, 1976) assessing overall marital relationship quality; the Modified Conflict Tactics Scale (Neidig & Friedman, 1984) assessing psychological and physical coercion in conflict; and the psychological control versus psychological autonomy, and the perception of acceptance versus rejection subscales of the Children's Report of Parental Behaviour Inventory (Schludermann & Schludermann, 1988) assessing quality of parent-child interactions.	Null for depressive symptoms and for depression diagnosis
Hammen et al. (2010)	Composite measure of family relationship stress based on 11 self-report and interview measures: parent and youth versions of UCLA Chronic Stress Interview (Hammen et al., 1987), covering quality of mother's marital/romantic relationship, mother's relationship with the youth, and the youth's relationship with immediate family members; the Satisfaction subscale of the Dyadic Adjustment Scale (Spanier, 1976) assessing overall marital relationship quality; the Modified Conflict Tactics Scale (Neidig & Friedman, 1984) assessing psychological and physical coercion in conflict; and the psychological control versus psychological autonomy, and the perception of acceptance versus rejection subscales of the Children's Report of Parental Behaviour Inventory (Schludermann & Schludermann, 1988) assessing quality of parent-child interactions.	Positive in girls, null in boys
Jenness et al. (2011)	Family relationship stress: Youth version of the Parent–child and household domains from the UCLA Chronic Stress Interview (Hammen et al., 1987), assessing the quality of the relationship between youth and parent figures and others in the household (e.g., siblings, grandparents).	Positive
Lavigne et al. (2013)	Composite measure of family conflict: The McCubbin Family Distress Index (McCubbin et al., 1996) which assesses both family stressors (e.g. substance use, divorce, emotional problems) and challenges which reflect family disharmony and family intolerance (e.g. conflict between children in the family, or with extended relatives or in-laws); the Family Environment Scale conflict scale (Moos & Moos, 1994), which measures the extent to which the family environment is characterised by conflictual interactions and open expressions of anger and aggression; and the McCubbin Family Problem Solving/Communication Scales (McCubbin et al., 1996) which assesses the degree to which affirming or incendiary communication strategies are used by families to manage and solve problems and conflicts in various types of stressful situations. All parent report.	Opposite for single-interaction analysis, null for multiple-interaction analysis

Li et al. (2013)	Youth's perceived family support measured by thirteen items assessing aspects that included parental closeness, quality of communication, connectedness, and feeling loved and wanted by family members, rated on an ordinal scale during an in-home structured interview at three time points (Borowsky, Ireland, & Resnick, 2001; Resnick et al., 1997).	Positive in boys for an additive and dominant but not recessive genetic model, null in boys (though non-significant opposite pattern of interaction present at trend for dominant genetic model)
Ritchie et al. (2009)	Single, study derived item measuring participant endorsement of 'excessive sharing of parental problems' (yes/no response)	Opposite
	Single, study-derived item measuring participant's retrospective endorsement of 'happy childhood' (yes/no response)	Null
	Single, study derived item measuring participant retrospective endorsement of 'impression parents' did their best' (yes/no response).	Null
Sen et al. (2010)	Adverse family environment: Risky Families Questionnaire (Taylor et al 2004) is based on participant report and was adapted from an instrument originally developed by Felitti et al (1998) measuring parental warmth and support, parental physical aggression and verbal hostility, violent arguments in the home, level of organisation, parental substance use, neglectful care.	Null
Sjoberg et al. (2006)	Conflicts within the family:Yes/no classification based on interviewer rating according to study-derived unstructured interview	Positive overall and for girls, null for boys
Taylor et al. (2006)	Adverse family environment: Risky Families Questionnaire (Taylor et al 2004) was adapted from an instrument originally developed by Felitti et al (1998)) measuring parental warmth and support, parental physical aggression and verbal hostility, violent arguments in the home, level of organisation, parental substance use, neglectful care.[d]	Positive
Vrshek-Schallhorn et al. (2014)	Chronic family relationship stress: Youth version of UCLA Chronic Stress Interview (Hammen et al., 1987) – domains not clearly specified[c]	Positive

[c] Likely to be the Parent-child and Household domains based on description in method

[d] Manuscript notes that the degree of adversity was fairly mild, consisting of some conflict, moderate household chaos, and/or cold, unaffectionate and distant behaviours. No instance of physical or sexual abuse was identified.

Interactions with socio-economic status

Table 3-6 shows that six studies based on five independent samples considered the possibility that serotonin transporter genotype might interact with socio-economic status. Results from each of the five samples provided some indication that L-homozygous individuals were more vulnerable to the depressogenic effects of socio-economic disadvantage, though most found this effect to be present in only a subset of participants or under certain conditions. Nobile et al. (2014) found the interaction was evident in only late (not early) adolescence and both Sjoberg et al. (2006) and Brummett et al. (2008) detected this interaction in boys. The interaction obtained by Sjoberg et al. (2006) also suggested the possibility that S-homozygous boys might be protected against depression in socio-economically advantaged homes. Brummett et al. (2008) identified that S-carrier girls from low socioeconomic backgrounds were at greater risk of depression. Lavigne et al. (2013) observed this L-allele effect was only significant when other family environment factors and their interactions with the serotonin transporter gene were not taken into account.

Table 3-6. *Studies testing interactions between 5-HTTLPR and socio-economic status*

Study	Measure	Finding
Brummett et al. (2008)	Father's educational level coded in years	Null overall, positive in girls, opposite in boys
Lavigne et al. (2013)	Education and employment coded into the Hollingshead Four-Factor Index of Social Status (Hollingshead, 1975)	Opposite in single-interaction analysis, null in multiple-interaction analysis
Nobile et al. (2009)	Parental employment coded by Hollingshead's 9 point scale for parental occupation (Hollingshead, 1975)	Null at 10-14 years
Nobile et al. (2014)	Parental employment coded by Hollingshead's 9 point scale for parental occupation (Hollingshead, 1975), dichotomised into a low SES 1–3 group and a medium to high SES 4–9 group.	Null for anxious depressed symptoms at 10-14 years, opposite for anxious/depressed symptoms at 15-19 years, null for withdrawn/depressed symptoms and overall internalising problems at both 10-14 years and 15-19 years.
Ritchie et al. (2009)	Single, study-derived item measuring participant's retrospective endorsement of 'poverty or financial difficulties' (yes/no response)	Opposite
Sjoberg et al. (2006)	Type of residence: Classified as 'owned own home' or 'multi-family home' based on interviewer rating according to study-derived unstructured interview	Opposite in boys, null overall and in girls

Interactions involving family structure

As can be seen from Table 3-7, five studies based on four independent samples considered a total of four different measures of family structure, indexed by either the report of parental marriage status, a change in primary caregiver for a child, or of a parental loss or separation. Findings were somewhat inconsistent regarding the presence of an interaction between the serotonin transporter gene and family structure. Both Fandino-Losada et al. (2013) and Fergusson et al. (2011) recorded null results, and did not find evidence for a moderating influence of gender. Sjoberg et al. (2006) also identified a null result in their sample of boys and girls together, but an opposite finding implicating L-homozygous boys (but not girls) with separated parents as more vulnerable to depression than those from nuclear families. Nobile et al. (2009) found evidence that early adolescents between the ages of 10-14 years from single-parent families who carried at least one copy of an S-allele reported significantly higher scores on the subscale of *Affective Problems* on the Child Behaviour Checklist (which has been found to correspond closely to DSM-IV Major Depressive Disorder and Dysthymia in referred children and adolescents; Ferdinand, 2008), compared to S-carriers and L-homozygous individuals from two parent families, but not L-homozygous individuals from single-parent families. The finding became non-significant (albeit present at trend level) however when SES was included as a covariate. In a follow up investigation, the authors tested this interaction with slightly different outcomes (the subscales of *Internalising problems, Anxious/Depressed* behaviours and *Withdrawn/Depressed* behaviours from the CBCL, which share a number of the same items) in approximately half of these participants who had gone on participate

longitudinally in the study (Nobile et al., 2014). Controlling for SES, they found significant effects of family structure in the S-carrier group but not the L-homozygous group at 10-14 years, but the difference between these associations turned out not to be significant. There was no indication of an interaction predicting outcomes at 15-19 years old.

Table 3-7. *Studies testing 5-HTTLPR x family structure (parental loss or separation)*

Study	Measure	Finding
Fandino-Losada et al. (2013)	Parental loss/separation (two study-derived items regarding the death of a parent or parental divorce/separation prior to 18 years old)	Null
Fergusson et al. (2011)	Change in parents (study-derived measure of change in family situation as part of annual parental assessments to age 16 years)	Null
Nobile et al. (2009)	Family structure (study-derived item regarding parental marital status – one parent home = 'divorced,' separated,' 'widowed,' and 'single parent,' and two-parent home = 'married' and 'cohabitating.'	Positive when SES not included in analyses as covariate
Nobile et al. (2014)	Family structure (study-derived item regarding parental marital status – one parent home = 'divorced,' separated,' 'widowed,' and 'single parent,' and two-parent home = 'married' and 'cohabitating.'	Null (though non-significant positive pattern of interaction present at 10-14 years).
Sjoberg et al. (2006)	Family constellation (nuclear versus separated)	Opposite in boys, null in overall sample and in girls

Table *3-8* shows that five studies have tested interactions between the serotonin transporter gene and parent psychopathology. Four of these studies focused specifically on interactions with parental depressive symptoms, according to self-report questionnaires. Findings of these four studies were somewhat inconsistent. Oppenheimer et al. (2013) recorded a significant interaction implicating the S-allele amongst early adolescents who experienced idiopathic increases in maternal depression (increased symptoms relative to their average or 'usual' level of symptoms). Lavigne et al. (2013) documented increased depressive

symptoms in young children homozygous for the L-allele relative to children carrying at least one copy of an S-allele, including when covariates of family conflict, socioeconomic status, life stress, parental support, hostility, and scaffolding skills, and their interactions with 5-HTTLPR, were added to the analysis. Two studies reported null findings; neither Van Roekel et al. (2011) or Araya et al. (2009) found evidence of a significant interaction implicating parental depression. Given these mixed findings, conclusions about the presence or direction of an interaction effect involving parental depression are difficult.

The remaining study by Ritchie et al. (2009) which considered parental mental health more broadly also did not identify a significant interaction. Significant methodological concerns, particularly around the retrospective measurement of parental psychopathology based on offspring report on a single item with unknown validity, arguably limits any conclusions that can be drawn about the veracity of this finding.

Table 3-8. *Studies testing 5-HTTLPR x parental psychopathology*

Study	Measure	Finding
Araya et al. (2009)	Maternal post-natal depressive symptoms: Self-rated Edinburgh Postnatal Depression Scale (EPDS; Cox, Holden, & Sagovsky, 1987)	Null
Lavigne et al. (2013)	Composite measure of caretaker depression: The Center for Epidemiological Studies–Depression Scale (Radloff, 1977), The Beck Depression Inventory (BDI; Beck, Ward, Mendelson, Mock, & Erbaugh, 1961)	Opposite
Oppenheimer et al. (2013)	Change in maternal depressive symptoms: Beck Depression Inventory (BDI; Beck et al., 1961) every 3 months over a 1-year period (five waves of data)	
	- Idiopathic changes (severity related to mother's average level of symptoms)	Positive
	- Normative changes (severity of symptoms compared to the sample as a whole)	Null
Ritchie et al. (2009)	Single, study-derived item measuring participant retrospective endorsement of 'father experienced mental problems' (yes/no response).	Null
	Single, study-derived item measuring participant retrospective endorsement of 'mother experienced mental problems' (yes/no response).	Null
Van Roekel et al. (2011)	Maternal depressive symptoms: Depressive Symptom Scale (DSS; Kandel & Davies, 1982)	Null
	Paternal depression: Depressive Symptom Scale (DSS; Kandel & Davies, 1982)	Null

Interactions with parenting behaviour

Table 3-9 shows that three studies considered the role that various parenting behaviours might play in interaction with the serotonin transporter gene in predicting depression. The possibility that the serotonin transporter gene might interact with hostile or punitive parenting (observational measure; Fergusson et al., 2011; parent-report questionnaire; Lavigne et al., 2013), reduced parental support/engagement (parent-report questionnaire; Lavigne et al., 2013; self-report questionnaire; Van Roekel et al., 2011) or parental scaffolding (observational measure; Lavigne et al., 2013) were considered. None

these studies found evidence that parenting behaviour interacted with the serotonin transporter gene to influence depression.

Table 3-9. *Studies measuring parenting behaviours*

Study	Measure	Findings
Fergusson et al. (2011)	Punitive parenting behaviour: Interviewer observations of mother–child interactions when the child was 3-5 years old, obtained using the Avoidance of Restriction and Punishment subscale of the HOME Inventory (Bradley & Caldwell, 1977) – based on yes/no ratings of items, (e.g., primary caregiver does not shout at child during visit; primary caregiver does not express overt annoyance with or hostility about the child).	Null
Lavigne et al. (2013)	Parental support/engagement: Factor-analytically derived subscale of parental support/engagement from The Parent Behaviour Inventory (Lovejoy, Weis, O'Hare, & Rubin, 1999) – parent report.	Null
	Parental hostility: Factor-analytically derived subscale of Hostility from The Parent Behaviour Inventory (Lovejoy et al., 1999) – parent report.	Null
	Composite measure of parental support/scaffolding: Sum of ratings from interviewer observations of mother-child interactions during the Three Boxes task (National Institute of Child Health and Human Development (NICHHD) Early Childhood Research Network (Network, 1999) on 7-point Likert scales of caretaker supportive presence, quality of assistance, cognitive stimulation, respect for autonomy, caretaker confidence, and hostility (reverse-coded).	Null
Van Roekel et al. (2011)	Perceived paternal support: 12-item version of the Relational Support Inventory (Scholte, Van Lieshout, & Van Aken, 2001) completed by adolescent, tapping several aspects of emotional support (e.g., 'This person shows me that he/she loves me') and instrumental support (e.g., 'This person explains or shows how I can make or do something').	Null
	Perceived maternal support: 12-item version of the Relational Support Inventory (Scholte et al., 2001), completed by adolescent, tapping several aspects of emotional support (e.g., 'This person shows me that he/she loves me') and instrumental support (e.g., 'This person explains or shows how I can make or do something')	Null

Coding of the serotonin transporter gene

Seventeen of the 22 studies sequenced the traditional biallelic variant of the serotonin transporter gene based on the VNTR polymorphism, which results in three possible genotypes of SS, SL or LL. Ten of the 22 studies sequenced the triallelic variant based on the rs25531 single-nucleotide polymorphism (where a base change from the A to a G reportedly renders the L allele functionally equivalent to an S-allele), which results in six possible genotypes of L_GS, L_GL_G, SS, L_AS, L_AL_G or L_AL_A. The findings by Fergusson et al. (2011) as well as findings of interactions that were not adequately described were not included in the following analyses.

Of the 93 interactions that were based on the biallelic VNTR polymorphism and had clearly reported findings, 62 (66.67%) were null, whilst 31 (33.33%) were significant (16 positive, 2 opposite). Of the 54 interactions that were based on the triallelic rs25531 single-nucleotide polymorphism and had clearly reported findings, 45 (66.67%) were non-significant, whilst 9 (16.66%) obtained significant findings (7 positive, 2 opposite). Whilst it is difficult to make any clear conclusions in the absence of statistical tests, it does seem that opposite findings are predominantly reported by studies based on the VNTR polymorphism.

Five studies completed analyses with both VNTR and rs25531 genotype coding. All of the studies identified the findings as generally comparable, though Laucht et al. (2009) recorded consistent findings implicating the L-allele for the outcome of depression/anxiety diagnosis only, and not for depressive symptoms, where a significant result was recorded with VNTR coding but not rs25531 coding. Nobile et al. (2009) recorded an interaction implicating the S-allele that was significant when the gene was sequenced according as the

VNTR but was present at trend level (p=.071) when analysed as the rs25531 single-nucleotide polymorphism.

Uher and McGuffin (2010) have recommended that the serotonin transporter gene should be analysed according to an additive, S-allele dominant and S-allele recessive model, with results of all three sets of analyses reported. In practice however, particularly given limits posed on the length of manuscripts, this rarely occurs. Indeed, only Fergusson and colleagues (2011) and Li and colleagues (2013) considered all three genetic models in their analyses with the serotonin transporter gene analysed according to the traditional biallelic VNTR coding. Choice of genetic model did not appear to impact the findings of the study by Fergusson et al. (2011) which were all non-significant. Li et al. (2013) however detected significant positive findings for boys when genotype was treated additively and dominantly, but not recessively, and a marginally significant opposite finding for girls when genotype was treated additively that was not evident when genotype was treated dominantly or recessively. Ritchie et al. (2009) reanalysed significant additive 5-HTTLPR VNTR findings according to a dominant genetic model, and concluded that results were largely comparable.

Table *3-10* provides the percentage of null and significant findings of the total number of interactions conducted for each genetic model for both the VNTR and rs25531 forms of the serotonin transporter gene (with the study by Fergusson et al. (2011) excluded). The table shows that studies tended to report analyses based on additive and S-allele dominant models, and rarely reported findings for S-allele recessive models. Given the small number of interactions tested according to each genetic model, it is difficult to discern any clear patterns of findings.

Table 3-10. *Comparison of studies testing different genetic models*

	Null	S-allele significant	L-allele significant	Total interactions	Studies
5-HTTLPR VNTR					
Additive (SS vs SL vs LL)	20 (50%)	12 (30%)	8 (20%)	40	Brummett et al. (2008); Chipman et al. (2007); Eley et al. (2004); Jenness et al. (2011); Laucht et al. (2009); Li et al. (2013); Oppenheimer et al. (2013); Petersen et al. (2012); Ritchie et al. (2009); Sjoberg et al. (2006); Taylor et al. (2006)
S-allele dominant (SS; SL vs LL)	40 (78.43%)	4 (7.84%)	7 (13.73%)	51	Lavigne et al. (2013); Li et al. (2013); Nobile et al. (2014); Nobile et al. (2009); Ritchie et al. (2009); Van Roekel et al. (2011); Vrshek-Schallhorn et al. (2014)
S-allele recessive (SS vs SL; LL)	2 (100%)	0 (0%)	0 (0%)	2	Li et al. (2013)
5-HTTLPR rs25531					
Additive (L_GS; L_GL_G; SS vs L_AS; L_AL_G vs L_AL_A)	20 (71.43%)	7 (25.00%)	1 (3.57%)	28	Araya et al. (2009); Fandino-Losada et al. (2013); Hammen et al. (2010); Jenness et al. (2011); Laucht et al. (2009); Petersen et al. (2012)
S'-allele dominant (L_GS, L_GL_G, SS, L_AS, L_AL_G vs L_AL_A)	20 (95.24%)	0 (0%)	1 (4.76%)	21	Conway et al. (2010); Nobile et al. (2014); Nobile et al. (2009); Sen et al. (2010)
S'-allele recessive (L_GS; L_GL_G; SS vs L_AS; L_AL_G; L_AL_A)	0 (0%)	0 (0%)	0 (0%)	0	-

Consideration of covariates of gender and ethnicity

Gender

Given the possibility of gender effects in the broad 5-HTTLPR x stress literature (Gressier et al., 2016), the extent to which gender might influence interactions between 5-HTTLPR and the family environment was explored in the current review. All of the studies except for the one by Fergusson et al. (2011) reported on the gender composition of their samples. An average of 45.83% of participants in these studies were male (range = 30.65% to 55.53%). The gender composition between studies with different outcomes appeared somewhat similar (S-allele support =44.35% male, L-allele support=48.73% male, null results = 44.84% male, mixed results=47.71% male), however it is possible that this difference could be statistically significant.

Thirteen of the 22 studies considered whether gender might influence an interaction involving 5-HTTLPR, adverse family environments and internalising symptoms, either by (1) conducting multi-group analyses and comparing the strengths of the 2-way interaction and model fit between males and females e.g., (e.g., Petersen et al., 2012), (2) including a three-way interaction term between gender x 5-HTTLPR x family environment, where a significant interaction resulted in post-hoc testing to clarify the nature of this interaction, and a non-significant finding was interpreted as indicating that results pertaining to the two-way interaction (both significant or non-significant) were not moderated by gender (e.g., Brummett et al., 2008; Hammen et al., 2010; Jenness et al., 2011; Laucht et al., 2009), or (3) conducting stratified analyses for males and females to identify whether the interactions in each group were significant (e.g., Araya et al., 2009; Eley et al., 2004; Fandino-Losada et

al., 2013; Fergusson et al., 2011; Sjoberg et al., 2006; Taylor et al., 2006; Van Roekel et al., 2011) though this latter method cannot ascertain statistically whether findings are significantly *different* in males or females.

As shown in Table *3-11*, six of the thirteen studies identified an effect of gender in their analyses. Three of these studies documented significant effects implicating the S-allele amongst girls only (Eley et al., 2004; Hammen et al., 2010; Taylor et al., 2006), one study documented significant effects implicating the S-allele as a risk allele amongst boys only (Fandino-Losada et al., 2013) though the L-allele was implicated in girls as a risk allele at trend, whilst two studies reported the S-allele acting as a risk allele in girls and the L-allele acting as a risk allele in boys (Brummett et al., 2008; Sjoberg et al., 2006). Seven studies did not identify any effect of gender on the findings they had obtained for their overall sample; findings from four of these studies suggested non-significant interactions in both boys and girls (Araya et al., 2009; Fandino-Losada et al., 2013; Fergusson et al., 2011; Van Roekel et al., 2011), whilst findings of two studies indicated the S-allele was implicated as the risk allele in both boys and girls (Jenness et al., 2011; Petersen et al., 2012), and findings of one study suggested the L-allele was implicated as the risk allele in both boys and girls (Laucht et al., 2009). Whilst clearly there is inconsistency in the findings across studies, when findings are considered at the study level, these numbers suggest that when a significant finding is identified, it is more likely to be positive amongst females, whilst positive and opposite findings appeared to be detected at a similar frequency in males.

Table 3-11. *Summary of findings of studies exploring the role of gender.*

Study	**Method to test interaction**	**Gender effect**	**Interaction present in females (and where present, which risk-allele implicated)**	**Interaction present in males (and where present, which risk-allele implicated)**
Araya et al. (2009)	Stratified analyses	No	No	No
Brummett et al. (2008)	3-way interaction	Yes	Yes (S-allele)	Yes (L-allele)
Eley et al. (2004)	Stratified analyses	Yes	Yes (S-allele)	No
Fergusson et al. (2011)	Stratified analyses	No	No	No
Fandino-Losada et al. (2013)	Stratified analyses	No	No	No
Hammen et al. (2010)	3-way interaction	Yes	Yes	No
Jenness et al. (2011)	3-way interaction	No	Yes (S-allele)	Yes (S-allele)
Laucht et al. (2009)	3-way interaction	No	Yes (L-allele)	Yes (L-allele)
Li et al. (2013)	Stratified analyses	Yes	No (though L-allele at trend)	Yes (S-allele)
Petersen et al. (2012)	Multi-group model	No	Yes (S-allele)	Yes (S-allele)
Sjoberg et al. (2006)	Stratified analyses	Yes	Yes (S-allele)	Yes (L-allele)
Taylor et al. (2006)	Stratified analyses	Yes	Yes (S-allele)	No
Van Roekel et al. (2011)	Stratified analyses	No	No	No

The average sample size of the seven studies that found no effect of gender was 1196.29 participants (*SD*=1483.94), which was approximately three times the average sample size of the six studies that identified an influence of gender (M=368.83 *SD*=340.77). The average sample size of the nine studies that did not consider gender as part of their analyses (M=442.89 *SD*=231.82) appeared similar to the sample size of studies that identified a gender effect. Of the studies that identified a gender effect, all three of the studies that identified positive findings in girls only were based on samples with fewer than 400 participants, as were the two studies that identified positive findings in girls but opposite findings in boys, whilst the study that identified an opposite effect in boys only was based on a sample of more than 400 participants. Of the studies that failed to identify a gender effect, three were based on samples of less than 400 participants, and four were based on samples of more than 400 participants. Studies identifying significant gender effects appear to be based on smaller samples. Thus, whilst it might seem that the S-allele is identified more commonly as a risk-allele for depression for females in adverse family circumstances, some caution about this interpretation may be warranted, given the possibility of publication bias.

Ethnicity

The distribution of 5-HTTLPR genotype is known to vary substantially across racial and ethnic groups (Gelernter et al., 1999; Hu et al., 2006; Nakamura et al., 2000) and it has been suggested that differences in the effect of the serotonin transporter gene may occur as a function of race/ethnicity (Davies & Cicchetti, 2014).

Nineteen of the 22 studies in the current systematic review provided some indication of the race/ethnicity composition of their sample, which allowed a classification of studies into those with samples of >80% White/Caucasian participants (n = 12) , and those with <80% White/Caucasian participants (n=7), as was performed by van Ijzendoorn et al. (2012). All four studies that found an opposite/partially opposite finding with data available on ethnicity were based on samples of primarily White/Caucasian participants. In contrast, only three of the eight studies with data available on ethnicity that found a positive/partially positive finding were conducted with samples of primarily White/Caucasian participants. Five studies with positive findings were based on samples containing greater variation in race/ethnic background. Four of the five studies with null-findings were based on primarily Caucasian samples, and one study with null findings was based on samples with greater ethnic/race variation. These comparisons of sample composition between studies with different outcomes do not appear to support the hypothesis that findings implicating the L-allele as a risk allele in adverse environments are more common in non-Caucasian, particularly African American, samples. Moreover, studies that re-analysed their overall findings with the largest race/ethic group sample (usually Caucasian) or performed analyses examining the moderating influence of ethnicity have found these findings to be comparable (Brummett et al., 2008; Fergusson et al., 2011; Jenness et al., 2011).

3.4 Discussion

The first part of this chapter reviewed the influence that specific parenting behaviours or practices might have on the emergence of depression across childhood and adolescence. It also considered evidence that several risk factors related to the broad family context

(specifically parental psychopathology, interparental conflict and low socioeconomic status) are thought to increase vulnerability to depression during this period through, at least in part, their effects on more proximal parenting behaviours.

Not all youth who receive suboptimal parenting or who live in adverse family environments go on to experience depression however. When there is robust overall evidence that a particular environment is pathogenic but there is also findings of variation in outcomes of people exposed to that same environment, it implies that individual differences in genetic susceptibility might be at play (Caspi et al., 2010; Moffitt et al., 2005). In these instances, gene-environment research assessing whether such environments might represent plausible candidate pathogens may be warranted, particularly when there is also evidence that biological mechanisms thought to underpin the disorder are sensitive to those particular environmental experiences (Moffitt et al., 2005), as has been shown to be the case for parenting and the family environment. The second part of this chapter therefore systematically reviewed 22 papers on gene-environment interactions between the serotonin transporter gene and elements of the family environment in predicting depression. This review has suggested that a sizeable number of these studies (approximately 40%) have identified at least one positive finding implicating the S-allele as increasing vulnerability to depression in adverse family environments. However, approximately 20% of studies reported contrasting findings suggesting that the L-allele could be associated with augmented risk for depression in adverse family contexts, and approximately one-quarter (27%) of studies failed to show any significant associations between 5-HTTLPR genotype, family adversity and depression. These findings suggested that outcomes of GxE studies involving the serotonin transporter gene may be more variable when family environment

rather than broad environmental stress or child maltreatment is considered. In particular, it seems that studies identifying an increased risk associated with an L-allele were more common than has been identified by previous reviews (Sharpley et al., 2014). At one-fifth of the total papers reviewed, this finding is not easily disregarded. Moreover, when findings were examined at the interaction level (with Fergusson et al. (2011) excluded from this analysis), only 15% of a total of 162 interactions across the studies provided support for the S-allele as the risk allele and 10% of the interactions provided support for the L-allele as the risk allele in unfavourable family environments. This analysis does not appear to strongly implicate the S-allele over the L-allele as conveying greater vulnerability to depression. Indeed, it rather seems to suggest that null findings predominate, and the available evidence for a moderating role of both the S-allele and the L-allele is possibly quite similar.

At face value, the number of significant findings for each allele, whilst not overwhelming, appear higher than the commonly accepted error chance of 5%. However, GxE researchers can analyse their genetic, environmental and outcome factors in a variety of forms, and undisclosed flexibility with testing and reporting of findings has been shown to dramatically increase false positive rates (Simmons, Nelson, & Simonsohn, 2011). Concerns have been raised about the extent to which researchers might examine a range of GxE models and "cherry pick" or include a biased selection of their significant findings that conform with expectations of the S-allele as the risk allele, and omit many more analyses of non-conforming, particularly null findings (Duncan & Keller, 2011; Heininga, Oldehinkel, Veenstra, & Nederhof, 2015). It is also plausible that the percentage of significant findings here has been overinflated by publication bias, whereby authors are more likely to submit,

and editors are more likely to accept, statistically significant GxE findings. The extent to which null or opposite findings sitting in researchers' 'file-drawers' might alter the figure obtained here is unknown. Interestingly, one study that has compared the findings of multiple tests involving varying operationalizations of 5-HTTLPR, childhood adversities and depression available within a single sample of adolescents followed longitudinally identified the percentage of significant interactions was 7.9% (of 2160 interactions) which is well below the 27% in the current analysis and only slightly above the 5% that might be expected based on chance (Heininga et al., 2015). This study did not go on to do post-hoc analyses to determine the direction of significant findings, however, so does not allow a comparison of the proportion of S-allele supportive versus L-allele supportive interaction findings.

Certainly, there was some indication of bias in the literature considered by this systematic review. In particular, it appeared that studies with S-allele supportive findings had the fewest analyses in their manuscripts whilst studies reporting L-allele supportive findings conducted the greatest number of analyses, perhaps reflecting some scepticism of reviewers or editors, who required further validation of the L-allele supportive findings before being willing to publish them. Further, editors may favour more consistent, "cleaner" results for publication over "untidy" findings (Nosek, Spies, & Motyl, 2012). Studies with null findings were also found to be based on larger sample sizes than those with significant findings, which tends to occur in the presence of publication bias (Duncan & Keller, 2011). Interestingly, a citation bias has also been found to exist for readers of the broader serotonin transporter gene-environment literature, with positive findings tending to be cited more frequently (de Vries, Roest, Franzen, Munafo, & Bastiaansen, 2016). In

addition, the positive aspects of inconclusive findings tend to receive more attention in published articles (de Vries, Roest, Franzen, Munafo, & Bastiaansen, 2016).

Importantly however, this systematic review also suggested the possibility that several factors related to methodology influenced studies' capacity to detect significant results. In particular, it appeared that interview-based approaches were more successful at identifying positive and opposite findings compared to questionnaire measures in terms of percentage of significant interactions. This finding appears to mirror the phenomenon that some investigators have argued exists in the broader GxE stress literature, involving enhanced detection of findings by studies relying on interviews compared to self-reported checklists of stressful life events (Caspi et al., 2010; Uher & McGuffin, 2010). Studies that drew on interviews or observation did appear to be based on fewer participants than studies that drew solely on questionnaire measures, lending some credence to the argument by Caspi and colleagues that many of the best-designed studies for testing GxE hypotheses have smaller samples (Moffitt & Caspi, 2014). Moreover, high quality measurement has been claimed to produce greater improvements in statistical power than increasing sample size (Manchia et al., 2013).

There was perhaps also some indication that opposite findings were more frequently identified by studies that had sequenced the bilallelic 5-HTTLPR VNTR, rather than the triallelic rs25531 polymorphism. Interestingly, the study by (Heininga et al., 2015) that tested 2160 interactions involving the serotonin transporter gene with all relevant measures available found the biallelic approach seemed to generate more significant interactions than the triallelic approach. The implications of this is somewhat unclear particularly given some uncertainty has been expressed about which genotyping approach should be preferred

(Hu et al., 2006; Martin, Cleak, Willis-Owen, Flint, & Shifman, 2007; Wendland et al., 2006).

In addition, there was some suggestion that the direction of the interaction might vary according to the specific feature of the family environment being investigated. Positive findings implicating the S-allele in depression were possibly more common amongst the group of studies that had considered the serotonin transporter gene in interaction with family relationship stress, though this might also have reflected that several of these studies relied on interview measures to capture the environment of interest. In contrast, the L-allele appeared to be associated with elevated risk for depression with some consistency in the group of studies that considered low socioeconomic status. There was some evidence that the serotonin transporter gene might interact with broad family adversity/stress and with parental psychopathology, but significant effects were not reliably detected and the direction of these interactions was unclear with similar numbers of studies reporting positive and opposite findings.

Parenting behaviour was the only domain of family environment where no significant interactions were noted. This group of studies is of particular interest given that parenting behaviours have been shown to have a robust association with adolescent depression (Yap et al., 2014), and the influence of many broad family factors on depression is thought to occur at least in part through the more proximal effect of parenting behaviour. Because proximal environmental influences are social or physical exposures that directly impact the individual, they would be expected to show stronger GxE interaction effects (Moffitt et al., 2005). It was therefore perhaps particularly surprising that no significant findings were identified by this group of studies. These null results raise questions about

the possibility that the serotonin transporter gene might not moderate the relationship between less optimal parenting and depression, or alternatively, these interactions might have been particularly susceptible to measurement or other design issues. Measures utilised here were both in the form of brief questionnaires (Lavigne et al., 2013; Van Roekel et al., 2011) and ratings based on interviewer observations of parent-child interactions (Fergusson et al., 2011; Lavigne et al., 2013). Whilst questionnaire measures of parenting are time and cost effective, and allow researchers to ask about a broad range of behaviours in a number of different contexts over an extended time period, they may not always accurately capture actual parenting behaviour, particularly as these ratings can influenced by the reporter's recall, their interpretation of the questions and by social desirability of certain responses (Locke & Prinz, 2002; Morsbach & Prinz, 2006). Interestingly, whilst internal consistency of the questionnaire measures appeared adequate (α ranging between .77 and .87), correlations with depression were relatively weak and frequently non-significant (ranging between .02 and -.26). Information regarding the extent of skewness in the data was also not available. It is possible that these relatively brief measures could not adequately encapsulate key parenting behaviour or variation in responses was insufficient to identify the interaction in the relatively small samples (175 participants in Lavigne et al., 2013; and 306 participants in Van Roekel et al., 2011). Data gathered by behavioural observations are considered to be more "objective" and have high face validity because they directly and systematically measure the behaviours of interest (though these may be affected by participants' reactivity to being observed, including behaving in socially desirable ways). The two observational measures considered here however were based on global ratings of behaviour for the entire observational period, on relatively brief Likert-like scales. The

apparent simplicity of this rating system can sometimes obscure a highly intricate judgement process involving a high level of abstraction from the behaviour and may not always adequately capture the variation in parenting behaviours (Alexander, Newell, Robbins, & Turner, 1995). Global ratings of behaviour which involve "judgements" of behaviour relative to norms established in training sessions are also potentially vulnerable to observer biases (Aspland & Gardner, 2003; Carlson & Grotevant, 1987). One alternative and arguably more objective form of analysing the data is to consider behaviour frequencies, determined by micro-analytic coding (Alexander et al., 1995; Aspland & Gardner, 2003; Carlson & Grotevant, 1987). It is possible that micro-analytic coding of parenting behaviour may be more sensitive to detecting an interaction between the serotonin transporter gene and parenting predicting depression.

The age at which parenting and depression were measured may also have consequences for detection of the interaction. Both Lavigne et al., (2013) and Fergusson et al., (2011) measured parenting in early childhood (approximately 4 years of age), whilst Lavigne et al., (2013) assessed depression at the same age, and Fergusson et al., (2011) assessed depression much later in life, during adulthood. Lavigne and colleagues may have had difficulties detecting an interaction predicting an outcome that likely had little variation, given depressive symptomatology is generally very low in early childhood, whilst Fergusson and colleagues (2011) may have had difficulties detecting an interaction predicting a distal outcome that was likely only weakly correlated to the predictor (though these descriptive statistics were not provided in their manuscript). Further consideration of this potential interaction in adolescence when depressive symptomatology becomes more

elevated, particularly by studies with micro-analytic coding of parenting behaviours, appears warranted.

In fact, after this systematic review was finalised, a paper based on a prospective longitudinal study by Koss, Cummings, Davies, Hetzel, and Cicchetti (2016) was identified that documented an interaction between 5-HTTLPR and harsh parenting predicting depression during adolescents. This study measured depressive symptomatology according to self-report questionnaire at three timepoints during adolescence and assessed rejecting, invalidating or coercive behaviour displayed by parents observed during a family problem-solving task completed at baseline, according to global ratings by trained observers of these phenomena. The outcomes of interest included initial depressive symptoms at baseline, change in adolescent depressive symptoms across the three timepoints, and the number of occasions that adolescents scored above clinical cut-off on questionnaire (referred to as frequency in elevated depressive symptoms). The sample of 206 adolescents was recruited from the community and was comprised primarily of Caucasian, middle-class families. The frequency of harsh parenting was noted to be low – more than half of parents (53%) displayed no occasions of harsh parenting, with the remainder (47%) known to display at least one instance of harsh parenting (the authors indicated that the global coding scale prohibited identification of the exact number of instances), which the authors discussed as likely related to the low risk, community nature of the sample. Intriguingly, amongst LL-homozygous individuals, there was an association between harsher parenting and a greater frequency of elevated depressive symptoms exceeding clinical cut-off over the three assessment timepoints. In contrast, adolescents with the functional S allele showed elevated symptoms regardless of the level of harsh parenting. The graph of the interaction suggested

potential differences between depressive symptomatology might be present at low levels of harsh parenting, with LL-homozygous individuals seeming relatively protected against symptomatology in these environments relative to carriers of a functional S-allele. There did not appear to be a difference in depressive symptomatology at high levels of harsh parenting, however, given the study did not evaluate the values of harsh parenting at which statistically significant differences were apparent (i.e., region of significance analyses), this interpretation is only speculative. Interactions predicting outcomes of initial depressive symptomatology and change in depressive symptoms were not significant. Importantly, this study did not control for positive parenting behaviour or test for an interaction between 5-HTTLPR and positive parenting predicting depression, leaving open the possibility that the finding that L-homozygous individuals' risk for depression varied as a function of harsh parenting whilst S-allele carriers' risk remained stable could be explained by an association between harsh parenting and positive parenting, particularly given suggestions that this was a relatively high functioning sample that appeared to experience low levels of harsh parenting.

A related relevant study is one by Hankin and colleagues (2011), which identified in three independent samples that youth with either one or two S-alleles who received more positive, warm parenting reported higher levels of positive affect than their L-allele homozygous counterparts, whereas youth (between 9-16 years old) carrying at least one S-allele who received less nurturant parenting reported lower levels of positive affect. Positive affectivity, which reflects an individual's disposition to experience a range of positive emotions, such as pleasure, enthusiasm, cheerfulness, joy and pride, and to feel actively and effectively engaged and alert (Watson, Clark, & Tellegen, 1988), has been

specifically associated with vulnerability and resilience to depression (Watson et al., 1995). Importantly, whilst low positive affect or anhedonia can be core feature of depressive disorders, which are characterized by disruptions to mood, and low positive affect would typically be expected to show some correlation with depressive symptomatology (Brown, Chorpita, & Barlow, 1998), low positive affectivity is not pathological or diagnostic of a mental health condition in and of itself. The study by Hankin and colleagues (2011) might therefore be said to focus on the association between the continuum of enriched-deprived environments and increased likelihood of positive outcomes (i.e., quadrant 2 illustrated in Figure 2-3 of CHAPTER 2) rather than negative/maladaptive outcomes, and findings may be consistent with the idea that S-carriers are more able to benefit from the nurturing aspects of positive parenting environments, potentially as a function of their putatively greater capacity for emotional responding and emotional engagement relative to L-homozygotes (Glenn, 2011; Homberg & Lesch, 2011). The replication of this finding across three distinct samples that drew on different methods for assessing parenting (both observational assessment based on global ratings and questionnaires) and different questionnaire measurement of positive affect represents an important methodological strength. Replication of candidate gene-environment interactions in independent samples is strongly recommended to reduce the probability that results are due to chance or to bias (Hewitt, 2012). This study however, also did not control for negative parenting or test whether the interaction between 5-HTTLPR and negative parenting predicted positive affect. Studies focusing on specific types of experiences (e.g., parenting lacking in warmth and nurturance) without adjusting for relevant co-occurring exposures (e.g., more negative, punitive parenting) can be limited in their conclusions regarding specific mechanisms that

might underpin gene-environment interactions involving these different dimensions of adverse experiences to psychopathology. Rather, studies able to measure and model both of these dimensions of experience are required to identify whether such specificity exists.

Indeed, an additional explanation for disparate results across studies evident in the current review, is that variation in findings reflects that particular types of environmental conditions differentially affect the relationship between 5-HTTLPR genotype and various outcomes. As reviewed in the previous chapter, McLaughlin and Sheridan (2014) have suggested that there might be dimensions of experience underlying a broad range of environmental exposures that might differentially influence developmental outcomes, including the emergence of psychopathology. The previous chapter considered evidence that possession of an S-allele might increase vulnerability to neurodevelopmental changes associated with environmental experiences high on the dimension of threat (the *presence* of actual or perceived harm to one's physical integrity, such as direct experience of abuse, or the witnessing of violent victimization), whilst possession of an L-allele might increase susceptibility to neurodevelopmental changes associated with experiences high on the dimension of deprivation (the *absence* of expected social or cognitive inputs and species- and age-typical complexity in environmental stimuli, such as emotional or physical neglect). It is possible that significant interactions implicating the S-allele reflect S-allele carriers' greater sensitivity to depressogenic effects of more threatening environments specifically, whilst significant interactions implicating the L-allele reflect L-homozygous individuals' sensitivity to the depressogenic effects of more deprived environments. Certainly, it seems plausible that measures of family relationship stress and conflict (which the S-allele appeared to interact with more consistently), might load on the dimension of

threat, whilst low socioeconomic status (which the L-allele appeared to interact with more consistently) has been discussed as a potential marker of both threat and deprivation, particularly deprivation involving enriching and cognitively complex environments (McLaughlin et al., 2014; Sheridan & McLaughlin, 2014).

The possibility that the S-allele and the L-allele are sensitive to the depressogenic effects of threat and deprivation respectively may explain some of the inconsistent findings between studies, including the presence of a large number of null findings. When measures of environment are used that capture both threat and deprivation (such as those capturing broad family adversity), a positive or opposite result may depend on the extent to which a particular group of participants has experienced more threat-related or deprivation-related exposures. Similarly, studies might report a null finding or even an interaction in the non-hypothesised direction when they have focused on specific types of exposure central to one dimension (e.g. family conflict which might load on the threat dimension and therefore be expected to interact with the S-allele), without controlling for correlated, co-occurring exposures that are central to the other dimension (e.g. deprivation related experiences such as low nurturance and parental involvement).

It is also important to remember that measures might also capture exposures that, in and of themselves, might not represent direct threat or deprivation, but are rather markers for these experiences. For example, parental psychopathology does not inherently involve dimensions of either threat or deprivation (i.e., it is possible to be child of a parent with a mental health condition, but still have no exposure to threatening experiences and typical and adequate exposure to cognitive, social and environmental complexity). However, parental psychopathology has been associated with lower parent-child synchrony, less

effective parental communication and facial emotional expressiveness, reduced responsivity to child behaviour, and greater parental hostility and aggression (Lovejoy et al., 2000). Thus, the degree of deprivation and threat may be heterogeneous between studies, and this could be one reason that findings regarding interactions with variables that represent markers of both these dimensions might be inconsistent.

Several findings regarding the putative effect of a number of other methodological aspects warrant discussion. First, there was no clear evidence in this review that the L-allele tends to be detected as the risk allele in studies with fewer Caucasian participants. In fact, all of the studies in this analysis that obtained opposite findings and provided information about ethnic background were based on samples of primarily Caucasian individuals, whilst the vast majority of studies with samples with a greater variety of participants from different ethnic backgrounds reported positive findings. This finding is important given some authors' claims that the L-allele in African-American or Black individuals may operate in a similar way to the S allele in Caucasian or White samples (Anderson & Mayes, 2010; Williams et al., 2003; Williams et al., 2008). This systematic review suggests that variation in the ethnic composition of the current samples is unlikely to account for variations in their outcomes.

Second, there was some indication that positive interactions implicating the S-allele may be more readily detected amongst females. This finding concurs with a recent review by Gressier et al. (2016) though this review cautioned that conclusions at this point in time were not possible given the limited number of studies that have systematically explored the role of gender. A much earlier review of a relatively small number of studies also noted that the serotonin transporter gene-environmental stress interaction might be stronger among

females than males, particularly during the adolescent period (Uher & McGuffin, 2008). This review suggested however that before it could be concluded that males might be protected from a pathogenic GxE effect, research needed to be able to address the extent to which these findings might be accounted for by the higher prevalence of depression and the stronger association between stressful life events and depression amongst females. In the current review, the most common methods to detect gender effects were three-way interactions and the investigation of the two-way interaction stratified by gender, dividing the overall sample into two groups. Analyses assessing the role of gender therefore requires larger samples to generate sufficient power to detect effects than tests of the two-way interaction that assume no sex-specific mechanisms. In the current review however there is evidence that gender effects implicating the S-allele in girls are being identified by smaller studies, which suggests a need for caution about the robustness of this finding in light of some indication of publication bias, and also given there was no clear indication that these analyses addressed the possibility that significant findings in females might relate to their greater experience of depression. The latter issue is a particular problem, given testing for the interaction in stratified samples does not provide a test of whether gender is a moderator of the effect (i.e., the strength of the interaction is *different* in males and females). A significant interaction effect in females but not in males demonstrated by stratified analyses might simply reveal the greater power to detect effects in the former group due to greater variance in their depressive symptomatology. Interestingly, recent meta-analyses have not detected effects of gender on the interaction (Culverhouse et al., 2017; Risch et al., 2009; Sharpley et al., 2014). Given concerns about false positives in this literature, it is important for future studies to have sufficient power before embarking on testing of gender effects

and to use appropriate statistical tests that clarify whether the strength of interactions in males and females are in fact significantly different from each other.

As in all research, there are some limitations to this narrative review. As already discussed, publication bias, both at a study level and a within-study analysis level may have influenced our findings, with positive results being reported and null or opposite ones potentially remaining in the 'file-drawer.' Furthermore, several studies from the same cohort were included in this review. Consequently, the results of these studies cannot be regarded as independent of each other in the same way that studies based on different cohorts can be. However, the findings of these studies were often considered of high quality, as they were often based on longitudinal data that was collected via gold-standard measures, such as interview approaches and hence seemed important to include.

Another limitation concerns the potential confounding influence of the differences between studies in the number of tests involving varying operationalisations of 5-HTTLPR, family environments and depression that were conducted. The number of these permutations did appear to vary substantially between individual studies. Different rates of multiple testing across the studies together with selective reporting has potential to artificially inflate or underestimate the proportion of significant findings in the current analyses. The small number of studies in this review meant that only exploratory investigations of potential patterns were possible as opposed to formal statistical testing that could have adjusted for the number of permutations of each analysis contained within each study. The problem of different numbers of analyses across studies arises in part from the tendency of observational studies, of which gene-environment investigations are a subset, to take a more wide-ranging focus rather than developing a clearly defined, a priori

primary set of analyses which has an identified core outcome, as is typically observed with randomised control clinical trials. Recognition of this issue has led to calls by researchers to adopt practices similar to those followed by clinical trials, including pre-specification of studies, their hypotheses, primary outcome and analytical approach to curtain the proliferation of approaches, and to encourage complete, unbiased reporting and publication of analyses (Chambers, 2013; Nosek et al., 2012). The proposed protocol of a collaborative meta-analysis (Culverhouse et al. 2013) of the serotonin transporter gene-environmental stress interaction is one recent relevant example. These recommendations might even be formalised into a following introduction of a policy that requires investigators to record information about their study design into a registry as a precondition for publication of the study's findings in member journals (DeAngelis et al., 2004), as is now required with clinical trials. Journals agreeing to publish findings based on soundness of the research proposal, regardless of the nature of the results, is likely to provide a significant incentive to investigators to preregister their studies.

3.5 Conclusions

This systematic review was conducted in an attempt to understand the state of the evidence on GxE research involving the serotonin transporter gene and family environments in predicting depression, focussing on the methodological approaches used across studies as these features have not been previously described for this particular group of studies. The results of this review suggest that the findings of GxE that pertain to the family environment are more mixed than those pertaining to stressful life events or adversities such as maltreatment: whilst the findings provide support for an association between the 5-

HTTLPR S-allele, adverse family contexts and depression, there is also potentially similar support for the opposite finding (i.e., the L-allele association with maladaptive family environments and depression). This does not mean that the hypothesis of association between the S-allele, family environments and depression should be dismissed, rather these findings suggest more complex relationships. One possibility is that the S-allele interacts with more threatening environments whilst the L-allele interacts with environments involving more deprivation, a notion that requires assessment. Certainly, the need for studies that are able to disentangle the effects of threat versus deprivation by measuring and appropriately controlling for the other has been emphasised (Sheridan & McLaughlin, 2014). A focus on parenting in particular appears warranted given this review indicates that the very limited number of studies that have considered interactions involving parenting behaviour failed to identify any significant associations (with one study with significant findings having emerged since completion of this review, to my knowledge). This limited number of significant findings is somewhat surprising given the literature identifying parenting behaviour as robust and proximal predictor of depression (Yap et al., 2014) and that parenting may influence biological systems involved in depression, which would be a good candidate environment to interact with the serotonin transporter gene. Moreover, the positive and negative dimensions of parenting lend themselves well to an assessment of the possibility of differential interactive effects of the S-allele and L-allele with threat (i.e. greater parental aggression and hostility) and deprivation (less parental warmth and nurturance) respectively. To this end, the next chapter of this thesis will comprise an empirical study of how the serotonin transporter gene might interact with parenting in

predicting the emergence of depression during adolescence, the developmental period when symptomatology is known to increase (e.g., Hankin, 1998).

CHAPTER 4: SOMETIMES IT'S GOOD TO BE SHORT: THE SEROTONIN TRANSPORTER GENE POLYMORPHISM, PARENTING AND ADOLESCENT DEPRESSION IN TWO LONGITUDINAL STUDIES

The following paper accepted at *Child Development* on 16th May 2017 is presented as CHAPTER 4 as the final version submitted to the journal.

Given that both the introductory chapters and the introduction of this publication needed to be able to stand independently, there is some direct duplication of content.

Sometimes It's Good to be Short: The Serotonin Transporter Gene, Positive Parenting and Adolescent Depression

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CHILD DEVELOPMENT

Sometimes it's Good to be Short: The Serotonin Transporter Gene, Positive Parenting and Adolescent Depression

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Title Page

Sometimes it's Good to be Short: The Serotonin Transporter Gene, Positive Parenting and Adolescent Depression

Abstract

In threatening environments, the short (S) allele of 5-HTTLPR is proposed to augment risk for depression. However, it is unknown whether 5-HTTLPR variation increases risk for depression in environments of deprivation, lacking positive or nurturant features. Two independent longitudinal studies examined whether 5-HTTLPR moderated associations between low levels of positive parenting at 11-13 years and subsequent depression at 17-19 years. In both studies only LL homozygous adolescents were at greater risk for depression with decreasing levels of positive parenting. Thus, while the S-allele has previously been identified as a susceptible genotype, these findings suggests that the L-allele may also confer sensitivity to depression in the face of specific environmental challenges.

Key Words: Gene-environment Interaction, Serotonin Transporter Gene, Depression, Adolescence, Positive Parenting

Depression is a common and debilitating disorder with a complex etiology that frequently has its initial onset during adolescence (Merikangas et al., 2010). The aggregation of depression within families has led to a focus on understanding how genetic contributions may interact with other factors to affect risk for the emergence of this disorder (Sullivan, Neale, & Kendler, 2000). One widely studied genetic risk factor for depression is a variable number tandem repeat located within the promoter of the serotonin transporter gene (5-HTTLPR), which has been shown to modify the effectiveness of the serotonin transporter enzyme in clearing the synaptic cleft (Heils et al., 1996).

The field is unclear, however, about the extent to which 5-HTTLPR modifies overall serotonin neurotransmission *in vivo*, and the extent to which this creates risk for, or protects against, depression. A seminal study by Caspi and colleagues (2003) found that individuals carrying the 'low expression' short (S) 5-HTTLPR allele (associated with reduced transcriptional efficiency and lower serotonin uptake activity) were more vulnerable to the depressogenic effects of childhood maltreatment or multiple negative stressful life events than were individuals homozygous for the long (L) allele. Attempts to replicate Caspi and colleagues' seminal findings have yielded mixed results, with two large meta-analyses showing no support for this gene by environment (GxE) interaction (Culverhouse et al., 2017; Risch et al., 2009) and two providing support for the GxE effect (Karg, Burmeister, Shedden, & Sen, 2011; Sharpley, Palanisamy, Glyded, Dillingham, & Agnew, 2014).

The largest of meta-analyses by Sharpley and colleagues (2014) however also noted that whilst the majority of studies in their analysis (65%) supported an association between the S-allele, adversity and depression, nearly 26% of the included studies failed to show a significant interaction, and approximately 10% found opposite results to those expected, implicating the L-

allele as conferring risk for depression in the presence of adversity. The authors suggested that these mixed findings do not necessarily deny a moderating role for this polymorphism; rather, they suggest that interactive effects may be more complex than originally conceptualized. Interestingly, a similar conclusion was reached in a recent meta-analysis by Weeland and colleagues (2015) that included 12 studies examining the interaction between the serotonin transporter gene, family adversity and externalising behaviors. Four studies found S-carriers to be more vulnerable to the deleterious effect of family adversity, whereas four studies found L-allele homozygous individuals to be more at risk as a result of adverse family environments, and a further four studies obtained null results. Both Sharpley and colleagues (2014) and Weeland and colleagues (2015) raised the possibility that the L-allele may too be associated with psychopathology in certain environmental contexts.

The interplay between allelic variation in the serotonin transporter gene and the environment in predicting outcomes such as depression has most commonly been discussed from a diathesis-stress, or vulnerability, perspective. This framework has often designated the S-allele as a "risk" allele that confers greater sensitivity to stress, which in turn increases susceptibility to disorder in contexts of high adversity (Caspi, Hariri, Holmes, Uher, & Moffitt, 2010). However, an alternative conceptualization has been suggested that proposes that the S-allele may be a "plasticity" allele that exhibits differing levels of adaptive fitness depending on the environmental context (Belsky et al., 2009; Belsky & Pluess, 2009). In this "differential susceptibility hypothesis", S-carrier status is not simply a risk factor for hypersensitivity to adversity, and hence psychiatric disorder, but is rather associated with a greater sensitivity to environmental influences more generally. In propitious environments, this sensitivity may

promote wellbeing or competence, whilst in adverse environments it may increase risk for negative outcomes.

The differential susceptibility hypothesis thus encompasses the notion of diathesis stress, as well as the notion of *vantage sensitivity,* the term that has been used to describe the potential for some individuals to derive more benefit from positive environmental experiences than others (Pluess & Belsky, 2013). Importantly, capturing the vantage sensitivity component of differential susceptibility phenomenon involves a consideration of the adaptive spectrum rather than simply the maladaptive spectrum, as the *absence* of negative outcomes (i.e. no psychopathology) may not the same as the *presence* of positive outcomes that would characterize thriving or optimal functioning. A smaller body of research has focused on the influence of positive environments, and a recent meta-analysis has suggested that S-carriers show a greater ability to capitalize on positive, supportive contexts to achieve positive developmental outcomes (van Ijzendoorn, Belsky, & Bakermans-Kranenburg, 2012), a finding consistent with the notion that the S allele may confer differential susceptibility.

The research focusing on the interaction between 5-HTTLPR and adversity, where significant variation in findings has been noted, has considered a broad set of exposures including physical or sexual abuse, institutional rearing, natural disasters, bullying victimization experiences, marital conflict, divorce, chronic poverty, and unresponsive or punitive parenting (Sharpley et al., 2014). Emerging evidence suggests that experiences of *threat*, involving the presence of experiences characterized by actual or threatened harm versus *deprivation,* involving impoverished expressive environments or the absence of expected environmental inputs and learning opportunities in cognitive, social or emotional domains, may have distinct influences on neurodevelopment and associated psychological outcomes (McLaughlin, Sheridan, & Lambert,

2014). In particular, McLaughlin and colleagues (2016; 2014) have argued that experiences of threat may alter the development of emotional processing by serving as potent learning experiences that may ultimately bias attention towards potential danger, increase reactivity to negative emotional information and decrease automatic down-modulation of emotional responses. In contrast, the authors have proposed that more deprived environments may adversely influence the development of other aspects of emotional processing, such as emotion recognition and discrimination as well as hamper development of executive functioning.

It is plausible that the serotonin transporter gene might be a marker of characteristics such as emotion processing and executive functioning that interact with these two forms of environmental experience in different ways. Interestingly, the strongest evidence for an interaction between the serotonin transporter gene and adversity appears to come from studies that have considered a single, specific exposure, such as childhood abuse or medical illness (Caspi et al., 2010). Whilst these studies may have considered different types of exposures, they are arguably united by their focus on threatening events, involving either the experience or anticipation of significant harm. In contrast, findings appear to be more mixed amongst the group of studies that have employed composite or count measures of adverse experiences, particularly based on checklists. This approach, which often includes experiences of both threat and deprivation, possibly obscures the distinct ways that the serotonin transporter gene interacts with particular environmental experiences to influence development.

Importantly, a component of the relationship between the serotonin transporter gene and environmental factors that has received relatively little systematic attention is whether 5-HTTLPR genotypes might interact with environments of deprivation to influence subsequent maladaptive psychological outcomes. It is well established that parenting and parent-child

interactions have an impact on young people's risk of developing depressive disorders during adolescence (Yap, Pilkington, Ryan, & Jorm, 2014). Whilst negative, harsh or aggressive parenting behaviour and positive, warm, nurturing behaviour could be conceived as falling on opposite ends of a single spectrum, research suggests that they represent distinct, albeit correlated, dimensions that make opposite and independent contributions to depression (Barrera, Chassin, & Rogosch, 1993; Dallaire et al., 2006). Indeed, although most parents are likely to be aware that critical or hostile parenting behaviors can be detrimental for children, there is some indication that failure to engage in positive, nurturing and affirming interactions with children may also have adverse effects (Schwartz et al., 2016).

Parents low in positivity may be offering their children fewer opportunities to learn about the nature of different positive emotions, the ways in which these emotions might be elicited by various stimuli, and the contextual appropriateness of emotional expression (Eisenberg et al., 2005; Morris, Silk, Steinberg, Myers, & Robinson, 2007). They may also be modeling poor emotion regulation strategies or a lack of emotion regulation strategies to their children. Reduced positive caregiving behaviors and less secure child attachment have been linked to less developed child executive function and related constructs such as self-regulation and effortful control (Bernier, Carlson, Deschenes, & Matte-Gagne, 2012; Eisenberg et al., 2005), which have been associated with greater risk of psychological disorders such as depression . Intriguingly the limited available literature suggests that in these more deprived environments, the *L allele* might be associated with a range of poor outcomes. For example, Sulik and colleagues (2012) reported a relationship between low levels of supportive parenting and noncompliance in young children that was evident only in the group of children with an LL genotype. Davis and Cicchetti (2013)

found that maternal unresponsiveness predicted greater externalizing problems, such as aggression and defiance, in children with the homozygous L genotype.

It is less clear whether this pattern of findings extends to internalizing disorders. Two studies provide evidence of an interaction between the serotonin transporter gene and the broader family climate that suggest L-homozygous individuals may be vulnerable to depression in less positive environments. Laucht and colleagues (2009) found that adolescents homozygous for the L-allele, but not adolescents with an S allele, showed increased vulnerability to depression and anxiety when they belonged to families that experienced a number of chronic adversities, such as early parenthood, low parental education, sole parenting or parental psychiatric disorder. Lavigne and colleagues (2013) found that LL-homozygous four year old children showed greater increases in depressive and anxiety symptoms in the context of greater caretaker depression and family conflict and lower socioeconomic status, as well as greater increases in symptoms of oppositional defiant disorder in the context of increases in family stress. Whilst these factors are known to impact on parenting behaviors (e.g., Lovejoy, Graczyk, O'Hare, & Neuman, 2000; Rao & Chen, 2009), and indeed, were correlated with measures of parental support/engagement and parental hostility in this study of much younger participants in early childhood, parental support/engagement and hostility did not interact significantly with 5-HTTLPR genotype. Moreover, Li and colleagues (2013) found a marginally significant interaction in girls, such that reduced family support predicted greater depression symptoms only among girls with the LL genotype. In contrast, they obtained a significant interaction for boys that conformed to a differential susceptibility model.

There is therefore some indication in the literature to suggest that possession of an L-allele may confer increased vulnerability to adverse effects of more deprived family

environments characterized by low support and nurturance. However, a systematic consideration of the differential effects of different genotypes in interaction with deprivation or threat has not been conducted within the same gene-environment study. Studies focusing on specific types of experiences (e.g., parenting lacking in warmth and nurturance) without adjusting for relevant co-occurring exposures (e.g., more punitive parenting) are limited in their conclusions regarding specific mechanisms that might underpin gene-environment interactions involving these different dimensions of adverse experiences to psychopathology. Rather, studies able to measure and model both of these dimensions of experience are required to identify whether such specificity exists.

Toward a More Nuanced Perspective on Moderation by the Serotonin Transporter Gene

One potential explanation for findings suggesting that carriage of either an S-allele or an L-allele might confer vulnerability to psychopathology depending on the environmental context might be related to potential psychological and behavioral characteristics associated with these different genotypes. Whilst these characteristics have not been conclusively identified, a number of reviews of the neuropsychological, psychophysiological, hormonal and brain imaging correlates of 5-HTTLPR genotype have posited that the S-allele may confer greater emotional reactivity and stress-responsivity (Canli & Lesch, 2007; Caspi et al., 2010; Hariri & Holmes, 2006; Homberg & Lesch, 2011), which may be associated with negative *or* positive outcomes, contingent on the environment. However, until relatively recently there has been little consideration of what these same studies might suggest about traits associated with an L-allele, and whether these characteristics might also affect vulnerability to psychopathology. Two reviews of the literature from this alternative perspective have argued that L-allele may be linked

to reduced emotionality (including shallow affect, lower levels of fearfulness, and reduced empathy, guilt and shame) and lower stress-sensitivity which may potentially increase risk for higher levels of callous-unemotional traits or psychopathy in the context of additional genetic and environmental factors (Glenn, 2011; Yildirim & Derksen, 2013). For example, compared to those with the LS or SS genotype, women with an LL genotype self-reported significantly greater difficulties with identifying feelings on a subscale measuring Alexithymia, a personality construct that captures problems with recognising, expressing emotions and understanding others' emotions (Kano et al., 2012). The L-allele may also be associated with a bias towards positive emotional stimuli and/or a bias away from negative stimuli (Fox, Ridgewell, & Ashwin, 2009), a pattern of attention that may be consistent with the reward-dominant response style or punishment insensitivity that is seen in individuals with psychopathy or who are high in callous-unemotional traits (Dadds & Salmon, 2003). L-homozygous individuals have also been found to display less emotionally expressive behaviours and reported less amusement, shame and anger when watching themselves in embarrassing situations (Gyurak et al., 2013). They also demonstrated reduced levels of prosocial emotional empathy and exhibited lower cardiovascular and electrodermal activity when watching others in serious distress (Gyurak et al., 2013). Individuals homozygous for the L-allele have been found to display higher levels of callous-unemotional traits compared to S-carriers (Brammer, Jezior, & Lee, 2016), though one study found this effect to be limited to the group of individuals brought up in socioeconomically disadvantaged environments, which can be a marker of deprived circumstances more broadly (Sadeh et al., 2010).

The potential link between the L-allele and higher callous-unemotional traits is perhaps particularly noteworthy given research suggesting that individuals high in callous-unemotional

traits who receive low levels of parental warmth may be at particular risk of behaviour symptoms (Pasalich, Dadds, Hawes, & Brennan, 2011) and that greater parental warmth/involvement predicts a decline in levels of callous-unemotional traits (Pardini, Lochman, & Powell, 2007). Furthermore, whilst callous-unemotional traits have typically been thought to be associated with low levels of anxiety and mood difficulties (Lykken, 1995), a number of studies have found that higher levels of callous-unemotional traits can in fact predict higher levels of internalizing problems (e.g., Hawes et al., 2014; Waller et al., 2015). One possible explanation for these findings is that restricted affect and reduced empathy may pose increased risk for depression via greater social withdrawal, isolation and anhedonia (Waller et al., 2015).

A number of genetic association studies have additionally suggested possible links between the 5HTTLPR L-allele and various aspects of executive functioning, including reduced cognitive flexibility (Borg et al., 2009; Tükel et al., 2016) and poorer sustained attention (Strobel et al., 2007). In addition, two studies provide some indication that the development of executive function of LL-homozygous individuals may be impeded by adverse family environments potentially high in deprivation, involving higher levels of maternal depressive symptomatology (Weikum et al., 2013) or lower levels of parental supervision (Li et al., 2015). Interestingly, LL-homozygous individuals also performed better than their S-allele counterparts on executive function tasks when their mothers endorsed few depression symptoms (Weikum et al., 2013).

In environments involving a high degree of threat, S-allele carriers, who are thought to be more emotionally reactive and especially sensitive to their context, may be at greater risk of stress-related psychopathologies, such as depression, than their less affectively responsive L-homozygotes. Moreover, in certain positive environments, these particular traits associated with S-allele carriage may promote of certain aspects of wellbeing, particularly those associated with

socio-emotional functioning. By contrast, in deprived environments that lack important nurturing features, the primary affective task may be to engage and extract nurturance and support from others in the interpersonal environment, a task for which S-carriers might be better suited than L-homozygous individuals, due to their greater capacity for affective engagement and social cognition. In such interpersonal environments where the primary challenge is to elicit care and support that is lacking, S-carriers' greater capacity for emotional responding and engagement with others may offer a buffer against psychopathology. In these contexts, it may therefore be the emotionally hyporesponsive L-homozygous individuals who are less adaptive, placing them at greater risk of psychopathology. Importantly, deficient emotional experiences, in the form of reduced emotional reactivity or low emotional responsiveness to changing contexts, have been associated with depressive disorders (Bylsma, Morris, & Rottenberg, 2008). Deficits in executive function have also been linked with depression (Snyder, 2013).

Thus, consideration of the L-allele as simply insulating individuals from all environments (both positive and negative), as per the differential susceptibility hypothesis, may present an incomplete picture. Instead it may be that *both* S-allele and L-allele individuals possess specific characteristics that may be advantageous or detrimental, depending on their environment. In other words, it is the *fit* (or lack thereof) between genetic or biological predispositions and environmental challenges that determines functioning and wellbeing. Importantly, this perspective does not suggest, for example, that S carriers do not require positive parenting or that L-homozygous individuals are not hurt by aggressive, critical parenting, but rather that there may be combinations of genotypes and environments that are particularly adaptive or unfavorable relative to other combinations. This paradigm has some parallels with Thomas, Chess and Birch's (1968) 'goodness-of-fit' theory, which suggests that the degree of match or mismatch

between a child's characteristics (temperament, capacities and motivations) and the demands and expectations of the caregiving environment in which he or she functions is an important determinant of behavioral adjustment.

Our aim in this study was to examine whether allelic variations in the 5-HTTLPR moderate risk for depression in the context of low levels of positive parenting (a form of deprivation), whilst controlling for the effect of high levels of negative, hostile parenting (a form of threat), in two longitudinal studies. This approach enabled us to test the same conceptual model of the relationship between positive parenting and depression in independent samples using different methods of measurement. There is a particular need for such replications given the inconsistencies in findings to date regarding GxE interactions involving the serotonin transporter gene. Based on previous studies indicating poor outcomes in LL genotype children and adolescents exposed to low nurturant environments, we predicted L-allele homozygous individuals would show greater vulnerability to depression in these contexts, relative to S-allele carriers.

Study 1

Method

Participants and Procedures. Participants were from the Australian Temperament Project (ATP). The original ATP cohort comprised 2,443 4-8 month old infants and their families, recruited through Maternal and Child Health centers in 1983. Families have been surveyed by mail generally every 1-2 years. Full descriptions of the background, sampling and design of the ATP can be found in Prior, Sanson, Smart & Oberklaid (2000). The subsample used for the current analysis consisted of the 681 participants (355 male) who had provided a DNA sample for genotyping purposes. Genetic samples were collected from participants who could

conveniently be visited at home. These participants therefore tended to be located in more urban areas and were of higher SES than participants who did not provide genetic samples, but the two groups did not differ on the variables of interest (parenting measures at 13-14 years and depressive symptoms at 17-18 years). Participants were identified as of either Anglo/European-Australian (96.8%) or non-Anglo/European Australian (3.2%) descent, based upon parental country of birth. The analysis also draws on survey data collected when participants were 13-14 years and 17-18 years old.

Measures.

Depressive Symptomatology at age 13-14 years and 17-18 years. Depressive symptomatology was measured by the self-report version of the Short Mood and Feelings Questionnaire (SMFQ; Angold et al., 1995), which has high reliability (**α = .87)** in the overall ATP sample.

Parenting. Positive parenting, in the form of parental warmth (e.g., I enjoy listening to and doing things with my child), and harsh, aversive parenting, in the form of physical punishment (e.g. how often do you hit, slap or spank your child?) at age 13-14 years were measured according to the ATP-devised Parenting Practices Questionnaire (Letcher et al., 2004), which is based on parent report. The Parental Warmth scale and the Physical Punishment scale have shown adequate reliability (α = .74 and α = .66 respectively) in the overall sample and have demonstrated good criterion validity (e.g., lower warmth and higher physical punishment have predicted higher levels of child internalizing and externalizing problems (Letcher et al., 2004)).

Genotyping. Buccal epithelial cells were collected via cotton swabs when participants were between 15 and 18 years old. Genomic DNA was isolated from the cells using QIA ampblood DNA kits (QIAGEN, Hilden, Germany). Polymerase chain reaction (PCR) primers

and conditions were as described by Heils et al. (1996). The method used for visualization of the PCR products in the ATP study has been described previously (Jorm et al., 2000). The genotype distribution for 5-HTTLPR (n = 222, LL, n = 346 SL, n = 113, SS) was in Hardy-Weinberg equilibrium χ^2 (1, N = 681) = 1.25, p=.263.

Analysis Plan

Primary analysis. As the majority of studies have converged on dominance of the S-allele over the L-allele (e.g., Canli & Lesch, 2007; Heils et al., 1996), we focused our analyses on a dominant genetic model (LL=0, SL+SS[i.e., S-carriers]=1).

Path models were specified to investigate the moderating effect of 5-HTTLPR genotype on the relationship between parental warmth and depressive symptoms, with adolescent gender, ethnicity and physical punishment as covariates. The hypothesized model outlining the tests for moderating effects, which also includes potential evocative gene-environment correlations (rGE) between genotype and parenting, is presented in Figure 1. A covarying path between gender and ethnicity was not specified in the model as gender and ethnicity would not be expected to be related. Path models were calculated using the maximum likelihood estimator in *Mplus* (Muthén & Muthén, 1998-2012) and were based on 5000 bias-corrected bootstrapped samples.

[INSERT FIGURE 1 HERE]

Prior to estimating the models, all continuous predictor variables and covariates were centered to reduce problems with multicollinearity. The interaction term was created by multiplying genotype and parental warmth. Significant interactions were clarified through post hoc analyses assessing whether the simple slopes representing associations between parental

warmth and depressive symptomatology were significantly different from zero for the different genotypes (Preacher, Curran, & Bauer, 2006).

In addition, to assess the possibility of differential susceptibility, Roisman and colleagues (2012) have recommended that investigators conduct regions of significance (RoS) tests to determine the full range of values of the predictor X, (i.e., parenting) for which the association between the moderator Z (i.e., 5-HTTLPR genotype) and Y (i.e., depressive symptoms) is significant. Roisman et al. (2012) recommend that results consistent with differential-susceptibility predictions would require a significant moderator-outcome association at both the low end of X and the high end of X. Roisman et al. (2012) suggested a guideline of bounding the range of X by +/-2 SD for the RoS tests to reduce the likelihood that values of X are not represented in the sample, however, they also note that this approach is sensitive to sample size, and that it is not uncommon for plots that look highly consistent with a pattern of differential susceptibility to be incorrectly classified as providing evidence for diathesis-stress as a result of low statistical power. The authors therefore additionally recommend the use of a metric named the Proportion of interaction (PoI) index, a measurement of the proportion of the total area between the two lines for each genotype group that comprise the interaction plot bounded by+2 SD on X, that is above the crossover point. In a prototypical interaction plot for differential susceptibility (i.e., a cross-over or disordinal interaction), the lines would be expected to cross over at the mean of X, resulting in 50% of the area bounded by the regression lines representing the "for better" region. In a prototypical plot for diathesis–stress (i.e., an ordinal interaction), the crossover point will occur on the far right side of the plot, such that 0% of the total area would represent the "for better" region. Roisman et al., (2012) initially specified that, as an approximate marker, interactions with values on the PoI metric between about 0.40 and 0.60

could be considered highly consistent with differential susceptibility. More recently Del Giudice (2017) has proposed a revision based on a .20-.80 range of PoI values given concerns that the narrower window of .40-.60 may be associated with a high likelihood of false negatives, whilst the .20-.80 window improves detection with little elevation in the rate of false positives. As noted by both Del Giudice (2017) and Salvatore and Dick (2015), there can however be difficulties with classifying variants as differential susceptibility loci by such methods, given measures of the environments typically do not have true zeros. As such, the range of environments captured for any given sample (i.e. high or low risk) will affect the shape of the observed interaction. To generate RoS on Z and PoI, we used a web-based program available at http://www.yourpersonality. net/interaction/ that is a supplement to the paper by Roisman and colleagues (2012) developed by the author Fraley.

Follow-up Analyses. The primary interest of this study was whether the lack of a positive environment (i.e. reduced warmth, or positive behaviors displayed by parents) would alter risk for depression differently in S-carriers versus L-homozygous individuals. A large literature however suggests that S-carriers are more susceptible to the presence of harsh, negative environments (such as those involving significant child maltreatment or stressful life events) compared to L-homozygous individuals. We therefore also examined whether 5-HTTLPR interacted with parental use of physical punishment, controlling for gender, ethnicity and parental warmth.

To further clarify the nature of the interactions, some additional exploratory analyses were conducted. First, due to possible variation in in allelic frequencies amongst different racial groups, analyses evaluating the interaction between 5-HTTLPR genotype and parenting were tested separately in the group of participants of Anglo-European background (n= 656). Second,

we completed a set of analyses that additionally controlled for baseline depressive symptoms at 13-14 years. Inclusion of baseline depressive symptomatology as a covariate allowed an examination of whether the interaction predicted prospective *change/growth* in depressive symptomatology over adolescence rather than *absolute* depressive symptomatology at the end of adolescence. The addition of this covariate introduces seven new paths into analyses. The reductions in power associated with this inclusion also means that these analyses should be interpreted with some caution.

Given that "dose-related" additive effects of the S-allele in addition to dominance effects have been documented by some studies (e.g., Caspi et al., 2003), with recessive effects being observed far less frequently (e.g., Williams et al., 2003), all of these analyses were rerun based on an additive genetic model (LL=0, SL=1, SS=2).

Missing data. Missing data averaged 12.1% (range 0-16.9%). Analyses presented in the Supplementary Section suggested that data were missing at random (MAR). Missing data were therefore accounted for by the Full Information Maximum Likelihood (FIML) method, to increase statistical power and to make optimal use of the data. FIML is recommended in situations where data are MAR, including when a large proportion of participants are missing data (Schlomer, Bauman, & Card, 2010), and has been found to be less biased and more efficient than deletion and single-imputation methods (Enders & Bandalos, 2001).

Results

Descriptive statistics, including intercorrelations between depression, variation in the serotonin transporter polymorphism, ethnicity, gender, parental warmth and physical punishment are shown in Table 1.

[INSERT TABLE 1 HERE]

The bivariate correlation between 5-HTTLPR genotype and parental warmth was not significant, suggesting that any GxE effects are not a function of an evocative gene-environment correlation (rGE).

Primary Analysis. Model fit indices showed that model provided an acceptable fit to the data (see Supplementary Table 1). Path model results are displayed in Table 2. For parsimony, only key relationships of interest between the independent variables (5-HTTLPR, parental warmth, and the 5-HTTLPR x parenting interaction term), covariates (gender, ethnicity and physical punishment) with the dependent variable (depressive symptomatology) and as well as the covarying association between 5-HTTLPR and positive parenting, are shown here. Results of the complete models, including other covarying paths between independent and covariate variables, are provided in Supplementary Table 2.

[INSERT TABLE 2 HERE]

The model explained 12% of the variance in depressive symptoms, as indicated by the R^2 value (0.12). Results indicated a significant path from lower parental warmth at 13-14 years to higher levels of depressive symptomatology at age 17-18 years. There was no main effect of physical punishment or 5-HTTLPR genotype on adolescent depression, nor was genotype related to parental warmth, physical punishment or to participant ethnic background. Female gender was significantly related to higher depressive symptomatology and parental warmth and to lower levels of physical punishment. Lower parental warmth was significantly associated with higher physical punishment. There was a significant 5-HTTLPR X parental warmth interaction effect on depressive symptomatology, which is shown in Figure 2.

[INSERT FIGURE 2 HERE]

The interaction indicated that parental warmth significantly predicted depressive symptoms for the L-homozygous group (b=-.29 [95% CI: -.43, -.15], S.E.=.07, β=-.29, p=.0001) but not the S-carrier group (b=-0.08 [95% CI: -0.19; 0.02], S.E.=.05, β=-.08, p=.126). S-carriers showed a stable risk for depressive symptoms that was independent of parental warmth, whereas L-homozygous individuals showed increasing risk for depressive symptoms as a function of decreasing levels of parental warmth.

For the RoS on X test, the regression of depressive symptoms on serotonin transporter genotype is statistically significant for all values of positive parenting that fall outside of the range of [-0.30; 2.53]. As the upper bound exceeds 2SD, this finding suggests the association between genotype and depression is predominantly significant when positive parenting is lower, and the interaction is considered to be more consistent with diathesis stress rather than differential susceptibility. However, the PoI = .36 may be interpreted as providing moderate support for a differential susceptibility model, given it is within the range of .20-.80 that is considered as consistent with differential susceptibility and only just outside of the range of 0.40-0.60 specified as providing strong support for differential susceptibility model.

Follow-up Analyses. There was no evidence of a significant interaction between 5-HTTLPR and negative parenting (parental use of physical punishment), as shown in Supplementary Table 3. The finding of an interaction between 5-HTTLPR and parental warmth predicting depression cannot be accounted for by an association between parental warmth and physical punishment.

The same patterns of findings involving a significant interaction between 5-HTTLPR (analysed according to an S-allele dominant model) and parental warmth were observed when

models were rerun for the largest ethnic subsample (n=656) of participants of Anglo-European background (Supplementary Table 4). The interaction involving physical punishment remained non-significant (Supplementary Table 5). The interaction between 5-HTTLPR and parental warmth was no longer significant when baseline depressive symptoms were included in the model (Supplementary Table 6). The interaction between 5-HTTLPR and physical punishment controlling for baseline depressive symptoms also failed to predict depressive symptoms at 17-18 years (Supplementary Table 7).

None of the interactions between 5-HTTLPR x parenting were significant when an additive genetic model was used, as shown in Supplementary Tables 2-7.

Study 2

Method

Participants and Procedures. The analyses in Study 2 are based on an initial subsample of 176 participants from the longitudinal Orygen Adolescent Development Study (ADS), conducted in Melbourne Australia, who had provided a genetic sample during the course of their participation. Of the 176 participants, one participant was diagnosed with Major Depressive Disorder at the diagnostic assessment during the first wave of the study (W1) and another was diagnosed with Major Depressive Episode within the context of a Bipolar I disorder during the course of the study. These two participants were excluded from this research to enable the study to be prospective in relation to MDD onset specifically (rather than affective disorders more broadly), leaving a total sample of 174 participants (71% of the total sample of 245 participants; 83 male).

The broad recruitment and screening of ADS participants has been fully reported previously (Yap, Whittle, Yucel, Sheeber, Pantelis, et al., 2008). Briefly, the sample, drawn from the general community of final year primary school students in metropolitan Melbourne, was risk-enriched based on scores on the temperament dimensions of Negative Emotionality and Effortful Control, measured according to the Early Adolescent Temperament Questionnaire-Revised (Ellis & Rothbart, 2001) given their hypothesized role as vulnerability factors for emotional and behavioral disorders. Participants in the current analyses were identified as of either Anglo-European (87.7%) or non-Anglo-European (12.3%) background, based upon their grandparents' country of birth.

The ADS involved four waves of data collection: W1 (*M* age 12.7 years, range 11.4 -13.7 years) included a diagnostic interview that assessed for current and lifetime episodes of MDD to exclude participants with a history of the disorder, and a family-interaction assessment, which allowed observation and coding of parenting behavior. Study 2 examines depressive symptoms at age 18-19 years collected via questionnaire at the fourth and final wave of the study (W4) as the outcome of interest, to closely replicate Study 1.

Measures.

Depressive symptomatology at 11-13 years and 18-19 years. Depressive symptomatology was measured according to the Centre for Epidemiological Symptoms Depression Scale (CESD; Radloff, 1977). The CESD consists of 20 items, rated on a 4-point scale from 0 (rarely or none of the time) to 3 (most or all of the time).

Parenting. The frequency of positive and aversive parenting behaviors displayed by mothers was assessed during two 20-minute parent-child interaction tasks at W1, which were videotaped for coding. An event-planning task was completed first, followed by a problem-

solving task. The tasks were intended to differentially elicit positive and negative behavior, respectively, thereby enabling an explicit examination of the effect of the interactional context on affective processes. Our previous work has indicated that negative parental behavior displayed during the positive EPI task and positive parental behavior during the negative PSI task may be particularly salient predictors of adolescent depression Schwartz et al. (2016). The ordering of tasks was fixed because of concern that negative affective states elicited by the problem-solving task had the potential to persist into the positive, event-planning task if the latter were conducted second.

For the event-planning interaction (EPI), mothers and adolescents were instructed to plan one or more pleasant events to do together, with up to five events chosen based on items that both the mother and adolescent rated as being '*very pleasant*' on the Pleasant Events Schedule (MacPhillamy & Lewinsohn, 1976). For the problem-solving interaction (PSI), up to five issues for discussion were selected that both the mother and adolescent endorsed as occurring the most frequently and generating the highest intensity of anger on the Issues Checklist (Prinz, Foster, Kent, & O'Leary, 1979). Parenting behavior from the tasks was coded according to the Living in Family Environments (LIFE) coding system. The LIFE (Hops, Biglan, Tolman, Arthur, & Longoria, 1995) is an observational, microsocial coding system that enables a detailed analysis of individual family members' behaviors and interactive family behaviors. In this study, the constructs of interest were the frequency of positive behaviors and aversive behaviors displayed by mothers on both the EPI and the PSI. Positive behavior included displays of happy, pleasant, and caring affect as well as approving, validating, affectionate or humorous comments made with neutral affect. Aversive behavior included all events with contemptuous, angry, and belligerent affect, as well as disapproving, threatening, or argumentative verbal content with

neutral affect. Approximately 20% of the interactions were coded by a second observer to provide an estimate of observer agreement. Kappa coefficients (a conservative index of interobserver reliability based on point-by-point agreement and corrected for chance) for the Positive and Aversive behavior constructs were 0.86 and 0.70 respectively. The validity of the LIFE system as a measure of family processes has been established in numerous studies (e.g., Sheeber, Davis, Leve, Hops & Tildesley, 2007).

Genotyping. Saliva was collected from participants for genetic analysis using Oragene DNA saliva collection kits (www.dnagenotek.com). Methods used for PCR amplification and visualization by gel electrophoresis were as described by Edenberg & Reynolds (1998). The genotype distribution for 5-HTTLPR (n = 54, LL, n = 83SL, n = 37, SS) was in Hardy-Weinberg equilibrium χ^2 (1, N = 174) = .24, p=.627.

Analysis Plan

The same analytic strategy employed in Study 1 was used to predict continuous depressive symptoms in Study 2, except that two separate path models were estimated to document effects of positive parenting in the EPI task and the PSI task.

Treatment of missing data. Levels of missing data averaged 13.3% (range 0-28.6%). Little's (1988) MCAR test was non-significant, $\chi^2(163)=179.54$, p=.178, therefore FIML was used to account for missing data.

Results

Correlations between variables in Study 2, namely depressive symptoms, serotonin transporter polymorphism variation, ethnic background, gender, positive parenting and aversive parenting in the two interaction tasks, are shown in Table 3. 5-HTTLPR genotype and positive

maternal behavior in the PSI (though not in the EPI) task were significantly correlated (r=.22, p<.05), indicating that a GxE effect between these two variables could be a function of evocative rGE. Aversive maternal behavior in the EPI and the PSI were not significantly correlated with 5-HTTLPR genotype.

[INSERT TABLE 3 HERE]

Primary Analyses. Model fit indices showed that all models in Study 2 provided an acceptable fit to the data (see Supplementary Table 1). Results for the paths from independent variables (5-HTTLPR, positive parenting, and the 5-HTTLPR x positive parenting interaction term) and covariates (gender, ethnicity and aversive parenting) predicting depressive symptoms, as well as the covarying association between 5-HTTLPR and positive parenting, are presented in Table 4. Results of the complete models are provided in Supplementary Table 8. The model for the EPI task explained 12% of the variance in risk for depressive symptomatology (R^2 =.12), whilst the model for the PSI task explained 9% of the variance in risk for depressive symptomatology (R^2 =.09). In the EPI, both lower frequencies of positive maternal behavior and higher frequencies of aversive maternal behavior at age 12-13 years was associated with higher levels of depressive symptomatology at 18-19 years. Lower positive maternal behavior was also related to higher aversive maternal behavior. Gender and ethnicity did not show significant associations with depressive symptoms, parenting or genotype. Neither 5-HTTLPR genotype nor the interaction between 5-HTTLPR genotype and positive maternal behavior were significant predictors of depressive symptomatology.

In the PSI, low frequencies of positive maternal behavior were associated with more frequent aversive maternal behavior as well as with higher levels of depressive symptomatology in late adolescence. Aversive maternal behavior however was not associated with later

depressive symptoms. Gender and ethnicity were also unrelated to depressive symptoms, genotype and parenting. 5-HTTLPR genotype was associated with positive maternal behavior at trend level (p=.054), but not with aversive maternal behavior. Critically, the interaction between 5-HTTLPR genotype and positive maternal behavior was significant.

The interaction, graphed in Figure 3, indicated that the frequency of positive maternal behavior was predictive of depressive symptoms for L-homozygous individuals (b=-6.28 [95% CI: -11.26 ; -1.30], S.E.=2.54, β=-.46, p=.014) but not S-carriers (b=-0.10 [95% CI: [-3.42 ; 3.22], S.E.=1.70, β=-.09, p=.953). S-carriers' risk for depressive symptoms was observed to remain stable, independent of the frequency of positive maternal behavior experienced, whilst L-homozygous individuals' risk increased as a function of decreased frequencies of positive maternal behavior. RoS analysis indicated that the association between serotonin transporter genotype and depressive symptoms was significant for all values of positive maternal behavior outside of the values of [-2.21, .72]. As the lower bound exceeds 2SD, this finding suggests the association between genotype and depression is predominantly significant when positive parenting is higher (indicative of a buffering effect of positive parenting on depression risk for L-homozygyous individuals relative to S-carriers). However, the PoI = .58, which may be interpreted as providing high support for a differential susceptibility model resembling a cross-over interaction with a cross-over close to the mean.

[INSERT FIGURE 3 HERE]

Follow-up analyses. As in study 1, we did not find evidence that 5-HTTLPR interacted with aversive parenting to predict depressive symptomatology (see Supplementary Table 9). The finding of an interaction between 5-HTTLPR and positive parenting predicting depression therefore cannot be accounted for by an association between positive parenting and aversive

parenting. Interactions were also non-significant when analyses were rerun according to an additive model (see Supplementary Table 8 and 9).

We additionally ran path models separately for the largest ethnic subsample of participants of Anglo-European background (n=150), which are displayed in Supplementary Tables 10 and 11. When the S-allele was treated as dominant, the size of the standardised coefficient of the interaction between positive maternal behaviour x 5-HTTLPR (β=.31) was very similar to that obtained for the overall sample, though this finding was no longer significant (*p*=.089), presumably reflecting the decrease in power associated with a smaller sample size.

As shown in Supplementary Tables 12 and 13, analyses were also rerun with the inclusion of baseline depressive symptomatology as a covariate to allow an examination of whether the interaction predicted prospective *change/growth* in depressive symptomatology over adolescence. The interaction between 5-HTTLPR and positive parenting in the PSI remained significant when an S-allele dominant genetic model was assumed and non-significant when an additive genetic model was assumed. In addition, significant interactions between 5-HTTLPR and positive parenting in the EPI emerged for both S-dominant and additive genotype models. Specifically, lower frequencies of positive maternal behaviour significantly predicted depressive symptoms for the L-homozygous group but not for S-carriers.

Discussion

The current results provide evidence of an interaction between 5-HTTLPR and low levels of positive parenting in predicting depression. In two independent cohorts, findings indicated that when the S-allele of the serotonin transporter gene was coded as dominant, adolescents carrying at least one copy of the S-allele showed little change in their risk of depression as a function of the positive parenting they received, whilst adolescents in the L-homozygous group were at

greater risk for depression with decreasing levels of positive parenting. Overall, the findings conflict somewhat with the more traditional view of the differential susceptibility hypothesis, which has suggested that the S-allele is a "plasticity" allele that increases general sensitivity to environmental effects whilst the homozygous L disposition is associated with more fixed outcomes across environments (Belsky, et al., 2009). This pattern of results is consistent with findings by other studies demonstrating that L-homozygous individuals who experience low maternal responsiveness or lack of supportive parenting may be more vulnerable to externalizing difficulties (e.g., Davies & Cicchetti, 2013; Lavigne et al., 2013), and with one previous study finding a trend suggesting that L-homozygous girls may exhibit higher depressive symptoms than S-carriers in family environments involving low levels of support (Li, et al., 2013). Taken together these studies constitute an emerging body of research that suggests that in certain contexts L-homozygous individuals may also be vulnerable to maladaptive outcomes.

It is noteworthy that the current findings were obtained in two longitudinal cohorts, based on independent samples, with different measures of depression (i.e., depressive symptomatology using a self-report scales at 17-18 years in study 1 and at 18-19 years in study 2) and different methods of measuring positive parenting (i.e., parental warmth according to parent-report versus an observational measure of positive parental behavior).

Somewhat surprisingly, the association between positive parenting and depression was non-significant for S-carriers in both Study 1 and Study 2, suggesting that S-carriers were neither at increased risk for depression in more deprived environments of lower positive parenting, but they also did not appear to be buffered from depression in arguably more, supportive environments of higher positive parenting. Whilst the former finding was in line with the hypotheses of the current study, the latter finding might be interpreted by some as a contradiction

of the hypothesis that S-carriers demonstrate vantage sensitivity – a proclivity to benefit from enriched environments. We would contend however, in line with positive development/adjustment research which views positive functioning and wellbeing as distinct from (albeit partly related to) the absence of mental ill-health (Tolan, Ross, Arkin, Godine, & Clark, 2016), that the lack of a protective effect of high positive parenting on depression risk for S-carriers relative to LL-homozygous individuals does not necessarily mean that enhancing effects of positive parenting on component behaviors, capabilities, and experiences of more positive functioning would not be present.

There was also evidence that S-carrier status was correlated with higher levels of observed positive parenting behaviors during the PSI in study 2 (though a similar association was not detected in study 1, which was based on parent report). This finding could indicate an evocative gene-environment correlation (rGE) that would be consistent with the possibility that S-carriers are better able to elicit warmth or nurturance from their parents. However, as parent genotype was not available in the current study, the possibility that genetic relatedness between the parent and the adolescent accounts for the observed correlation between adolescent genotype and positive parenting, such that parent genotype may in fact be predicting the levels of their own positive behavior (a passive rGE; Plomin, DeFries, & Loehlin, 1977) cannot be ruled out. It is noteworthy that a different study that also relied on observational methods of parenting found the S-allele of the serotonin transporter gene in boys predicted higher levels of mothers' positive parenting, with this effect being mediated by greater self-control exhibited by the child (Pener-Tessler et al., 2013). Interestingly, whilst there was also an association between mothers' serotonin transporter genotype and positive parenting, the effect of boys' 5-HTTLPR genotype on parenting remained significant following the inclusion of mothers' genotype in the model,

suggesting that the association between the child's genotype and parenting could not be solely attributed to a passive rGE and supporting a hypothesis for the role of an evocative rGE.

Contrary to expectations, the interaction between the serotonin transporter gene was not found to moderate risk for depression in family environments involving more hostile and punitive parenting in both samples. However, null findings in the broader serotonin transporter gene x environment literature are certainly not uncommon (Sharpley et al., 2014). Moreover, several studies have failed to identify an interaction between the serotonin transporter gene and negative parenting specifically in predicting depression (Fergusson, Horwood, Miller, & Kennedy, 2011; Lavigne et al., 2013). Recent reviews suggest that the interaction implicating S-carriers may be most readily detected when relatively extreme forms of adverse, threatening environments, such as those involving significant child maltreatment are considered (Caspi et al., 2010). It is possible that the degree of threat or adversity captured by the negative parenting measures in both the current and some other studies with null findings were not severe enough to reveal the interaction. We have identified however in the ADS sample that inclusion of hippocampal volume as an intermediate phenotype in a pathway from the serotonin transporter gene to MDD onset during the adolescent period reveals potential S-carrier vulnerability to depression in the context of negative parenting (Little et al., 2015). Specifically, possession of a greater number of S-alleles was associated with smaller hippocampal volume, and the specific variance in hippocampal volume accounted for by genotype was in turn associated with increased risk for MDD onset, but only in the context of more negative, punitive maternal behavior. This imaging gene-environment study suggests that inclusion of intermediate phenotypes such as brain structure in analyses may assist in the detection of otherwise unapparent relationships between genes, the environment and behavioral outcomes.

A strength of the current GxE study involving the serotonin transporter gene is the systematic investigation of the impact of an environment involving a form of *deprivation* on the maladaptive outcome of depression. This study is in contrast with the vast majority of research investigating GxE effects, which to date has tended to focus on the relationship between positive environments and positive outcomes, or threatening environments and negative outcomes. We believe that this study makes a valuable contribution to current theoretical understanding of associations involving the serotonin transporter gene, environments, and psychological outcomes by differentiating between interactions of deprivation versus threat. It may also offer a potential explanation for the sizable group of GxE studies that have identified null findings, some of which may have examined environments involving both deprivation and threat, and hence were not able to identify the effects of one allele over the other on risk for psychological difficulties. Future research would benefit from replicating the current findings in additional cohorts and extending them by considering other theoretically grounded environmental contexts that might be expected to show differential effects for S-carriers and L-homozygous individuals.

There are several limitations in the current study that should be noted. First, as noted above, although there is a body of *a priori* theoretical and empirical research supporting an association between 5-HTTLPR, stress sensitivity, emotional reactivity and social cognition (Canli & Lesch, 2007; Caspi et al., 2010; Glenn, 2011), which we have speculated may underlie the specific GxE interaction investigated here, this putative mechanism was not explicitly tested. A second limitation is our consideration of only one gene in the current research design, despite general acknowledgement that depression represents a highly complex polygenetic condition (Sullivan et al., 2000). We purposely selected 5-HTTLPR because the evidence supporting its involvement in GxE interactions is relatively advanced compared to other genes (Caspi et al.,

2010), whilst noting emerging evidence supporting its role in multilocus polygenetic profiles, gene–gene interactions and gene-gene-environment interactions in conferring risk for psychopathology (e.g., Ressler et al., 2010; Vrshek-Schallhorn et al., 2015). In addition, we did not analyze the minor allele rs25531, which comprises a single-nucleotide variant (A→G) within the L polymorphism that renders an L_g allele functionally similar to the S variant (Hu et al., 2006). Thus, it is possible that some LL or LS genotypes would have been better classified with the S-allele in the current study. However, the current classification would be expected to be associated with an attenuated effect or false negative rather than a false positive result.

Furthermore, whilst prior research has strongly implicated parenting factors in the development of child/adolescent depression, the exact degree to which parenting factors measured in the current study represent causal influences remains somewhat unclear due to issues regarding the direction of effects. It is conceivable that child depression could evoke, reinforce or shape particular parenting behaviors, and therefore that the parenting constructs in the current study may reflect a response to their adolescents' depressive behaviors to some extent. As we did not have information about parent genotype, we were also not able to rule out the possibility of a passive rGE. At least one previous study has noted the possibility of passive rGE processes in the association between parenting and children's depression, which may be underpinned by parental depressive symptomatology (Rice, Lewis, Harold, & Thapar, 2013). Finally, the samples in the current analyses are quite small for genetic analyses, and the number of participants in the analyses in Study 2 in particular might be considered preliminary. It is possible that our sample sizes may have limited power to detect smaller effects. Equally, there may be results that are "false positives". These results (perhaps particularly the nonsignificant

findings of small effect size), should be interpreted with caution until they are replicated by studies with larger samples.

In summary, results from two independent studies suggest that L-homozygous individuals may be more sensitive than S-allele carriers to the depressogenic effects of low positive parenting. This finding suggests that it is not only the S-allele that determines environmental sensitivity. Rather, consistent with a differential *capability* framework, both alleles can confer sensitivity to a maladaptive outcome such as depression (as well as potentially positive outcomes), dependent on the match or mismatch of the phenotypic characteristics of the individual and the challenges posed by the environment in which they are developing.

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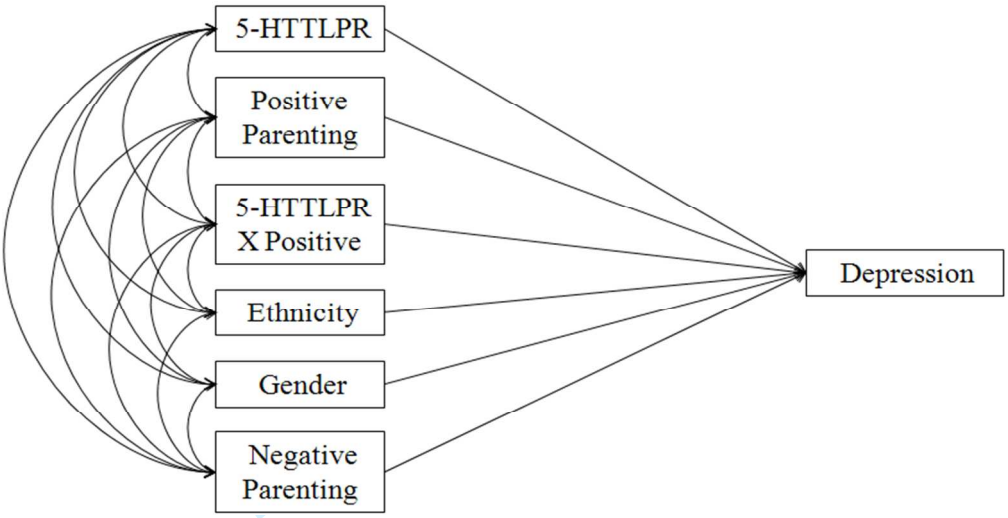

Figure 1. Hypothesized conceptual model outlining pathways examined in testing Gene X Parenting effects on adolescent depression

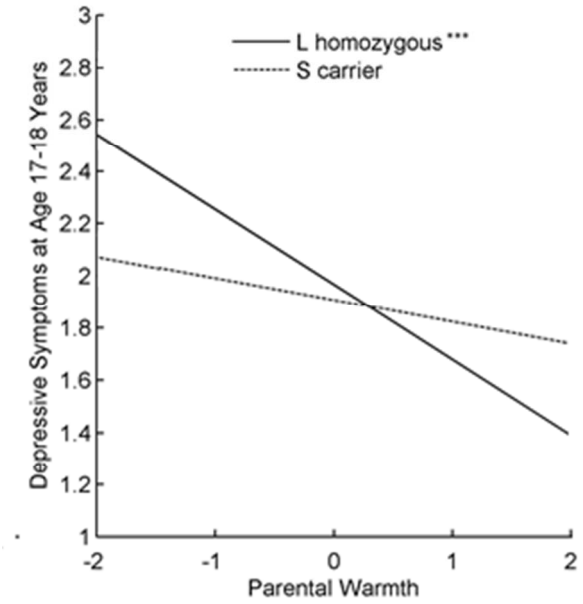

Figure 2. Influence of parental warmth at age 13-14 on depressive symptoms at 17-18 years for L homozygous individuals and *s*-carriers in Study 1. . *= p <.05; **= p <.01; ***= p <.001

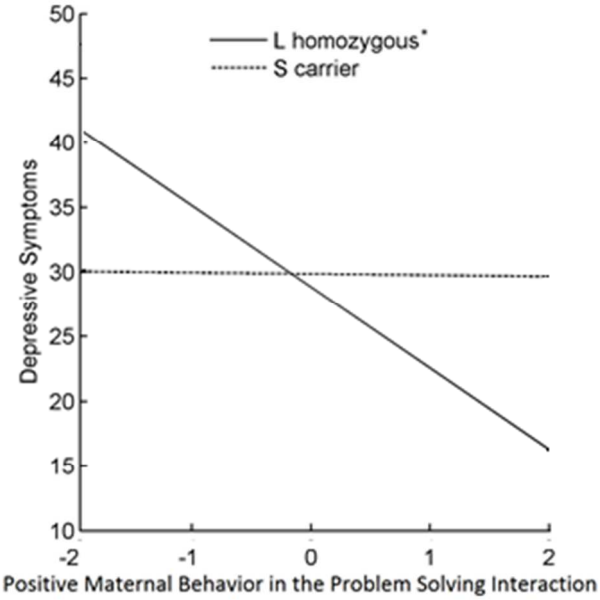

Figure 3. Influence of positive maternal behavior experienced at 11-13 years on depressive symptoms at 18-19 years for L homozygous individuals and *s*-carriers in Study 2. *= p <0.05; **= p <0.01; ***= p <.001

Table 1.

Descriptive statistics and intercorrelations for variables from the ATP in Study 1.

Variables	1	2	3	4	5	6	7
1. Depressive symptoms at 17-18 years	-	.33***	.11	-.02	-.12**	.06	0.46***
2. Gender (0=male, 1=female)		-	-.08	-.03	.15**	-.14**	0.137**
3. Ethnicity (0 = Aust-European descent, 1 = non-Aust-European descent)			-	-.08	-.10	-.12	0.191
4. Dominant serotonin transporter genotype (0 =LL, 1=SS or SL)				-	.00	-.02	-0.103*
5. Parental warmth					-	-.12***	-0.151***
6. Parental Physical Punishment						-	0.118**
7. Depressive symptoms at 13-14 years							-
Percentage of sample or *M* (SD)	2.10 (.60)	Males= 52.2%	Aust-Europe descent = 96.8%	LL=32.6%	4.21 (.60)	1.25 (.47)	4.30 (3.35)

$p \leq 05$ = *, $p \leq .01$ = **, $p \leq .001$ = ***

Table 2

Path model testing the interaction between 5-HTTLPR genotype x parental warmth at 13-14 years on depressive symptomatology at 17-18 years in Study 1.

Pathway	b	SE	Lower 95% CI	Upper 95% CI	β	*p*
5-HTTLPR → Depressive symptoms	-.06	.05	-.16	.04	-.05	.245
Parental warmth → Depressive symptoms	-.29	.07	-.43	-.14	-.29	.000
Physical punishment → Depressive symptoms	.10	.06	-.01	.21	.08	.080
Ethnicity → Depressive symptoms	.16	.15	-.11	.47	.05	.268
Gender → Depressive symptoms	.34	.05	.25	.44	.29	.000
5-HTTLPR X Parental warmth → Depressive symptoms	.20	.09	.02	.39	.16	.028
5-HTTLPR ↔Parental warmth	.00	.01	-.02	.03	.01	.777

Table 3

Descriptive statistics and intercorrelations for variables from the ADS in Study 2.

Variables	1	2	3	4	5	6	7	8	9
1. Depressive symptoms at 18-19 years	-	.15	.11	.03	-.19*	-.18	.27**	.13	.33***
2. Gender (male = 0, female = 1)		-	-.004	.13	-.08	-.05	.07	-.07	-.12
3. Ethnicity (0 = Aust-European descent, 1 = non-Aust-European descent)			-	.22	-.26	-.21	.05	.12	-.12
4. Dominant serotonin transporter genotype (0 =LL, 1=SS or SL)				-	.12	.24*	-.12	-.11	-.04
5. Positive Parent behavior EPI					-	.41***	-.31***	-.26**	.08
6. Positive Parent behavior PSI						-	-.43***	-.44***	-.14
7. Aversive Parent behavior EPI							-	.52***	.18*
8. Aversive Parent behavior PSI								-	.11
9. Depressive symptoms at 11-13 years									-
Percentage of sample or *M* (SD)	30.92 (9.29)	Male =47.70%	Aust-Europe descent = 87.70%	LL= 31%	2.368 (.64)	1.752 (.68)	.57 (.41)	1.26 (.61)	31.21 (9.50)

$p \leq 05$ = *, $p \leq .01$ = **, $p \leq .001$ = ***

Table 4

Path model testing the interaction between 5-HTTLPR genotype x positive maternal behaviour at 11-13 years on depressive symptomatology at 18-19 years.

	EPI Task						PSI Task					
	b	SE	95% CI		β	*p*	b	SE	95% CI		β	*p*
			Lower	Upper					Lower	Upper		
5-HTTLPR → Depressive symptoms	1.04	1.61	-2.18	4.19	.05	.520	1.02	1.58	-2.04	4.16	.05	.517
Positive parenting → Depressive symptoms	-5.19	2.39	-10.20	-.68	-.27	.030	-6.28	2.32	-11.36	-2.19	-.46	.007
Aversive parenting → Depressive symptoms	5.31	2.14	1.09	9.52	.23	.013	.80	1.88	-2.96	4.41	.05	.672
Ethnicity → Depressive symptoms	1.28	2.36	-3.33	5.92	.05	.587	1.19	2.35	-3.62	5.67	.04	.614
Gender → Depressive symptoms	1.82	1.54	-1.05	5.01	.10	.238	1.81	1.57	-1.15	5.00	.10	.248
5-HTTLPR X Positive parenting → Depressive symptoms	5.86	3.50	-.93	12.79	.23	.094	6.18	2.87	.58	11.94	.37	.031
5-HTTLPR ↔Positive parenting	.02	.02	-.03	.06	.09	.375	.05	.03	.00	.11	.17	.054

CHAPTER 5: INTERACTION BETWEEN THE L-ALLELE OF THE SEROTONIN TRANSPORTER GENE POLYMORPHISM AND POSITIVE PARENTING PREDICTS ONSET OF MAJOR DEPRESSIVE DISORDER: A PROSPECTIVE LONGITUDINAL STUDY

A set of analyses that focused on the prediction of first onset of Major Depressive Disorder during adolescence was included in the original submission of the paper that comprises CHAPTER 4 (which considers depressive symptomatology during adolescence) but was removed at the request of reviewers who had some concerns about power given the sample size. However, there is a particular need for such conceptual replications given the inconsistencies in findings to date regarding GxE interactions involving the serotonin transporter gene. The onset of MDD, which represent the presence of a clinically meaningful level of symptoms, is an important outcome to model that is arguably worthy of some trade-off in power, particularly when determined according to semi-structured interview, which is the 'gold-standard' assessment of psychiatric illness. This is relevant because as noted by Moffit and Caspi (2014), "doing [assessment] well can pay for itself by reducing sample size" (pg.,2). Moreover, as noted in CHAPTER 3, there are concerns within the serotonin transporter gene literature about the extent of publication bias, including cherry picking of analyses for inclusion in journal articles. Interestingly, the observation has been made that where publication bias exists, reviewers tend to be more concerned about power associated with sample size when opposite findings to what might be expected are identified than when consistent findings with the status quo are obtained, and that this can contribute towards a publication bias (Duncan & Keller, 2011). Given

these concerns, and given that these analyses are important to the arc of the current thesis, they are presented here as additional empirical chapter.

The aim was to examine whether allelic variations in the 5-HTTLPR moderate risk for Major Depressive Disorder in the context of low levels of positive parenting or high levels of negative parenting. Based on the differential capability theory, which suggests LL-homozygous individuals could be more vulnerable to deleterious effects of deprivation, the findings of our previous study (Little et al., accepted) as well as other studies indicating poor outcomes (both internalizing and externalizing) in LL genotype children and adolescents exposed to low nurturant environments (Davies & Cicchetti, 2014; Laucht et al., 2009; Lavigne et al., 2013; Sulik et al., 2012), we predicted L-allele homozygous individuals who would show greater vulnerability to depression in these contexts. Based on the differential capability theory, which would suggest that individuals carrying an S-allele would be more vulnerable to the adverse effects of threatening environments (Belsky & Pluess, 2009), and studies based upon highly threatening experiences such as child maltreatment, which identified particularly deleterious outcomes in S-carriers (Sharpley et al., 2014), we hypothesized that individuals carrying an S-allele would show greater vulnerability to depression in contexts of more negative parenting. Alternatively, based on findings from our previous study (Little et al., accepted), as well as other findings that failed to find significant interactive effects of the serotonin transporter gene and negative parenting within the more normative range (Fergusson et al., 2011; Li et al., 2013), we hypothesized no interaction would be evident between 5-HTTLPR and negative parenting.

5.1 Method

5.1.1 **Participants and Procedures**

Participants were the same individuals that comprised the ADS participant group of Study 2 in CHAPTER 4. As described by Yap et al. (2008), participants recruited to the study completed four waves of data collection (W1-W4) capturing the age range 11 to 19 years. Wave 1 (*M* age 12.7 years, range 11.4 -13.7 years) included a diagnostic interview that assessed for current and lifetime episodes of MDD to exclude participants with a history of the disorder, and a family-interaction assessment, which allowed observation and coding of parenting behaviour. The diagnostic interview was repeated at waves two, three and four (W2-W4), which were conducted approximately two-and-a-half, four and six years after W1, respectively. The W2-W4 diagnostic interviews assessed for current MDD and any new episodes of MDD since the date of the last assessment. This study examines the first onset of Major Depressive Disorder during adolescence based on diagnostic interview as the outcome of interest.

Measures

Genotyping, parenting and ethnicity measures are identical to those described in CHAPTER 4.

MDD Onset. MDD was measured at each of the four study waves by the Schedule for Affective Disorders and Schizophrenia for School-Age Children, Present and Lifetime version (K-SADS-PL; Kaufman et al., 1997), a semi-structured diagnostic interview that assesses current and lifetime symptoms and diagnoses of Axis I disorders in youths aged 6 to 18 years. Diagnostic interview data from each of the time points was used to construct a variable indicating whether participants had experienced an onset of MDD between the W1

and W4 time points. Due to attrition, this variable was available for 137 of the 174 participants in the current study, and there were no differences between these participants and the other 37 participants with missing data, according to gender, $\chi 2(1) = .25$, $p = .616$, socio-economic status, $t[172] = -.99$, $p = .325$ and W1 depression symptoms (as measured by the CESD scale; Radloff, 1977), $t[160] = .77$, $p = .441$.

5.2 Analysis Plan

Path models were specified to investigate the moderating effect of 5-HTTLPR genotype on the relationship between either positive parenting or negative parenting and the dichotomous outcome of a first onset of Major Depressive Disorder (present/absent), with adolescent gender, ethnicity and the other parenting variable of interest as covariates. The hypothesized model outlining the tests for moderating effects, which also includes potential evocative gene-environment correlations (rGE) between genotype and parenting, is presented in Figure *5-1*. A covarying path between gender and ethnicity was not specified in the model as gender and ethnicity would not be expected to be related.

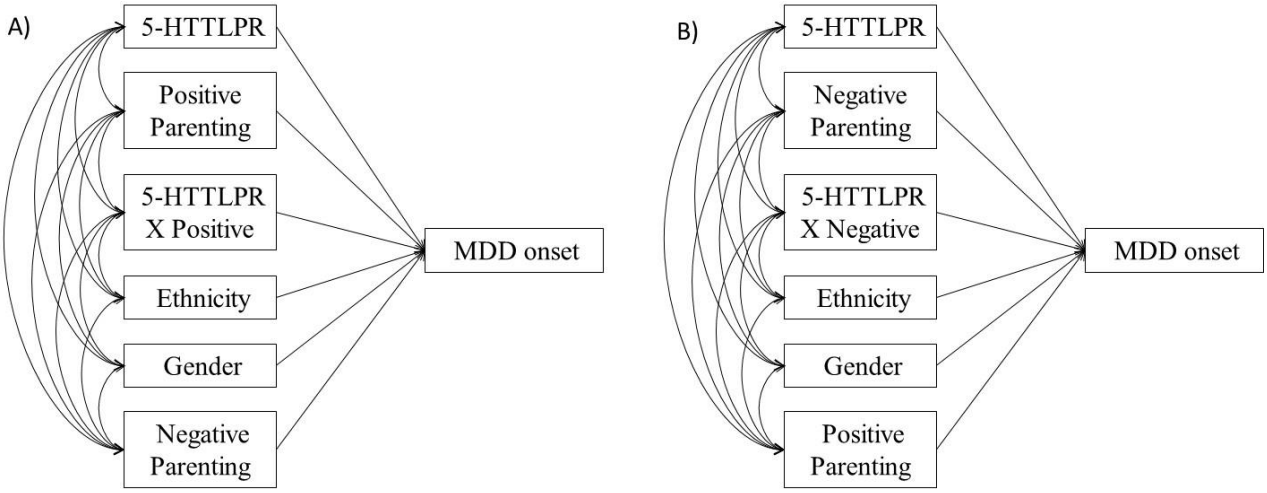

Figure 5-1. *Hypothesised conceptual model outlining pathways testing serotonin transporter gene x (A) positive parenting and (B) negative parenting effects on adolescent MDD onset.*

Consistent with the previous study, a dominant genetic model (LL=0, SL+SL[i.e., S-carriers]=1) and an additive genetic model (LL=0, SL=1, SS=2) was assessed in separate analyses. Eight path models were therefore estimated to document effects of positive parenting and negative parenting in the EPI task and the PSI task separately when the S allele of the 5-HTTLPR genotype was treated as dominant and additive.

Path models were estimated using weighted least squares with a mean- and variance-adjusted chi-square test statistic (WLSMV), which is an appropriate estimation method for models with categorical outcome variables (Muthén & Muthén, 1998-2012). As a limited number of fit statistics are available for the WLSMV estimator with bootstrapping, the models were first assessed with bootstrapping, whilst model fit was assessed for the same path models obtained without bootstrapping. Models were considered to have good fit at values of ≤ .06 for RMSEA, values <1 for the WRMR and values ≥ .95

for the CFI (Hu & Bentler, 1999). Path models were based on 5000 bias-corrected bootstrapped samples.

Prior to estimating the models, all continuous predictor variables and covariates were centered to reduce problems with multicollinearity. The interaction term was created by multiplying genotype and positive parenting. Significant interactions were clarified through post hoc analyses assessing whether the simple slopes representing associations between parental warmth and MDD onset were significantly different from zero for the different genotypes (Preacher, Curran, & Bauer, 2006).

Given Little's (1988) MCAR test was non-significant, $\chi^2(163)=179.54$, $p=.178$, we used pairwise deletion (the only option when using the WLSMV estimator and bootstrapping in *Mplus*) to account for missing data as FIML was not available with the WLSMV estimator in *Mplus*. Pairwise deletion has been shown to be unbiased when data is missing completely at random (Enders & Bandalos, 2001). Levels of missing data averaged 13.3% (range 0-28.6%).

5.3 Results

Correlations between variables are shown in Table 5-1. 5-HTTLPR genotype and positive maternal behavior in the PSI (though not in the EPI) task were significantly correlated ($r=.22$, $p<.05$), indicating that a GxE effect between these two variables could be a function of evocative rGE. Aversive parenting in the EPI and the PSI were not significantly correlated with 5-HTTLPR genotype.

Table 5-1 *Descriptive statistics and intercorrelations*

Variables	1	2	3	4	5	6	7	8
1. MDD onset (0=no onset, 1=MDD onset)	-	.05	-.17	-.14	-.11	-.28*	.34**	.09
2. Gender (male = 0, female = 1)		-	-.004	.13	-.08	-.05	.07	-.07
3. Ethnicity (0 = Aust-European descent, 1 = non-Aust-European descent)			-	.22	-.26	-.21	.05	.12
4. Dominant serotonin transporter genotype (0 =LL, 1=SS or SL)				-	.12	.24*	-.12	-.11
5. Positive Parent behavior EPI					-	.41***	-.31***	-.26**
6. Positive Parent behavior PSI						-	-.43***	-.44***
7. Aversive Parent behavior EPI							-	.52***
8. Aversive Parent behavior PSI								-
Percentage of sample or *M* (SD)	MDD onset = 26.30%	Male =47.70%	Aust-Europe descent = 87.70%	LL= 31%	2.368 (.64)	1.752 (.68)	.57 (.41)	1.26 (.61)

$p \leq 05$ = *, $p \leq .01$ = **, $p \leq .001$ = ***

Model fit indices displayed in Table *5-2* indicate that all models provided an acceptable fit to the data.

Table 5-2. *Fit Statistics of Path Models*

	χ^2	df	p-value	RMSEA	CFI	WRMR
Positive parenting						
Dominant						
EPI Task	.001	1	.980	.00	1.00	.004
PSI Task	.001	1	.980	.00	1.00	.004
Additive						
EPI Task	.001	1	.980	.00	1.00	.004
PSI Task	.001	1	.980	.00	1.00	.004
Negative parenting						
Dominant						
EPI Task	.001	1	.980	.00	1.00	.004
PSI Task	.001	1	.980	.00	1.00	.004
Additive						
EPI Task	.001	1	.980	.00	1.00	.004
PSI Task	.001	1	.980	.00	1.00	.004

df = degrees of freedom, RMSEA= Root Mean Square Error of Approximation, CFI= Comparative Fit Index SRMR= Standardized Root Mean Square Residual (available for continuous outcomes) WRMR= Weighted Root Mean Square Residual (available for categorical outcomes)

Thirty-six participants experienced an onset of MDD following the W1 assessment. Thirteen of these participants were L-homozygous at the 5-HTTLPR locus whilst 23 were S-carriers (17 SL genotype and 6 SS genotype).

5.3.1 **5-HTTLPR x Positive Parenting Interactions.**

Results of analyses modelling interactions between 5-HTTLPR and positive parenting predicting MDD onset are displayed in Table 5-3**.**

Dominant genetic model path analyses. The model for the EPI task explained 18% of the variance in risk for MDD onset (r^2 =.18), whilst the model for the PSI task explained 22% of the variance in risk for MDD onset (r^2 =.22). Results for the EPI task indicated no significant main effects of 5-HTTLPR genotype, gender, ethnicity or positive maternal behaviors on MDD onset, though higher frequencies of negative maternal behaviour were associated with greater risk of MDD onset. Neither the interaction effect between 5-HTTLPR and positive maternal behavior predicting MDD onset, nor the covarying relationship between 5-HTTLPR and positive maternal behavior was significant.

In contrast, lower levels of positive maternal behaviour in the PSI task were significantly associated with MDD onset, whilst negative maternal behavior did not show an association with MDD onset. In addition, the independent covariance between 5-HTTLPR genotype and positive maternal behavior was also significant and there was evidence of a significant interaction between 5-HTTLPR genotype and positive maternal behavior in the PSI task. Specifically, as illustrated in

Figure 5-2, the relationship between positive parental behaviors and MDD onset was significant for the L-homozygous group (b=-1.27 [95% CI: -2.15; -.40], S.E.=.45, β=-.86, p=.005) but not for the S-carrier group (b=-.15 [95 CI: -.68; .39], S.E.=.27, β=-.23, p=.592). L-homozygous individuals appeared to be more vulnerable to experiencing an onset of MDD in environments involving low frequencies of positive parenting behaviors.

Table 5-3. *Path model testing the interaction between 5-HTTLPR genotype x positive maternal behaviour at 11-13 years on MDD onset during adolescence.*

Specified Paths	EPI Task						PSI Task					
	b	S.E.	Lower 95% CI	Upper 95% CI	B	*p*	b	S.E.	Lower 95% CI	Upper 95% CI	β	*p*
5-HTTLPR S-allele Dominant												
5-HTTLPR → MDD onset	.12	.25	-.63	.36	-.06	.633	.03	.25	-.45	.51	.01	.903
Positive parenting → MDD onset	-.51	.37	-1.17	.30	-.25	.162	-1.27	.41	-2.14	-.49	-.86	.002
Aversive parenting → MDD onset	.83	.32	.16	1.42	.34	.009	-.14	.26	-.65	.34	-.09	.586
Ethnicity → MDD onset	-.29	.53	-1.32	.51	-.10	.579	-.44	.55	-1.91	.32	-.15	.419
Gender → MDD onset	.04	.24	-.43	.50	.02	.877	.00	.24	-.45	.49	.002	.987
5-HTTLPR X Positive parenting → MDD onset	.88	.52	-.12	1.96	.32	.090	1.13	.48	.23	2.16	.63	.019
5-HTTLPR ↔Positive parenting	.02	.02	-.03	.07	.10	.332	.06	.03	.002	.11	.18	.042
5-HTTLPR ↔ Aversive parenting	-.02	.02	-.05	.01	-.09	.307	-.02	.03	-.07	.02	-.08	.361
5-HTTLPR ↔ Gender	.02	.02	-.02	.05	.08	.287	.02	.02	-.02	.05	.08	.287
5-HTTLPR ↔ Ethnicity	.02	.01	-.01	.04	.10	.161	.02	.01	-.01	.04	.10	.161
5-HTTLPR ↔ 5-HTTLPR X Positive parenting	.01	.01	-.01	.03	.04	.494	.02	.02	-.01	.05	.07	.266
Positive parenting ↔ Aversive parenting	-.06	.02	-.11	-.02	-.32	.006	-.18	.03	-.25	-.12	-.44	.000
Positive parenting ↔ Gender	-.02	.02	-.06	.03	-.06	.497	-.02	.03	-.07	.05	-.04	.628
Positive parenting ↔ Ethnicity	-.03	.01	-.05	.000	-.15	.071	-.03	.02	-.08	.02	-.13	.217
Positive parenting ↔ 5-HTTLPR X Positive parenting	.13	.02	.09	.18	.74	.000	.32	.05	.23	.43	.83	.000
Aversive parenting ↔ Gender	.01	.02	-.03	.05	.05	.554	-.02	.03	-.07	.04	-.06	.538
Aversive parenting ↔ Ethnicity	.004	.02	-.03	.04	.03	.808	.01	.02	-.03	.06	.07	.491
Aversive parenting ↔ 5-HTTLPR X Positive parenting	-.04	.02	-.08	-.01	-.26	.026	-.10	.03	-.17	-.06	-.31	.000
5-HTTLPR X Positive parenting ↔ Gender	-.01	.02	-.04	.03	-.03	.720	.00	.03	-.05	.05	-.01	.879
5-HTTLPR X Positive parenting ↔ Ethnicity	-.02	.01	-.05	.00	-.17	.098	-.02	.02	-.07	.02	-.12	.346

5-HTTLPR Additive												
5-HTTLPR → MDD onset	-.16	.16	-.46	.16	-.11	.331	-.06	.17	-.38	.26	-.04	.726
Positive parenting → MDD onset	-.56	.35	-1.22	.16	-.27	.107	-1.18	.36	-1.88	-.48	-.80	.001
Aversive parenting → MDD onset	.84	.32	.18	1.42	.35	.008	-.18	.26	-.70	.32	-.11	.502
Ethnicity → MDD onset	-.20	.53	-1.22	.61	-.07	.707	-.32	.56	-1.78	.46	-.10	.570
Gender → MDD onset	.05	.24	-.42	.51	.02	.851	.03	.24	-.43	.50	.01	.915
5-HTTLPR X Positive parenting → MDD onset	.79	.37	.04	1.51	.38	.035	.74	.29	.16	1.32	.59	.011
5-HTTLPR ↔Positive parenting	.03	.03	-.04	.09	.09	.381	.07	.05	-.02	.16	.14	.133
5-HTTLPR ↔ Aversive parenting	-.01	.03	-.06	.05	-.02	.846	-.03	0.04	-0.11	0.04	-0.07	.397
5-HTTLPR ↔ Gender	.03	.03	-.02	.09	.10	.205	.03	.03	-.02	.09	.10	.205
5-HTTLPR ↔ Ethnicity	.03	.02	-.004	.07	.13	.109	.03	.02	.00	.07	.13	.109
5-HTTLPR ↔ 5-HTTLPR X Positive parenting	.02	.04	-.06	.09	.05	.667	.03	.07	-.10	.16	.05	.659
Positive parenting ↔ Aversive parenting	-.06	.02	-.11	-.02	-.32	.006	-.18	.03	-.25	-.12	-.44	.000
Positive parenting ↔ Gender	-.02	.02	-.06	.03	-.06	.497	-.02	.03	-.07	.05	-.04	.628
Positive parenting ↔ Ethnicity	-.03	.01	-.05	.000	-.15	.071	-.03	.02	-.08	.02	-.13	.217
Positive parenting ↔ 5-HTTLPR X Positive parenting	.17	.03	.12	.23	.70	.000	.43	.08	.29	.60	.78	.000
Aversive parenting ↔ Gender	.01	.02	-.03	.05	.05	.554	-.02	.03	-.07	.04	-.06	.538
Aversive parenting ↔ Ethnicity	.004	.02	-.03	.04	.03	.808	.01	.02	-.03	.06	.07	.491
Aversive parenting ↔ 5-HTTLPR X Positive parenting	-.05	.02	-.10	-.01	-.24	.032	-.12	.04	-.20	-.06	-.25	.001
5-HTTLPR X Positive parenting ↔ Gender	-.01	.02	-.05	.04	-.03	.780	-.01	.04	-.08	.07	-.02	.811
5-HTTLPR X Positive parenting ↔ Ethnicity	-.03	.02	-.07	-.003	-.21	.053	-.04	.03	-.11	.01	-.17	.170

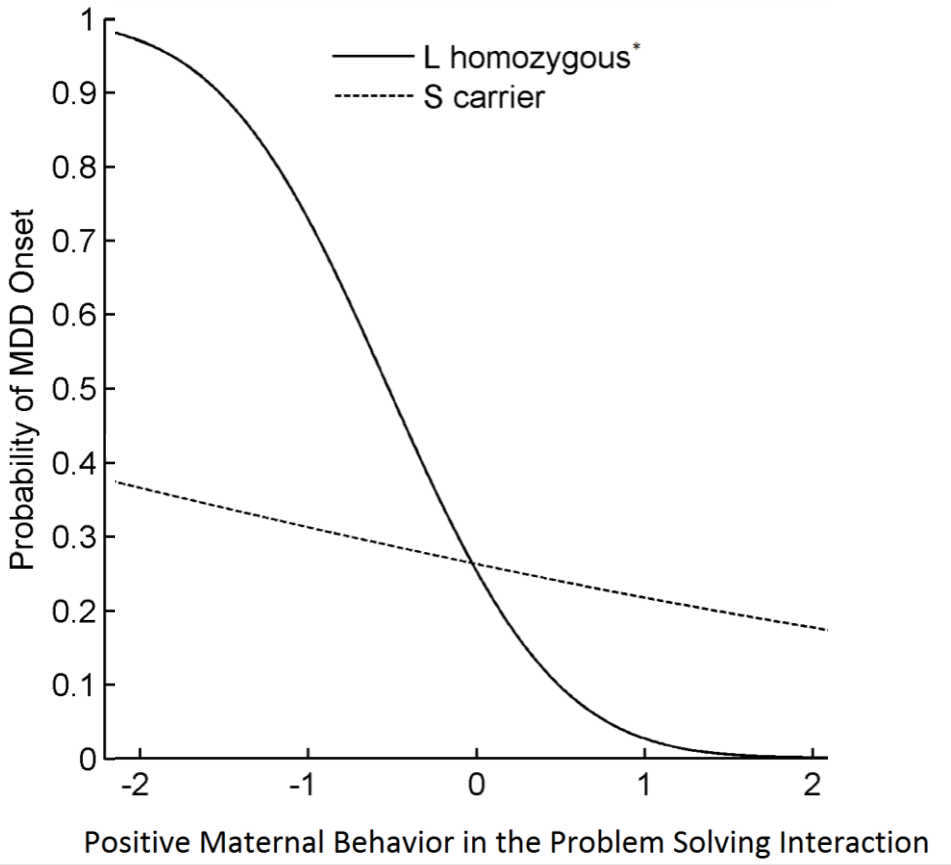

Figure 5-2. Influence of positive maternal behaviour measured during the PSI task when participants were 11-13 years on probability of MDD onset during adolescence for L homozygous individuals and S-carriers.

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

Additive genetic model path analysis. The model for the EPI task explained 22% of the variance in risk for MDD onset (R^2 =.22), whilst the model for the PSI task explained 24% of the variance in risk for MDD onset (R^2 =.24). With regards to the EPI model, there were no significant main effects of 5-HTTLPR genotype, gender, ethnicity or positive maternal behaviours on MDD onset, though higher frequencies of aversive maternal

behaviour was associated with greater risk of MDD onset. A significant negative relationship between positive and aversive maternal behaviour was also evident. Neither of the covarying relationships between 5-HTTLPR and maternal behaviour (positive or aversive) was significant however the interaction effect between 5-HTTLPR and positive maternal behaviour did significantly predict MDD onset.

Post hoc investigations, including positive maternal behaviour and risk for MDD onset was unrelated in both the LL-homozygous (b=-.56 [95 CI: -1.23; .11], S.E.=.34, β=-.27, p=.106) and SL heterozygous (b=.23, [95 CI: -.31; .77], S.E.=.28, β=.11, p=.408) groups (see Figure 5-3). There was a relationship however in the SS homozygous group, such that higher frequencies of positive maternal behaviour was associated with an increased probability of a later MDD onset (b=1.02 [95 CI: .02; 2.01], S.E.=.51, β=.49, p=.047).

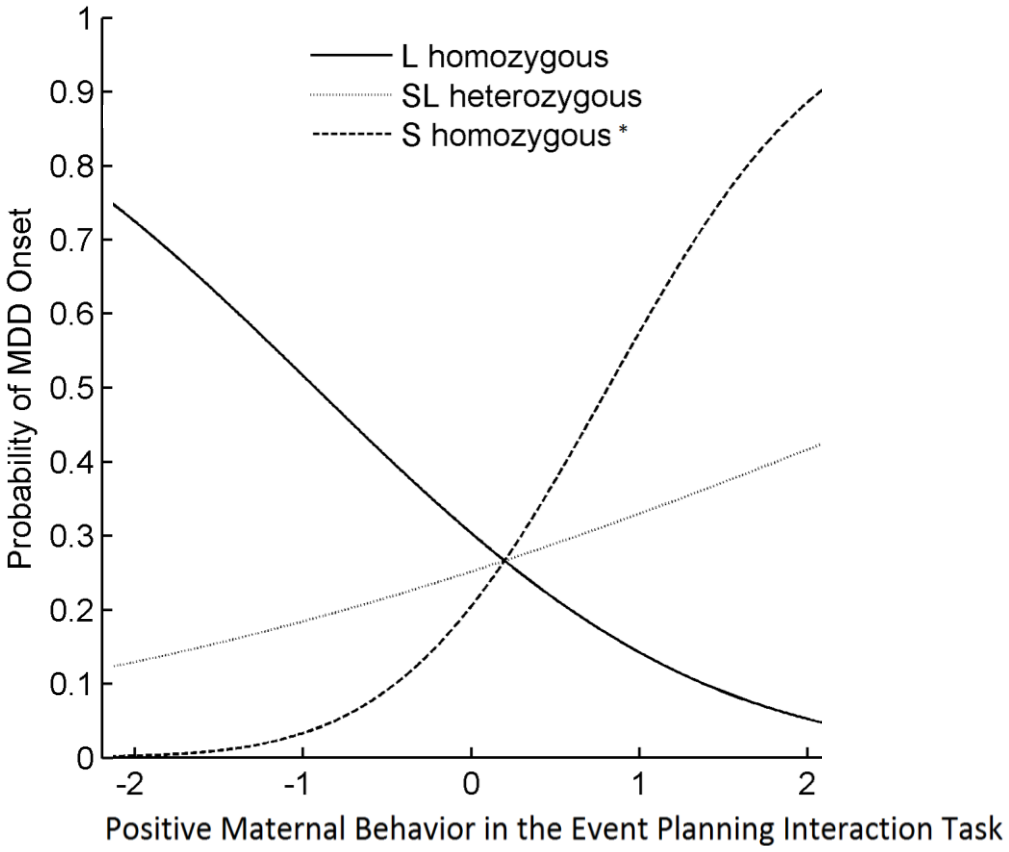

Figure 5-3. *Influence of positive maternal behaviour measured during the EPI task when participants were 11-13 years on probability of MDD onset during adolescence for LL homozygous, SL heterozygous and SS homozygous individuals.*
** = p <.05; ** = p <.01; ***= p <.001*

In the PSI model, positive maternal behaviour, but not aversive maternal behaviour was associated with increased risk of MDD onset. Positive and aversive maternal behaviour were negatively related, but neither positive or aversive maternal behaviour were associated with 5-HTTLPR genotype. Gender and ethnicity were unrelated to 5-HTTLPR genotype, risk of MDD onset or maternal behaviour. The interaction between 5-HTTLPR X positive maternal behaviour predicting MDD onset however was significant.

Post-hoc analyses suggested that the relationship between positive parental behaviours and MDD onset was significant for the L-homozygous group (b-1.18 [95% CI: -1.88,-.48], S.E.=.36, β=-.80, p=.001) and for the SL heterozygous group (b=-.44 [95 CI: -.82;-.06], S.E.=.19, β=-.21, p=.024) but not for the SS homozygous group (b = .30 [95%CI: -.48 ; 1.08], S.E.=.40, β =.39, p=.449). As shown in Figure *5-4*, the probability of experiencing an onset of MDD in both the L-homozygous and SL heterozygous groups appeared to be significantly greater in environments involving low frequencies of positive parenting behaviours.

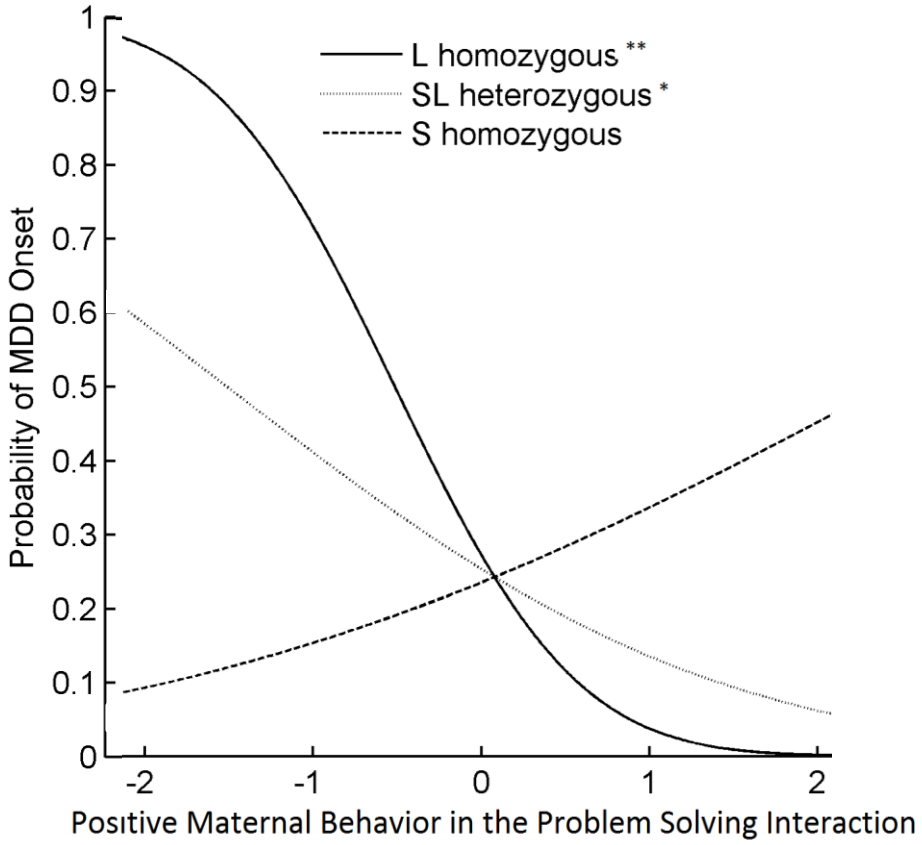

Figure 5-4. *Influence of positive maternal behaviour measured during the PSI task when participants were 11-13 years on probability of MDD onset during adolescence for LL homozygous, SL heterozygous and SS homozygous individuals.*
** = p <.05; ** = p <.01; ***= p <.001*

5.3.2 **5-HTTLPR x Negative Parenting Interactions.**

Results for path models testing for the presence of an interaction between the serotonin transporter gene and adverse maternal behaviour predicting MDD onset are displayed in Table *5-4*.

Dominant genetic model path analysis. The model for the EPI task explained 19% of the variance in risk for MDD onset (R^2 =.19), whilst the model for the PSI task explained 15% of the variance in risk for MDD onset (R^2 =.15). In the EPI model, aversive maternal behaviour but not positive maternal behaviour was significantly related to MDD onset. Genotype, gender and ethnicity were not associated with MDD onset, nor was genotype associated with either parenting variable. A significant negative covarying relationship between positive and aversive maternal behaviour was present. The interaction between serotonin transporter genotype and aversive behaviour was non-significant.

In the PSI task, serotonin transporter genotype, aversive maternal behaviour, positive maternal behaviour, gender and ethnicity were all unrelated to MDD onset. More frequent positive maternal behaviour was associated with S-carrier 5-HTTLPR status as well as with higher frequencies of aversive maternal behaviour. Aversive maternal behaviour did not vary significantly according to 5-HTTLPR genotype. The interaction between 5-HTTLPR and aversive maternal behaviour predicting MDD onset was also non-significant.

Additive genetic model path analysis. The model for the EPI task explained 17% of the variance in risk for MDD onset (R^2 =.17), whilst the model for the PSI task explained 14% of the variance in risk for MDD onset (R^2 =.14). Results for the EPI model showed no significant main effects of either parenting variable, genotype, gender or ethnicity on MDD

onset. A negative covarying relationship between positive maternal behaviour and aversive maternal behaviour was evident. Higher levels of positive maternal behaviour was also associated with adolescent S-allele 5-HTTLPR carrier status. There was no significant interaction between serotonin transporter genotype and aversive behaviour on MDD onset.

Table 5-4.*Path model testing the interaction between 5-HTTLPR genotype x negative parenting behaviour at 11-13 years on MDD onset during mid- to late-adolescence in Study 2B.*

	EPI Task						PSI Task					
Specified Paths	b	SE	Lower 95% CI	Upper 95% CI	β	p	b	SE	Lower 95% CI	Upper 95% CI	β	p
5-HTTLPR S-allele Dominant												
5-HTTLPR → MDD onset	-.11	.25	-.74	.29	-.05	.663	-.07	.25	-.56	.42	-.03	.779
Positive parenting → MDD onset	-.01	.25	-.68	.41	-.01	.964	-.41	.25	-.88	.09	-.28	.102
Aversive parenting → MDD onset	1.63	.48	.10	2.39	.66	.001	.51	.40	-.26	1.34	.31	.202
Ethnicity → MDD onset	-.25	.53	-2.32	.42	-.08	.634	-.45	.59	-2.12	.36	-.15	.445
Gender → MDD onset	.01	.24	-.57	.40	.01	.964	.09	.24	-.37	.57	.04	.720
5-HTTLPR X Aversive parenting → MDD onset	-1.23	.72	-3.54	-.17	-.41	.088	-.77	.46	-1.73	.07	-.39	.094
5-HTTLPR ↔Positive parenting	.02	.02	-.04	.06	.10	.331	.06	.03	.00	.11	.18	.042
5-HTTLPR ↔ Aversive parenting	-.02	.02	-.07	.01	-.09	.307	-.02	.03	-.07	.03	-.08	.361
5-HTTLPR ↔ Gender	.02	.02	-.03	.05	.08	.287	.02	.02	-.02	.05	.08	.287
5-HTTLPR ↔ Ethnicity	.02	.01	-.01	.03	.10	.161	.02	.01	-.01	.04	.10	.158
5-HTTLPR ↔ 5-HTTLPR X Aversive parenting	-.01	.01	-.03	.01	-.04	.555	-.01	.01	-.04	.02	-.03	.626
Positive parenting ↔ Aversive parenting	-.06	.02	-.13	-.03	-.32	.006	-.18	.03	-.25	-.12	-.44	.000
Positive parenting ↔ Gender	-.02	.02	-.07	.02	-.06	.497	-.02	.03	-.07	.04	-.04	.628
Positive parenting ↔ Ethnicity	-.03	.01	-.06	.00	-.15	.071	-.03	.02	-.08	.02	-.13	.220
Positive parenting ↔ 5-HTTLPR X Aversive parenting	-.04	.02	-.09	-.01	-.23	.024	-.10	.03	-.17	-.06	-.30	.000
Aversive parenting ↔ Gender	.01	.02	-.04	.04	.05	.554	-.02	.03	-.07	.04	-.06	.538
Aversive parenting ↔ Ethnicity	.00	.02	-.03	.04	.03	.808	.01	.02	-.03	.06	.07	.494
Aversive parenting ↔ 5-HTTLPR X Aversive parenting	.11	.02	.07	.15	.81	.000	.26	.04	.20	.34	.85	.000
5-HTTLPR X Aversive parenting ↔ Gender	.00	.02	-.04	.02	.00	.995	.00	.02	-.05	.04	.00	.964
5-HTTLPR X Aversive parenting ↔ Ethnicity	.01	.02	-.02	.04	.10	.509	.01	.02	-.03	.05	.05	.697

5-HTTLPR Additive												
5-HTTLPR → MDD onset	-.13	.16	-.46	.19	-.09	.428	-.10	.17	-.43	.23	-.08	.534
Positive parenting → MDD onset	.01	.26	-.49	.53	.00	.979	-.41	.24	-.86	.09	-.28	.096
Aversive parenting → MDD onset	1.34	.45	.34	2.17	.55	.003	.36	.39	-.42	1.12	.22	.355
Ethnicity → MDD onset	-.20	.56	-1.16	.64	-.07	.714	-.39	.59	-1.98	.44	-.13	.516
Gender → MDD onset	.04	.23	-.41	.52	.02	.869	.09	.24	-.36	.58	.05	.699
5-HTTLPR X Aversive parenting → MDD onset	-.57	.53	-1.77	.28	-.27	.280	-.47	.38	-1.25	.26	-.31	.216
5-HTTLPR ↔Positive parenting	.03	.03	-.04	.09	.09	.376	.07	.05	-.02	.16	.14	.131
5-HTTLPR ↔ Aversive parenting	-.01	.03	-.06	.05	-.02	.847	-.03	.04	-.10	.04	-.07	.396
5-HTTLPR ↔ Gender	.03	.03	-.02	.09	.10	.209	.03	.03	-.02	.09	.10	.209
5-HTTLPR ↔ Ethnicity	.03	.02	.00	.07	.13	.107	.03	.02	.00	.07	.13	.107
5-HTTLPR ↔ 5-HTTLPR X Aversive parenting	.03	.04	-.05	.11	.07	.540	-.02	.05	-.11	.07	-.04	.702
Positive parenting ↔ Aversive parenting	-.06	.02	-.11	-.02	-.32	.005	-.18	.03	-.25	-.12	-.44	.000
Positive parenting ↔ Gender	-.02	.02	-.06	.03	-.06	.500	-.02	.03	-.07	.04	-.04	.628
Positive parenting ↔ Ethnicity	-.03	.01	-.05	.00	-.15	.075	-.03	.02	-.08	.02	-.13	.220
Positive parenting ↔ 5-HTTLPR X Aversive parenting	-.05	.02	-.10	-.01	-.20	.026	-.12	.04	-.20	-.06	-.28	.001
Aversive parenting ↔ Gender	.01	.02	-.03	.05	.05	.556	-.02	.03	-.07	.04	-.06	.538
Aversive parenting ↔ Ethnicity	.00	.02	-.03	.04	.03	.807	.01	.02	-.03	.06	.07	.494
Aversive parenting ↔ 5-HTTLPR X Aversive parenting	.15	.03	.10	.23	.76	.000	.32	.05	.24	.41	.80	.000
5-HTTLPR X Aversive parenting ↔ Gender	.00	.02	-.04	.05	.01	.922	.00	.03	-.05	.06	.01	.928
5-HTTLPR X Aversive parenting ↔ Ethnicity	.03	.03	-.02	.10	.17	.349	.02	.02	-.03	.07	.08	.482

5.4 Discussion

The current results provide evidence of an interaction between 5-HTTLPR and low levels of positive parenting in predicting onset of Major Depressive Disorder. Findings indicated that adolescents carrying two copies of the S-allele who received less nurturant, positive parenting during more conflictual interactions appeared to be buffered against an onset of MDD, whilst adolescents carrying an L-allele were at greater risk for MDD onset with decreasing levels of positive parenting. There was also a barely significant finding suggesting that when 5-HTTLPR genotype was coded additively, SS homozygous individuals with parents who displayed greater warmth and positivity during more innocuous conversations centered on pleasant topics were more vulnerable to depression, however this finding was not replicated across the two studies. Given family environments involving high levels of positive, nurturing parental behaviors have consistently been identified as protective against depression (Yap, Pilkington, Ryan, & Jorm, 2014), this particular finding should be interpreted with significant caution until further replications are documented.

It is noteworthy that similar findings implicating the L-allele were obtained in the ADS sample when both depressive symptomatology at 18-19 years and the change in depressive symptomatology over adolescence were considered as outcomes, and as well as in the ATP sample for the outcome of depressive symptomatology at 17-18 years (based on a different self-report questionnaire for depression, as well as a parent report questionnaire measure, rather than observation, to assess parenting behaviour (Little et al., accepted). This pattern of results is also consistent with findings by other studies demonstrating that L-homozygous individuals who experience low maternal responsiveness or lack supportive parenting may be more vulnerable to externalizing and internalizing difficulties (Davies &

Cicchetti, 2014; Lavigne et al., 2013; Li et al., 2013 in girls). Taken together these studies provide some support for the possibility that L-homozygous individuals too may be vulnerable to maladaptive outcomes when exposed to particular environments.

Consistent with the previous study, we also did not find evidence that 5-HTTLPR interacted with negative parenting, therefore the finding of an interaction between 5-HTTLPR and positive parenting predicting onset of Major Depressive Disorder cannot be accounted for by an association between positive parenting and negative parenting.

The use of an observational measure of positive parenting across two different tasks provided further insight into the nature of this GxE interaction. Results indicated that reduced positive parental behaviours were associated with greater symptomatology amongst L-homozygous individuals when these behaviours occurred in conflictual interactional situations designed to elicit negative emotions (the PSI). In our previous study, the interaction between the serotonin transporter gene and positive parenting association also tended to emerge more consistently in the PSI, and in fact was only documented in situations intended to elicit positive emotion (the EPI), when the outcome of interest was a change in depressive symptoms over adolescence, despite positive parenting behaviours being more frequent in this task. The predictive importance of parental positive behavior may therefore be somewhat dependent on context, and not based simply on the rates of their occurrence.

These findings perhaps suggest that L-homozygous individuals whose mothers struggle to generate or maintain positivity in conflictual situations may be at particular risk of clinical depression during the adolescent period. One potential explanation for this finding might be related to previous observations suggesting that L-homozygous individuals are less emotionally reactive and perceptive (Glenn, 2011)(Yildirim & Derksen, 2013). As such they

may require more emotional coaching from parents in order to develop adequate social cognitive and interpersonal skills. Thus, L-homozygous individuals who do not receive this emotional scaffolding from parents may be less able to develop emotional regulation skills and good interpersonal connections, which in turn may increase their risk of depression (and potentially other psychological difficulties, such as externalizing disorders). Parents' displays of positivity and warmth during conflict are a particularly powerful demonstration of good skills in emotional expression and regulation. Thus, L-homozygous individuals who do not receive this emotional scaffolding from parents may be less able to develop emotional regulation skills and good interpersonal connections, which in turn may increase their risk of depression (and potentially other psychological difficulties, such as externalizing disorders). However, this explanation is admittedly speculative and would need to be specifically tested in future studies before conclusions could be drawn.

The limitations noted in the previous study (Little et al., accepted) are all relevant to the current analyses, including the consideration of only one gene and two specific environments in the current research design, and the lack of inclusion of the minor allele rs25531 of the serotonin transporter gene, which comprises a single-nucleotide variant (A→G) within the L polymorphism that renders an L_g allele functionally similar to the S variant (Hu et al., 2006). Epigenetic mechanisms, including DNA methylation of the serotonin transporter gene may also influence the current findings (Philibert et al., 2008). Moreover, the specific mechanisms underlying the current GxE interaction, including the potential for particular traits associated with an S-allele versus an L-allele to account for the associations, were not explored.

Nonetheless, this study adds to an emerging body of evidence suggesting that that L-homozygous individuals be vulnerable to adverse outcomes such as depression, and in particular that they may be more sensitive than S-allele carriers to the depressogenic effects of more deprived environments, such as those low positive parenting. This finding suggests that it is not only the S-allele that determines environmental sensitivity. Rather, as suggested by a differential capability framework, it is possible that, depending on the specific environmental challenges encountered, both alleles may confer sensitivity to particular outcomes, including depression.

CHAPTER 6: BRAIN STRUCTURES AS ENDOPHENOTYPES FOR DEPRESSION

CHAPTER 2 and CHAPTER 3 reviewed evidence for a potential gene-environment interaction, involving an association between the S-allele of the serotonin transporter gene and greater risk for stress-related psychiatric disorders such as depression, but only in the presence of threatening experiences. The possibility of a different gene-environment interaction between the L-allele and psychopathologies, including depression, in contexts involving more deprived environments, involving reduced levels of nurturance and support, was also introduced. Consistent with this, analyses presented in CHAPTER 4 and CHAPTER 5 indicated that adolescents homozygous for the L-allele who received less positive parenting showed greater vulnerability to depression than their S-carrier counterparts. The presence of this additional gene-environment interaction may account for variation in findings within the broader literature. This pattern of results may also be consistent with a *differential capability* theory, whereby the characteristics associated with either an S-allele or an L-allele may be advantageous or disadvantageous, depending on their fit with the current environment.

Critically, however, the underlying biological pathway from the serotonin transporter gene to behaviour is not fully understood. This chapter will therefore review the concept of the *endophenotype* and findings of imaging genetics studies, which may increase our understanding of these pathways. It will argue that differences in brain structure may offer one plausible mechanism/explanation for how the serotonin transporter gene variation may influence risk for depression. Differences in anterior cingulate cortex, orbitofrontal cortex, hippocampal and amygdala structure are likely to be particularly important, given

the research that has found these structures to be associated with depression and with 5-HTTLPR genotype.

6.1 The Endophenotype Concept and The Role of Imaging Genetics

The significant efforts that have been channelled towards determining the genetic basis of depression have so far have had limited success, often generating contradictory or null findings (Meyer-Lindenberg & Weinberger, 2006). Difficulty in identifying specific genes associated with depression is undoubtedly due in part to the complex mode of inheritance of the disorder and the heterogeneity of presentations. Nonetheless there is converging evidence to support the involvement of the serotonin transporter gene in depression (e.g., Caspi et al., 2010; Clarke et al., 2010; Karg et al., 2011), despite some acknowledged inconsistencies in findings. More specifically it appears that the serotonin transporter gene may have a role in emotion processing, stress responsivity and social cognition that have consequences for depression risk (Caspi et al., 2010; Glenn, 2011; Homberg & Lesch, 2011). In particular, it seems that the S-allele may confer greater emotional reactivity and sensitivity to threat, which may increase susceptibility to the disorder in the presence of highly aversive, threatening or stressful experiences. As reviewed in CHAPTER 2, an emerging literature suggests an intriguing possibility that the L-allele may be associated with emotional hyporesponsivity, low reactivity to stress and less developed higher-order cognitive processing, which may confer greater risk for depression in environments of greater deprivation. Currently, however, the underlying neurobiology of the mechanism of action of the serotonin transporter gene on depression remains poorly understood.

The challenges with clarifying the impact of genes on complicated, heterogeneous behavioural phenotypes such as depression have led to an increased focus on alternative methodologies, such as the "endophenotype approach," which is based on the premise that these complex psychiatric conditions can be deconstructed into more elementary components (Gottesman & Gould, 2003). These components are referred to as "intermediate phenotypes" or "endophenotypes" and are assumed to have simpler genetic underpinnings than the disorder syndrome. Endophenotypes occur at an intermediate stage in the causal pathway from a distal gene to overt expression of disease. They therefore allow exploration of both the "upstream" consequences of a set of genes (the association between particular genetic variants and the putative endophenotypes) and the "downstream" psychophysiology of a disorder (the association between endophenotypes and depression). It has been contended that focusing on endophenotypes that are more proximal to both the effect of genotype and the behavioural outcomes may allow improved biological characterisation of psychiatric conditions (Meyer-Lindenberg & Weinberger, 2006).

Endophenotypes may be assessed by neurophysiological, biochemical, endocrinological, neuroimaging, cognitive and neuropsychological measures (Gottesman & Gould, 2003). There should be clear, plausible biological or clinical rationale for selecting candidate endophenotypes for a condition of interest. In addition, there are five commonly accepted criteria for identification of an endophenotype in psychiatric genetics (Gottesman & Gould, 2003):

(1) association with the overall disease syndrome in the population,

(2) heritability (variance in the endophenotype appears to be underpinned by genetic variance)

(3) primarily state-independent (present regardless of whether or not the illness is currently being experienced) though it may only manifest after a certain developmental period or may require a "challenge" for its elicitation

(4) co-segregation with the illness within families (the endophenotype is more prevalent among the ill relatives of ill probands than the healthy relatives of the ill probands)

(5) shows familial association (there are higher rates of the endophenotype amongst non-affected relatives of probands than in the general population).

These criteria distinguish an endophenotype from a biomarker, a non-causal factor that may act as an indicator of risk or presence of the disorder, prognosis or likely response to treatment (Lenzenweger, 2013). Importantly, a biomarker may be stable or state-related and may also not necessarily reflect a genetic effect, but may rather be influenced by environmental or epigenetic factors or a combination.

The structure and function of brain regions responsible for specific emotional and cognitive processes have been identified as particularly promising endophenotypes of depression, given findings suggesting high heritability of these regions (Glahn, Thompson, & Blangero, 2007; Peper, Brouwer, Boomsma, Kahn, & Hulshoff Pol, 2007) and associations between their volume and activity with depression (Hasler, Drevets, Manji, & Charney, 2004; Savitz & Drevets, 2009). Genetic variation that alters the brain thus offers one plausible mechanism by which genes may affect risk for psychopathology. The research field of *imaging genetics* provides a means of linking candidate genes to brain

structure and function and offer some advantages in studying risk relationships between genes and mental health (Hariri & Weinberger, Bogdan, Nikolova, & Pizzagalli, 2013; Bogdan et al., 2017; 2003). In particular, the more continuous, tangible and relatively objective and quantifiable nature of brain imaging data makes it a preferable alternative to the dichotomous outcome of depression diagnosis, which may be based on biased self-report and involves heterogeneous symptoms. Because a depression diagnosis can encapsulate multiple distinct symptom profiles that might be underpinned by a variety of pathophysiologies, many neural circuits and a much larger number of genes are likely to be implicated in the disorder, and small effects conferred by single genetic variants on the depression behavioural phenotype will be highly difficult to detect. In contrast, an endophenotype approach promises to reveal the effects of genes more directly at a stage in the neurobiological pathway where fewer genes are involved in phenotypic expression and hence might be expected to account for a greater amount of trait variation (Goldman & Ducci, 2007 but see also Flint & Munafo, 2007). It has therefore been argued that power afforded by these imaging genetics studies may allow identification of gene effects with much smaller sample sizes than those required by traditional behaviour studies that examine the direct impact of genetic variation on distal behavioural outcomes (Hariri & Weinberger, 2003).

Given depression is regarded primarily as a disorder of emotion dysregulation, where key symptoms reflect distress (i.e., increased negative affect) or anhedonia (i.e., decreased positive affect), there has been a strong focus in imaging genetics research on identifying the impact that common genetic polymorphisms may have on brain circuitries that are related to emotional processing, particularly those within the prefrontal-limbic

network (Scharinger et al., 2010; Viding, Williamson, & Hariri, 2006; Won & Ham, 2016). The prefrontal cortex specifically comprises the medial PFC (mPFC), dorsal lateral PFC (dlPFC), dorsal medial PFC (dmPFC), and ventromedial PFC regions (vmPFC), as well as the orbital frontal cortex (OFC) and the anterior cingulate cortex (ACC), whilst the limbic network includes the amygdala, hippocampus, fornix, mammillary bodies, thalamus and insula. Broadly speaking, the PFC is thought to wield a top-down regulatory (inhibitory) control over the limbic system, which is involved in the rapid perception and appraisal of emotional stimuli and generation of more automatic affective responses (Hariri, Bookheimer, & Mazziotta, 2000). The high interconnectedness of the different regions within this system has made it very difficult to study as a whole. It has therefore been necessary to dissect this system into smaller neural networks or regions, and this has been found to be a fruitful method, though not without its limitations (Meyer-Lindenberg, 2009). The current thesis will focus on the ACC, OFC, hippocampus and amygdala as regions that have been strongly implicated in the occurrence of depression, as discussed below.

6.2 The anterior cingulate cortex (ACC).

Located bilaterally in the medial temporal lobe, the ACC forms the frontal part of cingulate cortex, a “collar-shaped” region surrounding the corpus collosum. The ACC is thought to have a role in various aspects of human behaviour, including executive, social, cognitive, affective and motor functions (Devinsky, Morrell, & Vogt, 1995; Paus, 2001). Connections to both the “emotional” limbic structures and the “cognitive” prefrontal regions allow the ACC to act as a point of integration and modulation of neural circuitry for affect regulation (Bush, Luu, & Posner). More recently, it has been suggested that the ACC

may have a key role in garnering and maintaining effortful control over extended goal-directed behaviours for long-term rewards (Holroyd & Umemoto).

The ACC can be divided cytoarchitectonically into the ventral limbic region (ACCL; Brodmann's Areas 24/24'), the dorsal paralimbic region (ACCP; Brodmann's Areas 32/32') and the subgenual cingulate (Brodmann's Area 25), which is located posterior to the subcallosal extension of area 24, ventral to the genu (Drevets, Ongur, & Price, 1998; Paus, 2001). Areas 24'/32' lie dorsal to the corpus callosum, while areas 24/32 occupy a pregenual position (Vogt, Nimchinsky, Vogt, & Hof, 1995).

Alternatively, the ACC can be partitioned into three subdivisions according to function – (i) a *rostral affective/visceral* region (aff-ACC; Brodmann's Areas 25, 24a-b, 32), located inferior and anterior to the genu of the callosum, which has extensive reciprocal connections with the orbitofrontal cortex and amygdala; (ii) a *dorsal cognitive* region (cog-ACC; Brodmann's Areas 24a'-b', 32'), superior to the collosum, with extensive reciprocal connections with other frontal and temporal regions particularly the dorsolateral prefrontal cortex and hippocampus, and (iii) a *caudal motor* region (mot-ACC; Brodmann's Areas 24c', 24c'g), which has reciprocal connections with the primary/supplementary motor and parietal regions (Stevens, Hurley, & Taber, 2011; Yücel et al., 2003). The cog-ACC is believed to form part of circuitry involved in the modulation of attention or executive functions, including working memory, monitoring of conflict of information or competition, detection of errors and processing novelty whilst the aff-ACC is thought to play a key role in appraising the importance of emotional and motivational information and regulating affective responses (Bush et al., 2000). The caudal motor region (mot-ACC) plays a role in premotor/skeletomotor functions (Picard & Strick, 1996).

An alternate division of the ACC that is often referred to in the depression literature has been termed the subgenual ACC (sgACC), which, according to Öngür and colleagues (2003), comprises Brodmann's areas 24b and, to a lesser extent, Brodmann's area 24a anteriorly and Brodmann's area 25 posteriorly. This cortical area shows particularly high densities of serotonin transporters (Varnäs, Halldin, & Håkan, 2004). The "perigenual" ACC region generally encompasses both the sgACC and the ACC situated anterior to the corpus callosum genu (ie, "pregenual" ACC) (Drevets, Savitz, & Trimble, 2008).

The literature is complicated however by inconsistent use of the terms used to describe these various divisions of the ACC. For example, one research group has described the "sgACC" as Brodmann's area 24 (Drevets et al., 1997), whilst another has referred to it as Brodmann's area 24 and 25 (Mayberg et al., 2000) and a further group as Brodmann's area 24 and sections of 32 and 33 (Kegeles et al., 2003). Further challenges in dividing the ACC into consistent regions arises from the variability in size and location of these regions as a result of differences in sulcal and gyral anatomy between individuals. Specifically, 30–60% of cases have a paracingulate sulcus, which runs dorsal and parallel to the cingulate sulcus (Fornito et al., 2006; Yucel et al., 2001). Presence of a paracingulate sulcus is associated with a relative expansion of Brodmann's area 32.

6.2.1 **ACC volume as a candidate endophenotype for depression.**

Meta-analyses indicate that the ACC may have particular importance amongst the various brain structures thought to be involved in depression, with smaller volumes consistently observed in patients with MDD compared to healthy controls (Bora, Harrison, Davey, Yücel, & Pantelis, 2012; Du et al., 2012; Koolschijn, Haren, Lensvelt-Mulders, Pol, & Kahn, 2009; Lai, 2013). The region of the ACC measured by different studies has varied

substantially, but significant findings have been obtained for "total" ACC volumes (e.g., Caetano et al., 2006; Frodl, Jager, Born, et al., 2008), as well as rostral (e.g., van Tol, van der Wee, van den Heuvel, & et al., 2010) and sgACC volumes (e.g.' Drevets et al., 1997) and, according to at least one meta-analysis, appear to be particularly pronounced for the left ACC (Koolschijn et al., 2009). These irregularities may not be necessarily specific to depression however, as similar deficits have been documented in other psychiatric conditions such as bipolar spectrum illnesses (e.g., Drevets et al., 1997; Haznedar, Roversi, & Pallanti, 2005; Hirayasu et al., 1999), obsessive compulsive disorder (Radua & Mataix-Cols, 2009). ACC structural abnormalities may therefore occur in psychiatric disorders more broadly.

Within the depression literature, there has been particular attention given to the role of the left sgACC, with findings of significant volumetric deficits associated with MDD that range from 19%-48% (Botteron, Drevets WC, Heath, & Todd, 2002; Drevets et al., 1997; Hastings, Parsey, Oquendo, Arango, & Mann, 2004). The study with the largest volume reduction comprised only patients with a family history of depressive disorder in one or more first-degree relatives (Drevets et al., 1997). Imaging findings are supported by post-mortem histopathological studies, which have indicated smaller neuronal soma, reduced numbers of glia and neuronal density increases in the subgenual region in patients with depression, particularly those with a family history of the disorder, compared to patients with schizophrenia and healthy individuals (Chana, Landau, Beasley, Everall, & Cotter, 2003; Cotter, Pariante, & Everall, 2001; Drevets et al., 1998).

The majority of research into differences in ACC volume associated with depression has been conducted in adults. Smaller ACC volumes in adolescents with clinical

depression have been documented by one study (e.g., Pannekoek et al., 2014). No sgACC volume differences were apparent between adolescents with or without MDD in another study, however smaller sgACC volumes were noted within the subgroup of individuals with MDD and comorbid anxiety (Jaworska et al., 2016). This study also identified an inverse association between sgACC volume and depressive symptomatology, suggesting that more severe manifestations of depression may be associated with sgACC volume reductions.

Findings from two studies also suggest grey matter reduction in the ACC may be present during childhood and adolescence before clinically significant illness onset, although gender may be implicated somewhat differently across the studies. Boes and colleagues (2008) demonstrated that boys aged 7-17 with subclinical depressive symptoms possessed smaller rostral ACC volumes compared to boys with no depressive symptoms. This finding was present bilaterally but was particularly pronounced for the left rostral ACC (14.6% reduction). The relationship was also stronger for the group of boys with a positive family history of depression. There was no significant relationship between rostral ACC structure and depressive symptoms amongst girls. Vulser et al. (2015) identified smaller gray matter volume in the right rostral ACC in adolescents with subthreshold depression at 14 years old compared to adolescents without depressive symptoms. Medial-prefrontal gray matter volume, which incorporated the rostral ACC, mediated an association between subthreshold depression at this baseline and high depression score two years later in girls only.

Preliminary evidence regarding the stability of a putative volume deficit following illness onset are somewhat conflicting and the current state of the literature does not yet

allow for the drawing of conclusions. Two studies suggest stability of the irregularities. Devrets and colleagues (1997) reported no change in mean volume reduction following three months of antidepressant treatment in their sample, but did not report whether there were any changes in volume associated with changes in diagnosis status or symptom levels. Botteron and colleagues (2002) observed smaller left sgACC volumes in two separate groups of younger females (between 17-23 years) with early onset depression and older females (24-52 years) with recurrent depression versus controls. The magnitude of difference (19%) was the same across the two groups, and there was no evidence of age effects when the groups were combined. The cross-sectional finding of similar volume deficits in individuals in an early phase of depression and those with recurrent depression suggest stability of these volume deficits, however without longitudinal analysis, this cannot be confirmed.

Indeed, in contrast to the above findings, three studies have provided some evidence of differences in volume associated with illness status or functioning more broadly (state-dependence), though the nature of these relationships differ somewhat between these studies. One cross-sectional study documented *greater* grey matter pregenual and subgenual ACC volumes in unmedicated patients with remitted MDD than in currently depressed unmedicated patients or healthy controls (Salvadore et al., 2011). The authors noted that the cross-sectional nature of the study meant that it was not possible to determine whether increased ACC volume arose during effective treatment or sustained remission as adaptive compensatory changes, or whether they might represent stable premorbid developmental differences present prior to illness-onset. In contrast to this study, a cross-sectional investigation by Bremner et al. (2002) identified a non-significant *reduction* (7%)

in overall ACC volumes (BA 24, subgenual gyrus and BA 32) in patients with remitted depression showing only mild, sub-threshold manifestations of the disorder compared with healthy individuals without a history of depression. One longitudinal study observed increases in left posterior sgACC volume in 6 out of 7 patients with psychotic major depression over a period of 2-8 years (the follow up period varied between participants) (Coryell, Nopoulos, Drevets, Wilson, & Andreasen, 2005). This group had shown reduced volumes in this region at baseline compared to patients with schizophrenia. Increases in volume were significantly correlated with improved outcome at follow up, according to the Global Assessment Scale (GAS), a rating scale for evaluating overall functioning on a continuum from psychological illness to health (Endicott, Spitzer, Fleiss, & Cohen, 1976), but no information regarding the level of depressive symptoms specifically was provided. The small sample size and the restriction of participants to those with psychotic symptoms limits the number of conclusions that can be made.

In summary, current findings support that (1) there are smaller volumes of the ACC, particularly the left subgenual region, in individuals with depression, (2) this relationship appears more robust amongst individuals with a family history of the disorder, suggesting a genetic basis, and (3) this abnormality may be present prior to clinical illness onset. Together these conclusions suggest that ACC volume is a promising endophenotype for depression, though queries about the stability of this abnormality remain.

6.2.2 **The impact of 5-HTTLPR genotype on ACC volume.**

There is preliminary evidence demonstrating an impact of 5-HTTLPR genotype on ACC structure. Pezawas and colleagues (2005) found that psychiatrically healthy *s* allele carriers showed significantly smaller subgenual and supragenual volumes compared to their

l/l homozygous counterparts. The volume reduction was particularly pronounced in the rostral part of the subgenual area, which included Brodmann's Area 24, one of the regions most strongly implicated in depression (Drevets et al., 1997). Canli and colleagues (2005) also observed reductions of the ACC in Brodmann's areas 24 and 32 bilaterally in non-depressed individuals carrying either one or two copies of the S allele in comparison to l/l individuals.

Currently, only one study has compared this relationship between patients with a diagnosis of MDD and healthy controls (Frodl, Jager, Born, et al., 2008). Consistent with past research, depressed patients showed smaller ACC volumes than non-depressed individuals. When the impact of genotype on ACC volume was considered in the depressed and non-depressed groups, participants of S/S, L_g/L_g and L_g/S genotypes were found to exhibit greater ACC volume deficits compared to L_AL_A homozygous participants, similar to the two previous studies involving healthy individuals only. There were no apparent genotypic effects amongst individuals experiencing MDD. However, when the impact of diagnosis within the different genotype groups was examined, MDD patients homozygous for the L_A allele showed reduced ACC volumes compared to their psychiatrically healthy L_AL_A genotype counterparts. Diagnosis status did not appear to impact ACC volume amongst individuals with other 5-HTTLPR genotypes. This finding suggests that L-allele homozygous individuals may be more susceptible to structural changes in the ACC associated with active depression however replication is warranted to demonstrate its reliability. These findings also need to be investigated longitudinally to determine their presence prior to illness onset. Furthermore whilst there is evidence suggesting smaller ACC volumes particularly in Brodmann's Area 24 in in patients with depression, it is not

yet known whether ACC volume mediates an association between 5-HTTLPR and depression.

6.3 The Orbitofrontal Cortex (OFC).

The orbitofrontal cortex (OFC) occupies the ventral region of the prefrontal cortex in the frontal lobes, immediately above the orbits in which the eyes are located and in humans comprises Brodmann area 10, 11 and 47 (Kringelbach, 2005). The OFC receives inputs from all five sensory modalities (auditory, visual, somatosensory, gustatory and olfactory) as well as visceral sensory information (Kringelbach & Rolls, 2004).It also has direct reciprocal connections with other brain regions, including the amygdala, hippocampus, dorsolateral prefrontal cortex, hypothalamus and cingulate cortex. These extensive connections allow the OFC to assume an important role in the executive control of information processing and in behaviour regulation by monitoring and integrating sensory and visceral motor information to modulate affect and affectively-driven behaviour (Kringelbach & Rolls, 2004).

The OFC can be subdivided into ‘medial’ and ‘lateral’ components. Connectivity studies have found that the medial OFC is characterised by strong connections with the ventrolateral section of the basal nucleus of the amygdala, the hippocampal formation, the anterior cingulate cortex, dorsolateral prefrontal cortex (DLPFC) and dorsomedial parts of mediodorsal thalamic nucleus (Carmichael & Price, 1995a, 1995b). The lateral OFC receives projections from visual, somatosensory, olfactory and gustatory modalities and shows connections with the ventromedial parts of the basal nucleus of amygdala, posterior cingulate cortex, DLPFC, entoperirhinal cortex, premotor and parietal cortex, and

ventromedial components of mediodorsal thalamic nucleus (Carmichael & Price, 1995a, 1995b; Öngür et al., 2003).

Medial and lateral regions of the OFC are believed to subserve different functions. The medial OFC has been implicated in the learning and monitoring the reward value of stimuli whilst the lateral OFC is thought to have a key role in the assessment of punishers and subsequent adaptation of behaviours (Kringelbach & Rolls, 2004). Moreover, whilst the medial OFC appears to be involved in a more 'pure' form of emotional processing, particularly of negative emotions, the lateral OFC may have a role in controlling emotional experience and expression via top-down regulation strategies such as reappraisal or suppression (Beauregard, Levesque, & Bourgouin, 2001; Blair, 2004; Lévesque et al., 2003), possibly by inhibiting neural activity in brain regions directly implicated in emotional feeling, such as the amygdala, medial OFC and insula (Banks, Eddy, Angstadt, Nathan, & Phan, 2007; Ochsner, Bunge, Gross, & Gabrieli, 2002; Ochsner et al., 2004; Piech et al., 2010). The lateral OFC may also facilitate selective attention by inhibiting irrelevant or unwanted emotional information (Vuilleumier, Armony, Driver, & Dolan, 2001).

6.3.1 **OFC volume as a candidate endophenotype for depression.**

The prefrontal cortex shows high heritability (90-95%) (Peper et al., 2007) and meta-analyses provide evidence that OFC volumes may be smaller in patients with MDD compared to healthy controls (Arnone, McIntosh, Ebmeier, Munafo, & Anderson, 2012; Kempton, Salvador, Munafò, & et al., 2011). Meta-regression analyses of the effect of illness characteristics and demographic variables did not suggest this relationship was influenced by gender, age of participants at the time of scanning, or their age at depression

onset (Arnone et al., 2012). Rather counterintuitively, there was some indication that as the proportion of life spent in illness (i.e. the ratio of duration of illness to age) increased, the effect of depression on right OFC grey matter became less pronounced. In contrast, treatment with antidepressants appeared to enhance this effect.

Findings are somewhat conflicting as to whether OFC volumetric differences might represent a vulnerability trait that precedes the illness or whether these might arise as a consequence of the disorder. Critically, prospective longitudinal studies that document OFC volumes prior to the emergence of depression are lacking. One longitudinal study of 193 participants initially aged 3-6 years old, enriched for early childhood depression, did not identify any initial differences in orbital gyrus volumes during a baseline MRI scan between 7-13 years, or change in volumes according to two follow up scans over an approximate 3 ½ year period that were associated with experiencing an episode of MDD prior to the first magnetic resonance imaging scan (Luby, Belden, Jackson, et al., 2016). Given approximately half of the participants had depression at recruitment, this study cannot speak to the question of whether there are differences in OFC (orbital gyrus) volumes *prior* to depression onset. It does suggest however that depression in early childhood does not affect the trajectory of orbital gyrus development in middle childhood and early adolescence. There is some suggestion however that childhood depression has a different risk profile to depression with onset in other developmental periods and therefore childhood depression may be associated with different underlying mechanisms (Scourfield et al., 2003). In particular, environmental factors may be more central to child depression whilst inherited genetic factors may play a less prominent role; twin studies consistently document *lower* heritability estimates for child-onset depression than adolescent-onset

depression (Thapar & Rice, 2006) and childhood depression also appears to show particularly strong associations with childhood family adversity and parental neglect, (Hill, Pickles, Rollinson, Davies, & Byatt, 2004; Jaffee et al., 2002). Interestingly, a twin study has also noted limited heritability of OFC volume in young children but considerably larger heritability during adolescence when this region undergoes substantial remodelling, consistent with increasingly pronounced genetic effects with maturation (Lenroot et al., 2009). Thus, the extent to which genetically-driven differences in OFC volumes associated with depression might become evident later in life and the extent to which OFC volumes remain stable in depression during a different development period remains unknown.

One cross-sectional study has documented larger right lateral (but not overall) OFC volumes in youth between 9 and 18 years old with MDD compared to healthy controls (Chen et al., 2008) though this finding did not survive correction for multiple analyses. This result, which given the participants' age, may reflect the state of the OFC in a relatively early stage of illness, contrasts with the more commonly obtained finding of a reduction in OFC volume in depressed patients. It is possible that this finding reflects an underlying pathological process whereby volumes increase in initial phases of the disorder but reduce as individuals experience longer durations or repeated episodes of depression, as suggested by the majority of research in adults (Bora et al., 2012). Alternatively, a deviation from the normative inverted U-shaped course of brain maturation, which typically follows a period of initial increase in cortical volume during childhood and a subsequent adolescent decline, could account for these findings (Giedd, 2004; Giedd et al., 1999; Gogtay et al., 2004; Shaw et al., 2008).

Bremner et al. (2002) identified smaller medial OFC volumes in patients with remitted MDD compared to psychiatrically healthy controls, consistent with diminishments being stable over time. In contrast, Lacerda et al. (2004) documented bilateral volumetric reductions of the medial OFC in currently depressed patients relative to controls but no difference in volumes between remitted patients and controls, or between currently depressed patients and remitted patients, raising the possibility that some reversal of the volume diminishment had occurred for remitted patients, and that OFC changes may therefore be state-dependent. The only longitudinal study of changes in OFC volume over the course of MDD identified greater decline in grey matter density over a three-year period in MDD patients compared to healthy controls (Frodl, Koutsouleris, Bottlender, Born, Jager, et al., 2008). There was no detectable difference in the rate of decline between MDD patients who achieved stable remission over the three years however and those that did not.

Findings to date regarding the degree to which OFC structural abnormalities show a familial association are also somewhat sparse and inconclusive. One study identified similar grey matter reductions in the orbitofrontal cortex in patients with MDD and healthy first-degree relatives of MDD patients (Opel et al., 2016). Similarly, a particularly large study documented diminished grey matter volumes of the right lateral orbitofrontal gyrus in the biological children or grandchildren (age range between 6 and 54 years old) of individuals with either moderate to severe, recurrent and functionally debilitating depression (Peterson et al., 2009) compared to those with no family history of the disorder. A limitation of this study however is the inclusion of a number of participants that had experienced MDD (both lifetime and current) in the sample, with a higher proportion in the familial high-risk group, which makes it difficult to know whether these findings reflect a

stable difference that had been present prior to depression onset or reflects changes that have occurred during illness. Another study failed to identify any differences in grey matter OFC density between healthy individuals with a first-degree relative with depression and a control group without a family history of the disorder (Macoveanu et al., 2014).

In summary, despite robust evidence from meta-analyses suggesting that adults with depression have smaller OFC volumes compared to non-depressed individuals, the small number of available studies and conflicting findings in the existing literature mean it is currently unclear whether this abnormality is present prior to illness onset or during remission. Moreover, the extent to which variations in OFC volume might be more common in individuals at high risk for depression as a result of a family history also remains difficult to assess. Further research is therefore needed to clarify the extent to which OFC volume might represent a candidate endophenotype for depression.

6.3.2 **The impact of 5-HTTLPR genotype on the OFC.**

Although there is robust evidence implicating smaller OFC volumes in depression, the literature that has examined the role of the serotonin transporter gene in accounting for OFC volume is scant. In healthy adults, carriage of an S-allele has been linked with diminishments in OFC volume (Canli et al., 2005) and prefrontal volumes more broadly (Frodl, Koutsouleris, Bottlender, Born, Jäger, et al., 2008), however another study failed to identify an effect of genotype on OFC volume (Atmaca et al., 2011). There does not appear to be any published research that has tested for differences in OFC volume in patients with depression of different serotonin transporter genotypes. Amongst patients with OCD however, smaller OFC volumes have been identified in S-carriers relative to LL-homozygotes (Atmaca et al., 2011).

6.4 The hippocampus

Also known as the cornu Ammonis, the hippocampus is a seahorse- shaped bilaminar grey-matter structure in the medial temporal lobe, which is comprised of distinct subregions, termed CA1-CA4, based on pyramidal neuron morphology and anoxia sensitivity (Campbell, Marriott, Nahmias, & MacQueen, 2004). The hippocampus has received significant attention in depression research for a number of reasons. First, the hippocampus has a critical role in learning, cognition and memory formation, particularly the consolidation of episodic or autobiographical memories into long-term storage (Eichenbaum, 2004; Squire, 1992) and the binding of contextual and affective elements of experience (Burgess, Maguire, & O'Keefe, 2002; Hassabis & Maguire, 2009). The experience of memory difficulties, including overgeneral autobiographic memories and biased recall of negative memories is a well-established symptom of depression (Dalgleish & Werner-Seidler, 2014; Hamilton & Gotlib, 2008; von Gunten, Fox, Cipolotti, & Ron, 2000). Second, the hippocampus has also been posited to have a critical function in the inhibitory regulation of the HPA axis and is also highly sensitive to the effects of stress (Sapolsky, 2000). This structure additionally appears to have a particularly important role in discerning between threat and safety (Ji & Maren, 2007; Lau et al., 2011). Third, the hippocampus is also highly connected to amygdala and orbitofrontal cortex (Fastenrath et al., 2014; Jin & Maren, 2015; Wikenheiser & Schoenbaum, 2016) suggesting an important broader contribution to emotional processing and motivation.

6.4.1 **Hippocampal volume as a candidate endophenotype for depression.**

Current estimates suggests hippocampal volume may have moderate-to high heritability, appearing to fall within a range of .40-.80 (den Braber et al., 2013; Glahn et al.,

2007; Peper et al., 2007; Stein et al., 2012). Whilst there have been some null findings amongst individual structural magnetic resonance imaging (MRI) studies (e.g., Caetano et al., 2004; Posener et al.; Vakili et al., 2000), a number of meta-analyses have demonstrated that patients with MDD show smaller hippocampi bilaterally relative to age and sex matched controls (Campbell et al., 2004; Videbech & Ravnkilde, 2004). Further support of hippocampal reductions in depressed participants has recently been provided by a large-scale international collaboration (ENIGMA consortium: 1728 MDD patients, 7199 controls; Schmaal et al., 2016).

These meta-analytic and collaborative studies have also considered the extent to which hippocampal volumes may be impacted by important clinical variables, particularly length of illness or illness state. Videbech and Ravnkilde (2004) reported that patients who had experienced more depressive episodes showed greater volume diminishments. Similarly, McKinnon and colleagues (2009) found evidence of smaller hippocampal volumes only amongst individuals who had experienced more than one episode of depression or who had experienced an illness duration of more than two years. Schmaal et al. (2016) also failed to detect any volume differences between first episode patients and controls. There was however an association between earlier age of onset ($\leqslant$21 years and a smaller hippocampus. Contrasting somewhat with these findings, Kempton et al. (2011) found equivalent hippocampal volumes between patients with first episode versus multiple episodes and between patients with early versus late-onset depression. The reduction in hippocampal volume also remained significant when the meta-analysis was limited to studies with first-episode patients. However, patients with MDD in remission were found to

have increased volumes relative to those who were currently depressed, and there was no significant difference in volume between patients in remission and healthy controls.

Whilst the finding of reduced hippocampal volume in depression appears relatively robust, other findings regarding the degree to which hippocampal volume might vary as a function of illness factors are somewhat inconsistent. Importantly, the studies by Videbech and Ravnkilde (2004), McKinnon et al. (2009), Kempton et al. (2011) and Schmaal et al. (2016) were based on cross-sectional data which merely allow speculations about stability or state-dependence.

Longitudinal studies have therefore been important in shedding further light on the relationship between hippocampal alterations and the course of MDD. One prospective longitudinal investigation followed a group of adolescents who, at the start of the study, had never experienced a depressive episode but who had been sampled so that there was an overrepresentation of individuals at high and low temperamental risk for psychopathology (Whittle, Lichter, et al., 2014). This study found evidence of larger hippocampal volumes in early adolescence (11-13 years) and then an apparent attenuation of the normative pattern of growth over mid-adolescence (until 15-16 years) to be associated with onset of depression over the late adolescent period (16-19 years). This finding suggests abnormal patterns of hippocampal development might predate the occurrence of clinically significant depression. (Isikli et al., 2013) detected no apparent differences in hippocampal volume between first-episode MDD patients and healthy controls, both at baseline and at five-year follow up, though shape analyses indicated some structural changes in the CA1 and subiculum regions of the head and tail of the hippocampal formation in patients. Frodl, Koutsouleris, Bottlender, Born, Jager, et al. (2008) identified significantly more decline in

grey matter density in the hippocampus over a three year period in MDD patients compared to controls. Remission during the three year period was associated with less volume decline in the left hippocampus however. Phillips and colleagues (2015) did not identify any structural differences in the hippocampus between patients and controls at baseline but patients who remitted and did not remit within six months showed subtle changes in volume over this follow up period; increased hippocampal volume was documented in remitters, whilst decreased volume was documented in non-remitters. Somewhat similarly, Ahdidan et al. (2011) found that diminished hippocampal volumes in MDD patients that had been observed at baseline relative to controls were no longer evident at an 11 year follow up, where remission had been achieved (non-remitted patients were not included in the follow up). Hou et al. (2012) found that whilst there was evidence of a bilateral reduction in hippocampal volume in first-episode geriatric MDD patients (with a duration of less than 6 months) currently in remission compared to healthy controls, there was no difference in right hippocampal volume between the groups after 21 months. There is also some indication that diminished hippocampal volumes may be associated with poorer prognosis (MacQueen & Frodl, 2011), including reduced likelihood of remission after 1 year (Frodl, Meisenzahl, Zetzsche, et al., 2004) and a longer duration of illness (MacQueen et al., 2003).

Reduced neurogenesis in the dendate gyrus as well as loss of pre-existing glial cells and retraction of dendrites have been implicated as proximal causes of this hippocampal atrophy, and it has been hypothesised that both of these processes may be the result of an increase in glucocorticoids from a stress-induced increased activity of the HPA axis (Czeh & Lucassen, 2007; Sapolsky, 2000). Given that the hippocampus is involved in HPA axis

regulation, hippocampal structural changes may affect negative feedback inhibition of this system, leading to further damage of the hippocampus (Sapolsky et al., 1986). Animal models show that antidepressants as well as non-pharmacological treatments for depression such as electroconvulsive therapy and exercise stimulate neurogenesis in the hippocampus (Kiuchi, Lee, & Mikami, 2012; Madsen et al., 2000; Malberg, Eisch, Nestler, & Duman, 2000; Perera et al., 2007; van Praag, Christie, Sejnowski, & Gage, 1999) and the timecourse of the therapeutic effects of antidepressants appears to coincide with the formation of new dendate neurons (Espósito et al., 2005; Ngwenya, Peters, & Rosene, 2006).

Findings that the extent of hippocampal atrophy may change over the course of the depressive disorder and that the basis of this persistent atrophy may be HPA-induced neurotoxity and/or reduced neurogenesis suggests this volume deficit is a consequence or correlate of the condition (a biomarker) rather than a pre-disposing vulnerability factor or endophenotype (Savitz & Drevets, 2009). There are however several important inconsistencies noted in the literature that challenge this conclusion somewhat. First, a number of studies have observed differences in hippocampal volumes in healthy individuals at high risk for the disorder by virtue of their family history of depression, compared to those without this history. The vast majority of these studies have detected reduced volumes at-risk individuals (e.g., Amico et al., 2011; Baaré et al., 2010; Carballedo et al., 2012; Chen, Hamilton, & Gotlib, 2010; Rao et al., 2010). For example, a study by Amico and colleagues (2011) found that healthy adults with a family history of depression showed smaller right hippocampal grey matter volumes compared to those without a family history and, somewhat surprisingly, even smaller right hippocampal volumes than patients

with MDD. One study however identified enlarged volumes associated with high risk status (Romanczuk-Seiferth et al., 2014). Second, smaller hippocampi have been reported in depressed children and adolescents, many of whom had experienced relatively short periods of illness (e.g., Caetano et al., 2007; MacMaster & Kusumakar, 2004; MacMaster, Mirza, et al., 2008; Rao et al., 2010 but see MacMillan et al., 2003; Rosso et al., 2005 for null findings) and hence limited time for changes to occur. A correlation between smaller volumes and increased depressive symptoms has also been noted in psychiatrically healthy children but did not predict changes in depressive symptomatology over an 18 month period (Pagliaccio, Luby, Luking, Belden, & Barch, 2014).

The potential involvement of the HPA-axis in the occurrence of hippocampal atrophy additionally raises questions about whether these volume deficits are associated with stress more broadly, rather than being specifically related to depression. Indeed, research indicates that hippocampal volume reductions are associated with stress and trauma, such as a history of childhood maltreatment, particularly in patients with MDD but also (to a somewhat lesser extent) in psychiatrically healthy individuals (Calem, Bromis, McGuire, Morgan, & Kempton, 2017; Paquola, Bennett, & Lagopoulos, 2016). Additionally, analyses have suggested that childhood trauma may account for the difference in hippocampal volume between healthy and depressed individuals (Opel et al., 2014). Reduced hippocampi have also been demonstrated in other stress-related disorders, including schizophrenia, OCD, PTSD and Borderline Personality Disorder (Geuze, Vermetten, & Bremner, 2005; Ruocco, Amirthavasagam, & Zakzanis, 2012; Smith, 2005).

Extensive studies in animals and a smaller body of research in humans suggest that stress encountered early in development, during a particularly sensitive period of high

neural plasticity, may cause alterations to this structure that may not be immediately visible but are rather expressed in a later period of development. In humans, there are consistent findings of reduced hippocampal volumes in adults with a history of childhood maltreatment but these reductions are identified less consistently in children who have been maltreated (Teicher & Samson, 2016). Furthermore, one study has suggested that young adults who experienced sexual abuse between the ages of 3-5 years and 11-13 years show the largest hippocampal reductions (Andersen et al., 2008). In rodents, exposure to stress in early life was associated with hippocampal volume deficits that emerge only during puberty and early adulthood (Andersen & Teicher, 2004). Taken together, these findings could suggest that early stress or trauma may sensitize the HPA axis to even mild stress later in life and alter hippocampal development in such a way that creates a broad susceptibility to stress-associated emotional disorders such as depression.

Importantly, individuals may be differentially sensitive to the effects of early stress on the hippocampus and this sensitivity may be genetically based (Frodl et al., 2010). This hypothesis has been supported from findings by a longitudinal study that examined the relationships between early adversity, hippocampal volume and vulnerability to depressive disorder (Rao et al., 2010). The study showed that both currently depressed adolescents and never-depressed adolescents with a parental history of the disorder had smaller hippocampal volumes bilaterally than healthy controls with no family history. Greater early-life adversity was associated with smaller hippocampal volumes in both the controls and in the high-risk participants. In the depressed cohort, this relationship was moderated by parental depression, such that there was a strong negative correlation between early adversity and hippocampal volume in adolescents with a parental history of depression, and

only a small correlation in those without a parental history of the disorder. Importantly, after controlling for early adversity, hippocampal volume predicted depression diagnosis during a five year follow-up period. Hippocampal volume failed to significantly mediate a relationship between early adversity and depression however, though the authors acknowledged this might have been due to low power to detect effects.

Further support for the possibility that hippocampal sensitivity to the environment might vary as a function of genetics comes from a study by Carballedo et al. (2012) which reported that psychiatrically healthy individuals with a family history of the disorder had diminished hippocampal volumes relative to those without a family history, but that within the positive family history group, those who reported more frequent experiences of child trauma showed smaller hippocampal volumes than their counterparts without a significant trauma history. Importantly, differences in hippocampal volume between individuals with and without significant experiences of trauma were not apparent in the absence of a family history of depression. Similarly, smaller hippocampal volumes have been found to confer vulnerability to PTSD. A study of monozygotic twins discordant for exposure to combat found that hippocampal volume in both the trauma-exposed twin and in the non-exposed asymptomatic twin showed a significant negative correlation with PTSD symptom severity in the twin who had experienced combat (Gilbertson et al., 2002). These studies indicate that deficits in hippocampal volume may precede the onset of stress-related illness and that environmental factors may contribute towards further deterioration in volume, particularly in high risk individuals by virtue of their family history.

In summary, findings from numerous studies support an association between smaller hippocampal volume and depression, however it is unclear whether hippocampal

volume deficits arises as a result of the disorder, or represents a pre-existing, stable vulnerability to depression that is more common in individuals with a family history of the disorder. It is also possible that there may be a genetically-based dormant *predisposition* to HPA-induced hippocampal atrophy that only manifests in adverse or stressful environments, such as those involving child maltreatment, which in turn creates a depression susceptibility (Frodl et al., 2010).

6.4.2 **The impact of 5-HTTLPR genotype on hippocampal volume.**

A high density of serotonergic neurons within the hippocampus (Jacobs & Azmitia, 1992) suggests that genes with the potential to alter serotonin levels, such as the serotonin transporter gene, could have an effect on hippocampal structure, and that this in turn could impact on susceptibility to depression.

With regards to healthy individuals, whilst there has been one report of diminished volumes in S-allele carriers compared to individuals homozygous for the L allele (Frodl, Koutsouleris, Bottlender, Born, Jäger, et al., 2008), the majority of studies to date have generally detected no effect of 5-HTTLPR genotype, either in isolation or in interaction with early adversity, on hippocampal volumes (e.g., Cole et al., 2011; Dutt et al., 2009; Eker et al., 2011; Frodl, Meisenzahl, Zill, et al., 2004; Frodl et al., 2010; Pezawas et al., 2005). Everaed and colleagues (2012) however found evidence for a three-way interaction, such that the association between serotonin transporter genotype and hippocampal morphology was affected by gender, particularly under stress conditions. Whilst female S-allele carriers had smaller hippocampal volumes than L-allele carriers overall, only male S-allele carriers who had experienced severe childhood adversity showed diminished hippocampi. Thus in healthy individuals, the S-allele may be associated with hippocampal

volume independent of childhood adversity in women whilst the S-allele may only predict reduced hippocampal volumes in men who have experience childhood adversity.

Reduced hippocampal N-acetylaspartate (NAA) concentration in healthy adult S-allele carriers compared to L-homozygotes has also been observed. Lower NAA concentrations, which is considered as a marker of neuronal or axonal damage, have also been documented in youth and adults with depression (de Diego-Adelino et al., 2013; MacMaster, Moore, et al., 2008).

There is also evidence of a genetic effect for 5-HTTLPR on hippocampal morphology amongst patients with MDD, though it is somewhat unclear which allele is associated with smaller structure. Frodl and colleagues (2004) found that patients homozygous for the L-allele possessed significantly reduced right hippocampal white matter compared to patients carrying at least one S-allele. Patients of L/L genotype also showed smaller left hippocampal white matter volumes compared those homozygous with the S/S genotype (but not the S/L genotype). Significantly smaller hippocampal grey and white matter volumes were also detected only in the group of patients with the L/L genotype as compared with controls who carried this genotype. Patients and controls heterozygous for the L/S genotype and patients and controls homozygous for the S/S genotype showed comparable hippocampal volumes. The same research group replicated these findings in a later study with a different sample using the functional triallelic as well as the diallelic polymorphisms of the serotonin transporter (Frodl, Koutsouleris, Bottlender, Born, Jäger, et al., 2008). In contrast to these findings, Eker and colleagues (2011) reported smaller hippocampal morphologies in patients of S/S genotype compared to both S/L carriers and L/L homozygotes. Age of illness onset may interact with 5-HTTLPR genotype

to impact hippocampal volumes in depression; one study has found that S/S genotype predicted reduced hippocampal volumes in early onset patients (those experiencing a first episode prior to age 50), whilst the opposite pattern was obtained in late-onset patients (Taylor, Steffens, Payne, & et al., 2005). This relationship may also be further complicated by the experience of high stress, such as childhood adversity, according to findings by Frodl and colleagues (2010). They reported that MDD patients carrying an S-allele who had also experienced emotional childhood hood neglect showed smaller hippocampal volumes than both L-allele carriers with a similar history and S-allele carriers who had not encountered emotional childhood neglect.

This literature arguably provides preliminary evidence for an effect of 5-HTTLPR genotype on hippocampal volume in both healthy individuals and patients with MDD, particularly under circumstances of environmental adversity. It is important to note though that the relationships between 5-HTTLPR genotype, hippocampal volume, environmental variables such as early adversity, and depression in all of these studies have to date involved explorations of their interactional effects. As reviewed in the previous section however, the genetic basis to hippocampal morphology as well as findings that indicate healthy individuals with a family history of depression show smaller hippocampal volumes than individuals without a family history of the disorder, hint at an alternate possibility; that differences in hippocampal volume might account for or mediate a relationship between 5-HTTLPR genotype and depression. Moreover, given the findings linking early adversity or trauma with hippocampal volume, this mediated relationship proceeding from serotonin transporter genotype to depression through hippocampal volume may be moderated by environments involving varying degrees of adversity.

6.5 The Amygdala.

The amygdala is the almond shaped structure located in medial temporal lobe and is highly connected to other brain regions, including cortical and subcortical regions, such as the sensory (visual, auditory, olfactory, taste and somatosensory, including pain) systems, as well as hippocampus, and medial prefrontal cortex (LeDoux, 2007). Whilst understanding of the role of this structure is constantly being refined, current consensus is that the amygdala has a central role in both normal and pathological emotion processing, particularly in emotionally mediated attention, assigning biological relevance (either reward- or threat-related) or emotional significance to stimuli and emotional memory (Adolphs, 2008, 2010; Hooker, Germine, Knight, & D'Esposito, 2006; Phelps, 2006; Phelps & Sharot, 2008). It has been suggested that fearful facial expressions may engage these processes more reliably than other facial expressions. Enhanced amygdala activity in response to fearful faces has been documented more consistently than in response to other expressions such as anger and happiness (Morris et al., 1996; Whalen et al., 2001).

6.5.1 **Amygdala volume as a candidate endophenotype for depression.**

Twin studies indicate moderate-to high heritability of amygdala volume within the range of .48-.83 (den Braber et al., 2013; Hulshoff Pol et al., 2006; Kremen et al., 2010; Peper et al., 2009), suggesting a significant genetic basis to amygdala morphology. Structural imaging studies also indicate that morphological abnormalities of the amygdala may be associated with major depressive disorder however the exact nature of the relationship has been difficult to determine (Bellani, Baiano, & Brambilla, 2011). Findings of structural imaging studies investigating the association between amygdala volume and depression have often been conflicting, with two meta-analyses suggesting no difference in

amygdala morphology between patients with MDD and healthy individuals (Kempton et al., 2011; Koolschijn et al., 2009) and two meta-analyses suggesting alterations (Hamilton, Siemer, & Gotlib, 2008; Sacher et al., 2012). The meta-analysis by Sacher et al. (2012) identified smaller left amygdala volumes in patients with MDD, whilst the meta-analysis by Hamilton et al. (2008), which also addressed the impact of antidepressant treatment and illness duration, revealed that amygdala volume was significantly decreased in samples comprising unmedicated depressed individuals in studies, and significantly increased in depression in samples containing medicated individuals compared to controls. Importantly, the relationship between medication and illness chronicity also approached significance (such that medication was associated with shorter lengths of illness), and when medication was *not* taken into account in analyses, chronicity of depression also accounted for significant variation in average amygdala volumes between depressed individuals and controls.

Whilst these two meta-analyses perhaps provide some cross-sectional evidence for smaller amygdala structures in depression, it is unclear whether any potential amgydala irregularities are permanent, consistent with an endophenotype, or whether they are state-dependent, seen only during the acute phase of the disorder, which would be more suggestive of a biomarker. Longitudinal studies are better placed to answer such questions. One prospective longitudinal study that examined how absolute amgydala volumes and volumetric changes might be associated with depression onset identified that adolescent females who experienced a first onset of depression during late adolescence (16-19 years) showed initially smaller amygdala volumes in early adolescence (11-13 years) followed by increased growth of the amygdala over early to mid-adolescence (11-16 years) relative to

individuals who remained depression free over the course of adolescence (Whittle, Lichter, et al., 2014). In contrast, males who experienced a first onset of depression during late adolescence showed initially larger amygdala volumes and then attenuated amgydala growth during early-mid-adolescence compared to healthy adolescents. Gender may therefore also play a role in explaining inconsistent findings. Interestingly, volume differences at early-adolescence were not apparent during mid-adolescence (15-16 years) the period before documented depression onset. Significant findings remained when participants who developed a depressive disorder before mid-adolescence were excluded. Disruptions to more typical patterns of amygdala development may represent a risk factor for depression that is present before disorder emergence, with differences in absolute volume only evident at certain points in the course of the disorder. This study did not examine whether further changes occurred later in adolescence which may have led to differences in amgydala volume that were apparent at disorder onset or after a longer duration of active illness.

There are several longitudinal studies that have considered changes occurring illness. Longitudinal follow up of a group of adult MDD patients and healthy controls did not identify any initial differences between these groups at baseline or changes in amygdala volumes over a 1 year period (Frodl, Meisenzahl, Zetzsche, et al., 2004), or a three year period (Frodl, Jager, Smajstrlova, et al., 2008). Similarly, Weber and colleagues failed to detect any volumetric changes involving the amygdala over a two year period. A different longitudinal study by Frodl, Koutsouleris, Bottlender, Born, Jager, et al. (2008) identified greater volume decline in the left amygdala over three years in MDD patients versus healthy individuals. Cross-sectional analyses have provided findings of both reduced

volumes (e.g., Sheline, Sanghavi, Mintun, & Gado, 1999) and preserved volumes in remitted patients (e.g., Frodl, Meisenzahl, Zetzsche, et al., 2004; Lorenzetti, Allen, Whittle, & Yücel, 2010; Sheline et al., 1999; van Eijndhoven et al., 2009) relative to healthy controls.

Moreover, some though not all studies suggest that enlarged amygdale may be present in the initial stages of the condition. Several cross-sectional studies have reported increased amygdala volumes in first episode patients compared to healthy individuals and recovered patients (Frodl et al., 2002; van Eijndhoven et al., 2009) and compared to patients with recurrent depression (Frodl et al., 2003). Frodl and colleagues (2002) have proposed this finding may reflect greater amygdala metabolism and blood flow to the area in early phases of depression. In contrast, Rosso and colleagues (2005) documented decreased amygdala volumes in paediatric patients with MDD, who presumably were also in very early phases of the disorder compared to healthy controls, whilst MacMaster and colleagues (2008) did not identify any differences between first episode child and adolescent patients who also had a family history of the disorder and healthy participants. Saleh and colleagues (2012) reported that female patients with a negative family history were found to have larger right amygdala volumes than healthy females with a negative family history and female patients with a positive family history. Findings of smaller amygdala volume in patients with a positive family history relative to patients without a family history raises the possibility that progressive volume decline during the disorder could be specific to individuals with a stronger genetic loading for the disorder.

Interestingly, studies have more consistently demonstrated increased and longer lasting amygdala reactivity to negative stimuli in patients with MDD relative to controls

(Drevets, 2001; Hamilton et al., 2012; Siegle, Steinhauer, Thase, Stenger, & Carter, 2002). The finding of intense amygdala activity has been shown to hold across a number of different negative stimuli, including faces with negative expressions, negative words and negative emotion-inducing pictures (Savitz & Drevets, 2009). This altered amygdala activity been interpreted to represent a bias in the processing of negative emotional stimuli (Hariri & Holmes, 2006). Problems with disengagement from negative stimuli has repeatedly been identified as present in MDD (Disner, Beevers, Haigh, & Beck, 2011; Gotlib & Joormann, 2010). Increased amygdala activity has also frequently been shown to be associated with individual differences in trait anxiety (e.g., Etkin et al., 2004; Haas, Omura, Constable, & Canli, 2007; Stein, Simmons, Feinstein, & Paulus, 2007), which is a well-recognised risk factor for depression. A bias in the deployment of attention to negative emotional stimuli has been noted in anxiety (Mogg & Bradley, 2005).

The questions regarding the etiology of potential structural abnormalities of the amygdala in depression, including how they might be associated with functional abnormalities in the amygdala may be important to resolving whether or not amygdala volume meet the criteria for a depression endophenotype. Surprisingly, very little research attention has been given to the issue of how reactivity and structure might be related. One study has suggested an association between heightened, more sustained amygdala responsivity and smaller amygdala structure in both depressed and non-depressed patients (Siegle, Konecky, Thase, & Carter, 2003). A similar relationship has been observed in patients with Bipolar Disorder, which was independent of current mood state (mania or depression) (Kalmar et al., 2009). The cross-sectional nature of these studies however means that it is not known whether amygdala reactivity drives structure or whether

structure drives reactivity. It has been suggested that sustained, excessive emotion-related amygdala activity may facilitate excitotoxic effects of glucocorticoids or glutamate, and that this may be a factor contributing to amygdala volume reductions (Sheline, Gado, & Price, 1998). Alternatively, pre-existing differences in amygdala structure, another brain region such as the ventromedial prefrontal cortex, or connectivity between these regions, might drive increased amygdala reactivity (Foland-Ross et al., 2010; Kim & Whalen, 2009).

Further contributing to this debate are conflicting findings as to whether amygdala structural abnormalities may represent a pre-existing familially-based vulnerability to depression that is evident without illness onset. Romanczuk-Seiferth et al. (2014) found larger amygdala volumes in healthy first degree relatives of patients with MDD compared to healthy individuals without a family history, suggestive of a possible vulnerability factor in MDD aetiology. Van der Plas and colleagues (2010) reported a positive association between fearfulness and larger right amygdala volume in an overall sample of girls between 7-17 years old and that this relationship was more robust and present bilaterally in girls with a positive family history for depression. There was no significant association in boys. Further supporting these findings are results from a small pedigree study involving 5 affected and 10 non-affected members of a family with mood disorders who were compared to 15 healthy, unrelated matched controls (Boccardi et al., 2010). Four of the 5 affected family members had a diagnosis of major depression and one had a diagnosis of bipolar disorder. The study showed that both affected and non-affected relatives possessed larger left amygdale than the control participants, suggesting enlarged amygdala volume may be present prior to illness onset and that volumes may be, at least in part, genetically-

determined. In contrast, a study by Saleh et al. (2012) did not identify any differences in amygdala volume between healthy individuals with and without a family history of depression. There were also no apparent differences between the individuals with a family history of the disorder that had gone on to experience an onset of depression themselves and those that remained healthy.

In summary, there is some suggestion that larger amygdala structure could be associated with an early phase of the disorder, possibly due to greater amygdala metabolism and blood flow, and may also occur with antidepressant treatment but that smaller amygdala volumes may be present in chronic, untreated depression. The limited number of studies to date that identified differences in amygdala volume in children and adolescents with and without a family history of depression have tended to identify enlarged structures in higher risk individuals.

It has been suggested that this volume abnormality may be related to increased amygdala reactivity, which has also been associated with a well-established depression vulnerability trait of fearfulness or trait anxiety. It is still premature to draw any definite conclusions, particularly given the few studies that have considered longitudinal changes. These current findings leave open the possibility that abnormal amygdala structure represents a candidate endophenotype for depression.

6.5.2 **The impact of 5-HTTLPR genotype on the amygdala.**

Dense innervation by serotonergic neurons, particularly from the dorsal raphe (Varnäs et al., 2004) as well as a high concentration of serotonin receptors in the amygdala subnuclei (Pazos, Probst, & Palacios, 1987) supports the hypothesis that abnormal

serotonergic neurotransmission within the amygdala is likely to have important consequences for emotional behaviour, including mood.

A small literature suggests an association between the serotonin transporter gene and the amgydala. Pezawas and colleagues(2005; 2008) found evidence for decreased grey matter volumes in the amgydala (as well as reduced functional connectivity to the perigenual anterior cingulate cortex) in S-allele carriers, compared to L-allele homozygotes. Frodl and colleagues (Frodl, Jager, Born, et al., 2008) also reported reduced amygdala volumes in individuals homozygous for the S-allele. However, opposite findings (Scherk et al., 2009) and null findings (Canli et al., 2005; Stjepanović, Lorenzetti, Yücel, Hawi, & Bellgrove, 2013) have also been documented.

Previously, it was thought that the most robust support of a link between 5-HTTLPR and the amygdala came from functional imaging studies, which appeared to consistently indicate that healthy S-allele carriers show higher levels of amygdala activity in response to negative stimuli in comparison to individuals homozygous for the L allele, according to two meta-analyses (Munafò et al., 2008; Murphy et al., 2013). This finding has been somewhat called into question however by the most recent meta-analysis, which included the largest replication study of this relationship to date, as well as additional unpublished studies, and found the pooled meta-analytic effect to be only marginally significant (p=.06), suggesting that the strength of the association may be smaller than originally thought (Bastiaansen et al., 2014).

Functional connectivity studies however have identified that 5- HTTLPR S-allele carriers may exhibit reduced coupling of amygdala and sACC activity (e.g., Heinz et al., 2005; Lemogne et al., 2011; Pezawas et al., 2005; Roiser et al., 2009 but c.f. O'Nions,

Dolan, & Roiser, 2011), and that this reduction of amygdala- sACC coupling leads to disinhibition of a feedback loop that results in increased amygdala activity (Hariri et al., 2000; Heinz et al., 2005; Pezawas et al., 2005).

Interestingly, there is some suggestion that 5-HTTLPR genotype effect on amygdala activity may be mediated by amygdala structure, with smaller volumes being associated with both S-allele genotype and increased amygdala response to negative stimuli and the direct relationship between 5-HTTLPR and amygdala activation no longer significant once amgydala volume was taken into account (Kobiella et al., 2011). Taken together, these studies could suggest that S-allele carriers may show amygdala hyperresponsivity to negative stimuli as a result of smaller amgydala volumes, or decreased decoupling between the amygalda and the ACC, mechanisms that could partly underlie a greater sensitivity to stress. However, given some evidence of inconsistencies between findings of an association between the serotonin transporter gene and the amygdala (both structure and function), this area would benefit from further research.

6.6 Conclusion

The current chapter has reviewed findings of studies that have explored potential *brain-behaviour* associations between the anterior cingulate cortex, orbitofrontal cortex, hippocampus and amygdala volumes and depression. There is relatively robust evidence to support the presence of reduced ACC, OFC and hippocampal volumes in depression, however evidence is less consistent regarding the presence of altered amygdala volumes in the disorder. This chapter has also reviewed imaging genetics (*gene-brain*) studies that have considered how variation in the serotonin transporter gene might be associated with variation in these same structures. Studies so far have rather remained siloed, investigating

either gene-brain structure or brain structure-depression relationships in independent samples, and have not yet addressed whether serotonin transporter gene might account for variation in brain structure associated with depression.

Conflicting findings in the currently available research about the timing of the emergence of volumetric differences, including their relationship with active illness also mean that it is unclear whether abnormalities represent a stable, premorbid trait vulnerability for disorder or a transformation associated with changes in illness state. Prospective longitudinal studies that cover the transition from wellness to active illness are particularly needed to provide answers to these questions. Given these current gaps in the literature, the next chapter will examine whether 5-HTTLPR genotypes predict variations in ACC, OFC, hippocampal and amgydala volumes in early adolescence, and whether these variations in turn prospectively predicted an onset of MDD in a 6 year follow up period.

CHAPTER 7: ASSOCIATION BETWEEN SEROTONIN TRANSPORTER GENOTYPE, BRAIN STRUCTURE AND ADOLESCENT-ONSET MAJOR DEPRESSIVE DISORDER: A LONGITUDINAL PROSPECTIVE STUDY.

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ORIGINAL ARTICLE

Association between serotonin transporter genotype, brain structure and adolescent-onset major depressive disorder: a longitudinal prospective study

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The extent to which brain structural abnormalities might serve as neurobiological endophenotypes that mediate the link between the variation in the promoter of the serotonin transporter gene (5-HTTLPR) and depression is currently unknown. We therefore investigated whether variation in hippocampus, amygdala, orbitofrontal cortex (OFC) and anterior cingulate cortex volumes at age 12 years mediated a putative association between 5-HTTLPR genotype and first onset of major depressive disorder (MDD) between age 13–19 years, in a longitudinal study of 174 adolescents (48% males). Increasing copies of S-alleles were found to predict smaller left hippocampal volume, which in turn was associated with increased risk of experiencing a first onset of MDD. Increasing copies of S-alleles also predicted both smaller left and right medial OFC volumes, although neither left nor right medial OFC volumes were prospectively associated with a first episode of MDD during adolescence. The findings therefore suggest that structural abnormalities in the left hippocampus may be present prior to the onset of depression during adolescence and may be partly responsible for an indirect association between 5-HTTLPR genotype and depressive illness. 5-HTTLPR genotype may also impact upon other regions of the brain, such as the OFC, but structural differences in these regions in early adolescence may not necessarily alter the risk for onset of depression during later adolescence.

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INTRODUCTION

Depressive disorders are common and debilitating, have a multifaceted etiology and often emerge during adolescence.[1,2] Recent efforts to understand the underlying biological basis of susceptibility to depression have focused on genetic risk factors.[3,4] However, comprehensive genome-wide association studies have had little success in identifying risk loci, with no replicated findings to date.[5] Increasingly, researchers are returning to more theoretically guided approaches based on biological systems implicated in depression. Such an approach can extend from candidate gene to whole-pathway analyses.[6,7] It is widely accepted that abnormal serotonergic function is implicated in the onset and course of depressive disorders.[8] The serotonin transporter gene (*SLC6A4,* synonyms: 5-HTT, SERT) controls transporter enzyme production and is a key regulator of serotonergic neurotransmission. Furthermore, the effects of genetic variation at this loci have been shown to interact with environmental stressors, such as child maltreatment,[9,10] however, this has not been consistently demonstrated,[11] suggesting a need for further refinement of research methodologies.

Detection of genetic risk could be enhanced by consideration of endophenotypes that occur at an intermediate stage in the causal pathway from a distal gene to the overt expression of disease.[12,13] Brain structure and brain function have been identified as particularly promising endophenotypes for depression, given the findings suggesting they are highly heritable[14,15] and the reported associations between the volume and activity of specific brain regions and the disorder.[16,17] In particular, variation in the volume of brain structures involved in emotional processing and stress responses, including the hippocampus, anterior cingulate cortex (ACC), orbitofrontal cortex (OFC) and amygdala, have been theorized to have a role in mood disorders.[18,19] Specifically, volume reductions in the hippocampi,[20–23] the ACC[24] and the OFC[23,25] have been consistently documented in patients with major depressive disorder (MDD). Smaller hippocampal and ACC volumes have also been linked to poorer clinical outcomes longitudinally.[26–28] Studies of the association between amygdala volume and depression have been somewhat more conflicting, with a recent meta-analysis indicating volume deficits in MDD patients compared to healthy controls,[29] although some earlier meta-analyses have indicated no structural difference between these groups.[23,30] These brain regions are also densely innervated by serotonergic neurons originating primarily in the dorsal and median raphe nuclei.[31] Emerging evidence from imaging genetics studies of mood disorders suggests that variations in serotonergic neurotransmission, due in part to 5-HTTLPR genotype, may be associated with variations in these brain structures, although current findings present a somewhat inconsistent picture.[18] Findings on the hippocampus have been equivocal, with the majority of studies failing to identify differences in hippocampal

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volumes associated with the 5-HTTLPR genotype in healthy individuals (for example, Eker *et al*.,[32] Taylor *et al*.,[33] Frodl *et al*.[34,35]). However, one study with a large sample has reported that individuals homozygous for the S-allele had significantly smaller left hippocampal volumes than those homozygous for the L-allele.[36] With regard to MDD, there have been reports of smaller,[32] larger[34–36] and equivalent volumes[33] in S-allele carriers compared to their L-allele homozygous counterparts.

There have been more consistent reports of smaller ACC structures in psychiatrically healthy S-allele carriers compared to L-homozygous individuals.[36–38] No apparent genotypic effects have been observed in individuals currently experiencing MDD; however, MDD patients homozygous for the L allele have been found to have reduced ACC volumes compared to psychiatrically healthy controls with the same genotype.[36]

Furthermore, there is some evidence suggesting decreased amygdala volumes (as well as reduced functional connectivity between the amydgala and the perigenual ACC) in S-allele carriers.[37,39] However, opposite[40] and null[38] findings have also been documented, albeit in smaller samples. Evidence of an impact of 5-HTTLPR on OFC volumes in humans is currently limited, with only one study to date showing S-allele-associated volume deficits in the left OFC, in psychiatrically healthy individuals.[38]

A key unresolved issue is the extent to which these brain structural abnormalities might serve as endophenotypes that mediate the putative link between 5-HTTLPR and depression. In general, a given variable may be regarded as a mediator to the extent that it accounts for the relationship between the predictor and the outcome. Because endophenotypes occur at an intermediate stage in the causal pathway from a distal gene to overt expression of disease, a mediation model is often assumed (for example, Waldman,[41] Munafò,[42] and Hyde *et al*.[43]) but has rarely been tested explicitly within the field of imaging genetics (see Nikolova *et al*.[44] for a notable exception). To our knowledge, there are no imaging genetic studies of this nature that have examined depression as an outcome. Studies so far have rather remained siloed, investigating either gene–brain structure *or* brain structure–depression relationships, and have not systematically tested mediation relationships within the same sample. There are also a limited number of longitudinal studies that have been able to examine whether neuroanatomic abnormalities are prospectively associated with later occurrence of the disorder (for example, Rao *et al*.[45]).

Thus, the purpose of the current study was to examine whether 5-HTTLPR genotypes predict variations in brain volumes in early adolescence, and whether these variations in turn prospectively predicted an onset of MDD in a 6-year follow-up period. We directly tested the hypotheses that (i) S-allele carriers would demonstrate reduced volumes of the hippocampus, ACC, amygdala and OFC, (ii) that smaller volumes of each of these structures would be prospectively associated with MDD onset, and, critically, (iii) that variation in brain structure would statistically mediate the association between 5-HTTLPR genotype and MDD onset.

MATERIALS AND METHODS

Participants and procedures

The current analyses are based on a subsample of 174 participants (71% of the total sample, 83 male) from the longitudinal Orygen Adolescent Development Study (ADS), conducted in Melbourne, Australia, who had provided a genetic sample during the course of their participation. The recruitment and screening of ADS participants has been reported previously.[46] These analyses draw on all four waves of ADS data collection: wave 1 (W1; *M* age 12.7 years, range 11.4–13.7 years) included a structural magnetic resonance scan and a diagnostic interview that assessed for current and lifetime mood disorders to exclude participants with a history of an episode of major depression. The diagnostic interview was repeated at waves 2, 3 and 4 (W2–W4), which were conducted ~2.5, 4 and 6 years after W1, respectively. The W2–W4 diagnostic interviews assessed for current MDD and any new episodes since the date of the last assessment.

Measures

MDD onset. MDD was measured at each of the four study waves by the Kiddie-Schedule for Affective Disorders and Schizophrenia for School-Age Children, Present and Lifetime version (K-SADS-PL), [47] a semistructured diagnostic interview that assesses current and lifetime symptoms and diagnoses of Axis I disorders in youths aged 6–18 years. Diagnostic interview data from each of the time points were used to construct a variable indicating whether participants had experienced their first occurrence of an episode of MDD between the W1 and W4 time points. Owing to attrition, this variable was able to be calculated for 138 of the 174 participants in the current study, and there were no differences between these participants and the 37 participants with missing data according to gender, $\chi^2(1) = 0.25$, $P > 0.05$, socio-economic status, $t[172] = -0.99$, $P > 0.05$, and W1 depression symptoms (as measured by the Centre for Epidemiological Symptoms – Depression scale), $t[160] = 0.77$, $P > 0.05$. A total of 36 participants had experienced their first onset of MDD between W1 and W4. Of these participants, 30 met criteria for one (or more) other lifetime psychiatric disorders compared to 34 of the 101 participants who did not experience an onset of MDD during adolescence (Supplementary Table 1). Intelligence (IQ) was assessed by a short form of the Wechsler Intelligence scale for Children, Fourth Version (Wechsler 2003).

Neuroimaging

One-hundred and twenty-five participants of the current sample completed a structural magnetic resonance imaging (MRI) scan at W1, using a 3-Tesla GE scanner. Details regarding image acquisition, image pre-processing and tracing protocols for morphometric analysis can be found in Supplementary Information. Briefly, the guidelines for tracing the amygdala and hippocampus were adapted from those described by Velakoulis *et al*.[48,49] Watson *et al*.'s protocol[50] was used to separate the amygdala from the hippocampus (see Supplementary Figure 1). The boundaries of the OFC were based on a previously published method by Riffkin *et al*.[51] In accordance with Bartholomeusz *et al*.,[52] medial and lateral OFC regions were separated with the medial orbital sulcus[53] (see Supplementary Figure 2). The boundaries of the ACC were based on a previously published method,[54] which defines separate limbic and paralimbic regions according to individual differences in the morphology of the cingulate, paracingulate and superior rostral sulci (see Supplementary Figure 3).

Interrater and intrarater reliabilities were assessed by means of the intraclass correlation coefficient (absolute agreement) using 10 brain images from a separate MRI database established for this purpose. Intraclass correlation coefficient values were deemed acceptable for all ROIs (29 of the 36 ROIs were < 0.90 and none < 0.75), as shown in Supplementary Table 1. All brain structural measures were corrected for whole-brain size separately by gender by means of a covariance adjustment method[55] and converted from mm^3 to cm^3.

Genotyping

Saliva was collected from participants for genetic analysis using an ORAGENE saliva pot (www.dnagenotek.com). The methods used for PCR amplification and visualization by gel electrophoresis were as described by Edenberg and Reynolds.[56] The genotype distribution for 5-HTTLPR (LL: $n = 54$, SL: $n = 83$, SS: $n = 37$) was in Hardy–Weinberg equilibrium ($\chi^2(1, N = 174) = 0.24$, NS).

Statistical analysis

We used path analysis to test a multiple mediator model, with serotonin transporter genotype as an ordinal independent variable (IV), the left and right structures of a specific brain region of interest (corrected for whole brain volume) as continuous mediators, and MDD onset as the binary dependent variable (DV). Alterations in the normal asymmetry of brain regions, particularly limbic structures such as the hippocampus, have been implicated in depression, generally evidenced by greater reductions in the left, compared to the right, structure (for example, Mervaala *et al*.[57] and Bremner *et al*.[58]). Research, however, has tended to examine left and right structures separately, making it difficult to know whether asymmetrical changes have occurred, or whether there are bilateral changes that

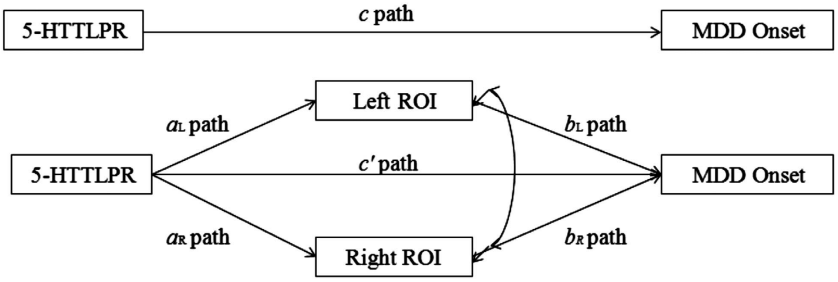

Figure 1. Hypothesized model outlining the tests for meditational effects. Path *a* (L or R) is the effect of 5-HTTLPR on the volume of a particular (left or right) brain region of interest (ROI), path *b* (L or R) is the effect of the volume of a particular (left or right) ROI on major depressive disorder (MDD) onset, path *c* is the total effect of 5-HTTLPR on MDD onset (that is, not controlling for left and right region of interest (ROI) volume), and path *c* is the direct effect of 5-HTTLPR on MDD onset (that is, controlling for left and right ROI volume).

Table 1. Means and standard deviations (s.d.) of regional brain volumes (before correction for whole brain volume) in cm^3

	Full sample (N = *125*)		*MDD onset status*				*Serotonin transporter genotype*					
	M	*s.d.*	*MDD onset* (n = *26*)		*No MDD onset* (n = *73*)		*SS* (n = *29*)		*SL* (n = *59*)		*LL* (n = *37*)	
			M	*s.d.*	M	*s.d.*	M	*s.d.*	M	*s.d.*	M	*s.d.*
Left hippocampus	2.77	0.33	2.70	0.35	2.77	0.33	2.65	0.25	2.80	0.35	2.81	0.35
Right hippocampus	2.95	0.34	2.94	0.35	2.91	0.33	2.88	0.28	2.96	0.36	2.99	0.35
Left amygdala	1.89	0.26	1.86	0.25	1.89	0.25	1.89	0.23	1.89	0.28	1.88	0.26
Right amygdala	1.83	0.28	1.75	0.24	1.85	0.29	1.85	0.27	1.85	0.26	1.80	0.31
Left medial OFC	7.55	1.80	7.13	1.47	7.62	2.00	6.96	1.78	7.58	1.60	7.94	2.05
Right medial OFC	7.19	1.71	6.80	1.27	7.27	1.87	6.62	1.92	7.16	1.54	7.66	1.70
Left lateral OFC	12.41	3.04	11.81	3.31	12.63	3.06	12.00	3.83	12.70	2.46	12.26	3.25
Right lateral OFC	13.33	2.75	13.00	2.63	13.50	2.92	13.11	3.07	13.57	2.33	13.11	3.16
Left limbic ACC	4.98	1.68	5.44	1.38	4.77	1.68	4.55	1.55	5.02	1.67	5.27	1.79
Right limbic ACC	5.77	1.91	5.51	1.99	5.99	1.87	5.98	1.79	5.60	2.01	5.88	1.88
Left paralimbic ACC	5.33	1.99	4.73	1.72	5.47	2.14	5.57	2.23	5.22	2.01	5.33	1.77
Right paralimbic ACC	4.79	1.80	4.79	1.55	4.67	1.89	4.67	2.02	4.91	1.82	4.67	1.63

Abbreviations: ACC, anterior cingulate cortex; MDD, major depressive disorder; OFC, orbitofrontal cortex.

happened to be significant for only one side. While an investigation of the presence of asymmetry was not a focus of the current study, we included left and right structures of a specific region of interest in the same path model to better understand the relative contribution of each structure to the risk for depression. Acceptable tolerance (>0.2) and variation inflation factor (<5) values indicated no significant multicolinearity between the left and right structures for any of the regions of interest. Separate mediation analyses were conducted for the hippocampus, the amygdala, the medial OFC, the lateral OFC, and the limbic and paralimbic ACC. Path models were estimated in *Mplus*[59] using weighted least squares with a mean- and variance-adjusted chi-square test statistic (WLSMV). Fit statistics are not reported as the models of interest were just identified.

A hypothesized model outlining the tests for mediational effects is presented in Figure 1. When both the relationship between the IV and the mediator (the *a* path) and the relationship between the mediator and the DV controlling for the IV (the *b* path) were significant, mediation was tested by assessing the significance of the cross product of the coefficients for these two paths (that is, the *ab* cross product). The product of coefficients method has been shown to yield more accurate results compared to other methods when the DV is binary,[60] and also allowed us to test for significant mediation in the absence of a direct effect of the IV on the DV.

The current analyses were based on 5000 bootstrapped samples and bias-corrected bootstrapped parameter estimates were used to test the significance level of the indirect effects, according to current recommendations for determining mediation.[61–64] If the 95% and 90% confidence intervals for these estimates of an indirect effect do not contain 0, it can be concluded that the indirect effect is statistically significant at the 0.05 and 0.10 level, respectively.[65] As both the left and riight structures of a specific brain region were included in the model, two specific indirect effects (a_Lb_L and a_Rb_R) were investigated. Given that the left and right volumes of a particular brain region would be expected to be related, their residuals were covaried in the model. Additional mediational analyses that included the covariates of adolescent gender, ethnicity, full-scale IQ and age at time of the MRI scan were conducted, but did not alter the pattern of results and hence are not reported.

Listwise deletion because of missing data would have resulted in only 98 cases remaining in the analysis due to non-participation in either the MRI at wave 1 or the psychiatric interview at waves 2, 3 or 4. Little's MCAR test[66] was non-significant, $\chi^2(163) = 179.54$, $P = 0.178$. We therefore used pairwise deletion (the default when using the WLSMV estimator in *Mplus*) to account for missing data. Pairwise deletion has been shown to be unbiased when data are missing completely at random.[67]

RESULTS

Table 1 presents mean brain volumes for each brain region considered in the current analyses before correction for whole brain volume.

For all analyses, the total effect of 5-HTTLPR on MDD onset (path *c*, that is, not controlling for ROI volumes) was non-significant (95% CI: −0.49 to 0.14, $\beta = -0.18$, s.e. = 0.16, $P > 0.05$). Each of the direct associations between 5-HTTLPR and MDD onset (path *c*′, that is, controlling for the relevant ROI volumes), 5-HTTLPR and the ROI volumes (path *a*), as well as between the ROI volumes and MDD onset (path *b*), can be seen in Table 2. In all path models, the direct effect of 5-HTTLPR on MDD onset (path *c*′) was non-significant.

Table 2. Path model of the effects of 5-HTTLPR genotype and brain ROIs on MDD onset

	b	*s.e.*	β	P
Hippocampus				
5-HTTLPR → MDD onset (path *c'*)	−0.21	0.17	−0.15	0.22
5-HTTLPR → left hippocampus (path *a*)	−0.08	0.03	−0.18	0.03
Left hippocampus → MDD onset (path *b*)	−1.79	0.79	−0.53	0.02
5-HTTLPR → right hippocampus (path *a*)	−0.05	0.04	−0.12	0.16
Right hippocampus → MDD onset (path *b*)	2.10	0.72	0.63	0.004
Amygdala				
5-HTTLPR → MDD onset (path *c'*)	−0.17	0.16	−0.12	0.30
5-HTTLPR → left amygdala (path *a*)	0.01	0.02	0.04	0.66
Left amygdala → MDD onset (path *b*)	1.44	1.08	0.31	0.18
5-HTTLPR → right amygdala (path *a*)	0.02	0.03	0.07	0.42
Right amygdala → MDD onset (path *b*)	−1.23	0.88	−0.29	0.16
Medial OFC				
5-HTTLPR → MDD onset (path *c'*)	−0.21	0.17	−0.15	0.23
5-HTTLPR → left medial OFC (path *a*)	−0.46	0.21	−0.21	0.03
Left medial OFC → MDD onset (path *b*)	−0.02	0.15	−0.04	0.88
5-HTTLPR → right medial OFC (path *a*)	−0.51	0.19	−0.25	0.006
Right medial OFC → MDD onset (path *b*)	−0.04	0.16	−0.05	0.83
Lateral OFC				
5-HTTLPR → MDD onset (path *c'*)	−0.19	0.16	−0.13	0.25
5-HTTLPR → left lateral OFC (path *a*)	−0.10	0.37	−0.03	0.79
Left lateral OFC → MDD onset (path *b*)	−0.06	0.11	−0.15	0.59
5-HTTLPR → right lateral OFC (path *a*)	−0.01	0.32	−0.003	0.98
Right lateral OFC → MDD onset (path *b*)	0.06	0.12	0.14	0.61
Limbic ACC				
5-HTTLPR → MDD onset (path *c'*)	−0.12	0.17	−0.08	0.49
5-HTTLPR → left limbic ACC (path *a*)	−0.39	0.20	−0.17	0.06
Left limbic ACC → MDD onset (path *b*)	0.16	0.08	0.27	0.04
5-HTTLPR → right limbic ACC (path *a*)	−0.33	0.22	0.01	0.87
Right limbic ACC → MDD onset (path *b*)	−0.02	0.08	−0.04	0.79
Paralimbic ACC				
5-HTTLPR → MDD onset (path *c'*)	−0.17	0.16	−0.12	0.29
5-HTTLPR → left paralimbic ACC (path *a*)	0.13	0.23	0.05	0.58
Left paralimbic ACC → MDD onset (path *b*)	−0.09	0.08	−0.16	0.25
5-HTTLPR → right paralimbic ACC (path *a*)	0.05	0.22	0.02	0.82
Right paralimbic ACC → MDD onset (path *b*)	0.09	0.08	0.15	0.28

Abbreviations: ACC, anterior cingulate cortex; MDD, major depressive disorder; ROI, region of interest.

Increasing copies of the S-allele predicted smaller left hippocampal volume (path a_L). Smaller left hippocampal volumes also predicted increased risk for MDD onset (path b_L). Bias-corrected 95% confidence intervals showed that smaller left hippocampal volume significantly mediated the relationship between S-allele copies and risk for MDD onset (indirect effect = 0.14, 95% CI = 0.009–0.42, s.e. = 0.10).

The association between S-allele copies and right hippocampal volume (path a_R) was not significant; however, larger right hippocampal volumes were predictive of increased risk for depression (path b_R).

Increasing copies of the S-allele of 5-HTTLPR predicted both smaller left and right medial OFC volumes (paths a_L and a_R); however, the associations between left medial OFC volume and MDD onset (path b_L) and between right medial OFC volume and MDD onset (path b_L) were non-significant; therefore mediation analyses were not conducted.

There was a trend ($P < 0.10$) towards increasing copies of the S-allele predicting smaller left limbic ACC volume, and a significant relationship ($P < 0.05$) between smaller left limbic ACC volume and decreased risk for MDD onset. Bias-corrected 90% confidence intervals indicated that left limbic ACC volume mediated the relationship between serotonin transporter genotype and risk for MDD onset (indirect effect = −0.06, 90% CI: −0.17 to −0.01, s.e. = 0.05), which is statistically significant at the 0.10 level. There were no significant findings relating to the right limbic ACC.

Given these results, further analyses were conducted on rostral, dorsal and ventral regions of the limbic ACC, which indicated that the finding obtained for the left limbic ACC was localized to the rostral region, such that a greater number of S-alleles was associated with smaller volumes of the left rostral limbic ACC, and that, in turn, smaller rostral limbic ACC volumes were associated with decreased risk for depression onset at trend level. The indirect pathway was also significant at trend level according to bias-corrected confidence intervals (indirect effect = −0.06, 90% CI: −0.17 to −0.003, s.e. = 0.05), suggesting possible mediation of the relationship between serotonin transporter genotype and risk for MDD onset by rostral limbic ACC volume. There were no significant findings relating to the right rostral limbic ACC. 5-HTTLPR did not predict left or right dorsal or ventral limbic ACC volumes, nor were these volumes related to risk for MDD onset. Mediation analyses for these regions were therefore not conducted.

5-HTTLPR did not predict left or right amygdala volume, left or right lateral OFC volumes, and left or right paralimbic ACC volume,

nor were these volumes related to risk for MDD onset. Mediation analyses were therefore not conducted for these ROIs.

Scatter plots of significant gene–ROI and ROI–MDD onset associations are provided in Supplementary Figures 4.

DISCUSSION

The aim of the current study was to investigate whether the volume of the hippocampus, ACC, amygdala and OFC mediated an association between variation in the serotonin transporter gene and a first onset of MDD in a large sample of adolescents using a longitudinal, prospective design. The findings are summarized in Figure 2. Our results support the role of left hippocampal volume deficits in early adolescence as salient mediators of the link between serotonin transporter genotype and increased risk for MDD onset in later adolescence. Specifically, we found that an increasing number of S-allele copies were associated with smaller left hippocampal volume, and smaller left hippocampal volume was in turn associated with increased risk of experiencing a first onset of MDD. Right hippocampal volume did not significantly mediate the pathway from 5-HTTLPR genotype to MDD onset, although larger right hippocampal volume did predict an increased risk of a depressive episode.

These results provide evidence that neurobiological factors may partly underlie the link between serotonin transporter genotype and depression. Furthermore, our finding that the S-allele predicted smaller left hippocampal volumes in early adolescence prior to illness onset is consistent with previous findings of a volume deficit in these structures in S-allele carriers.[32,33,36] Our finding that volume reductions in the hippocampus are associated with depression onset, but also predate its occurrence, also concords with suggestions that hippocampal volume deficits are one of the most consistently observed structural aberrations in depression,[19–23] and that this anomaly may represent a vulnerability factor that is present prior to emergence of mood disorder.[45,68]

The hippocampal region has been found to have moderate concentrations of the serotonin transporter.[69] An *in vivo* positron emission tomography study has revealed a strong leftward asymmetry in serotonin transporter distribution in the hippocampus,[70] suggesting greater expression of the serotonin transporter gene in the left hippocampal structure. Higher concentrations of serotonin transporters in the left compared to the right hemisphere may explain why serotonin transporter genotype was predictive of left hippocampal volume only in the current study. The hippocampus is known to be involved in the regulation of the stress response, specifically in the inhibition of the hypothalamic–pituitary–adrenal (HPA) axis.[71–73] Smaller hippocampal volumes associated with S-carrier status may affect negative feedback inhibition of the HPA axis, which could result in HPA hyperactivity. Alternatively, the S-allele may be associated with greater stress responsivity in the form of higher basal cortisol or a greater cortisol response,[74] which may have neurotoxic, atrophying effects on the hippocampus,[75] in turn increasing the risk for depression.

The finding that left and right volumes have opposite effects on the onset of MDD may initially seem inconsistent with previous studies that have found bilateral reductions in hippocampal volume that were predictive of depression. As far as we are aware, however, our study is unique in having considered the relative contribution of the left and right hippocampi to depression (that is, controlling for hippocampal volume in one hemisphere while assessing the effect of the volume in the other hemisphere). This renders it difficult to directly compare our findings with those of previous studies, which have focused on absolute volume in each hemisphere. It may still be worth noting that a number of these studies documented substantially greater left hippocampal volume reductions compared to the right in depression,[57,58] including child- or adolescent-onset depression,[76,77] raising the possibility that the presence of asymmetry in this region may have a role in the disorder. The implication of the finding of a difference in the directionality of the relationship between the left and right hippocampal volume with depression onset is unclear but is intriguing given suggestions that asymmetries in the limbic system, including the hippocampus, are associated with hemisphere asymmetries,[78] and there are suggestions that the right hemisphere may be more dominant in processing of negative emotions while the left hemisphere may be more dominant in processing of positive emotions.[79,80] It is not implausible that changes to asymmetry may have consequences for emotional processing that alters the risk for depression.

Possession of a greater number of S-allele copies also predicted both smaller left and right medial OFC volumes, although neither medial nor lateral OFC volumes (whether on the left or on the right) were prospectively associated with a MDD during adolescence. The finding that serotonin transporter genotype was associated with variation in medial but not lateral OFC volumes is consistent with the fact that the medial region of the OFC shows strong connections to limbic structures involved in emotion processing and reward, such as the amygdala, dorsolateral prefrontal cortex and ACC.[81,82] One factor that may be relevant to the lack of a prospective relationship between OFC volume and onset of depression is the time at which OFC volumes were measured. The OFC, which is thought to have an important role in inhibitory control and reward-based decision-making,[83] undergoes significant remodelling throughout adolescence and early

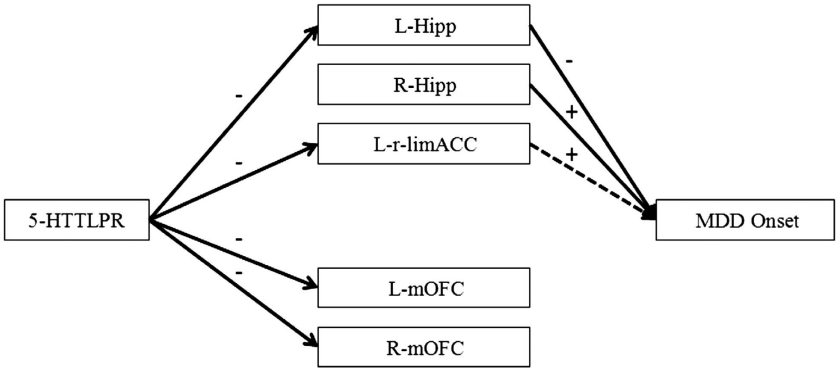

Figure 2. Summary of significant findings. A greater S-allele load was found to predict smaller left hippocampal volume, smaller left rostral limbic anterior cingulate cortex (ACC) volume, and smaller left and right medial orbitofrontal cortex (OFC) volumes. Smaller left but larger right hippocampal volumes predicted an increased probability of major depressive disorder (MDD) onset. There was a trend for smaller left rostral ACC volume to be associated with a decreased probability of MDD onset.

adulthood,[84] and it has been suggested that abnormalities in the maturation in this region may contribute to the etiology of depression.[85] Given that the OFC has not yet fully developed at 11–13 years old, it is possible that differences in OFC volume across adolescence may be more predictive of depression at a later age.

There was also evidence that an increasing number of S-allele copies predicted smaller left (but not right) rostral limbic ACC volume, a finding that accords with the results of previous investigations of this particular gene–brain linkage.[37,38] Somewhat surprisingly, there was a trend for smaller left (but not right) rostral limbic ACC volume to be associated with decreased risk of depression onset during adolescence (or, alternatively, that larger left rostral limbic ACC volumes were associated with increased risk for depression onset), and the mediating pathway from the 5-HTTLPR genotype to the left rostral limbic ACC volume to depression onset was also significant at the trend level. The presence of an association between larger rostral limbic ACC volume and depression onset in the current study is somewhat inconsistent with past research, which has generally suggested that volume deficits are associated with depression.[24] It is important to note, however, that evidence supporting the presence of smaller ACC volumes prior to illness onset comes exclusively from a few studies that have examined brain structure in high-risk samples, which are defined by the presence of a family history of depressive disorder (for example, Boes *et al*.).[86]

The lack of evidence supporting amygdala volume as an intermediate phenotype between serotonin transporter gene and depression onset is perhaps somewhat unsurprising, given the heterogeneous findings regarding the association between 5-HTTLPR and amygdala structure,[37–40] and between amygdala structure and depression.[23,30] These null findings may reflect a need to take additional mediating or moderating factors, such as psychosocial risks (for example, stressful life events, trauma, family environment and peer relationships), into account. Our research group has previously found that amygdala volume and parenting interact to predict depressive symptoms.[87] The structure of the amygdala is thought to be highly plastic to environmental changes and behavioral manipulations,[88–90] and there is also indication that alterations in amygdala volume may occur during the course of depression,[19,30,91] raising the possibility that structural differences in this region could represent the epiphenomena of, or consequential change associated with, the disorder rather than a premorbid vulnerability factor.

A number of study limitations must be acknowledged. First, examining brain structure in an adolescent sample at only one time point renders it impossible to determine whether these findings reflect stable differences present prior to illness onset or abnormal developmental changes that emerge during early adolescence. Second, the current investigation also did not take into account the contribution of environmental factors, such as stressful life events, trauma, parenting and peer relationships to these associations. Hippocampal volume has been found to be affected by environments that are regarded as often having an etiological role in the development of depression, including early life adversity, such as abuse or neglect,[92,93] as well as more normative caregiving experiences.[94] Both increased depression risk [95] and hippocampus diminishments [96,97] have been documented in S-carriers who have experienced severe childhood adversity. Future studies may wish to consider how potential mediating paths such as those documented here might be moderated by these relevant developmental risk or protective factors. A third point for consideration is the higher rates of other lifetime psychiatric conditions in the group of participants who experienced an onset of MDD compared with participants who did not. Although comorbidity with depression is extremely common (for example, Merikangas *et al*.,[2] and Rohde *et al*.[98]) it limits our ability to attribute the observed relationships to depression specifically as opposed to the presence of psychopathology more generally. Finally, it should be noted that, although these results would not survive Bonferroni adjustment, the magnitude of the difference in left hippocampus volume between individuals who experienced an onset of depression and those who did not is comparable to that found by a meta-analysis examining hippocampal atrophy in first episode depression patients.[22] Given the large effect sizes required to survive the loss of power associated with such a conservative test as the Bonferroni adjusted significance test[99,100] and that the effects of individual genes on the risk for psychiatric disorder tend to be small,[101] we would contend that uncorrected results retain valuable information that would otherwise potentially be lost to Type 2 error.

In summary, despite much supposition about the extent to which brain structures involved in the stress response and emotion regulation might serve as intermediate phenotypes in the pathway from the serotonin transporter gene to depression, for example, Savitz and Drevets,[17] and Schaninger *et al*.[18]), these indirect relationships had not been formally assessed prior to the present study. Our results provide evidence that during early adolescence structural abnormalities in the left hippocampus and, potentially, the left rostral limbic ACC may exist prior to onset of depression and may be partly responsible for the link between 5-HTTLPR genotype and depressive illness.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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Supplementary Information accompanies the paper on the Translational Psychiatry website (http://www.nature.com/tp)

CHAPTER 8: EMPLOYING AN IMAGING GENE-ENVIRONMENT INTERACTION FRAMEWORK TO UNDERSTAND RISK FOR DEPRESSION

Previous chapters have reviewed *gene-environment interaction* literature and *imaging genetics* literature that implicates the serotonin transporter gene in the development of depression during adolescence. The current chapter will consider an approach proposed by Hyde and colleagues (2011) that incorporates the methods employed by both these groups of studies within a single imaging gene-environment (IGxE) interaction framework, with illustrative examples. The following chapter (CHAPTER 9) will provide an application of an IGxE framework that considers how hippocampal volume may play a role in linking the interaction of the serotonin transporter gene and family experiences to the emergence of depression during adolescence. In that chapter, a brief review of studies that suggest cross-level integrations between these specific variables will be provided.

8.1 The important but distinct roles of gene-environment studies and imaging genetic studies

Both gene-environment interaction studies and imaging genetics studies have made valuable contributions to the understanding of psychopathology more broadly and depression specifically (Hyde et al., 2011). Specifically, gene-environment interaction elucidates for whom (i.e. individuals of particular genotypes) an environmental experience might incur particular vulnerability for a behavioural outcome, such as depression, or conversely, what under what environmental circumstances might a specific genotype have capacity to influence behaviour (Moffitt et al., 2005), as illustrated in Figure 8-1a. Whilst

GxE studies highlight the contingent nature of the relationships between the genome, experiences and behaviour, this research in isolation cannot identify specific biological mechanisms underpinning these relationships (Hyde et al., 2011).

One important avenue in clarifying these mechanisms is to identify the pathways from genes to behaviour. Imaging genetics studies aim to link common genetic polymorphisms to variability in brain structure (Hariri & Weinberger, 2003; Scharinger et al., 2010), as shown in Figure 8-1b. Given the serotonin transporter gene is known to affect both neurodevelopment (Daubert & Condron, 2010; Gaspar et al., 2003; Oberlander, 2012) and neurotransmission (Lesch et al., 1996), effects of this gene on brain structure would certainly be anticipated (Bogdan et al., 2013). Indeed, imaging genetics studies have identified associations between 5-HTTLPR variation and ACC, OFC, hippocampal and amygdala structural differences (Scharinger et al., 2010; Won & Ham, 2016). As demonstrated in the previous chapter (Little et al., 2014), structural abnormalities in the left hippocampus in particular may be present prior to the onset of depression during adolescence and represent an intermediate stage in a neurobiological pathway from 5-HTTLPR genotype to depressive illness.

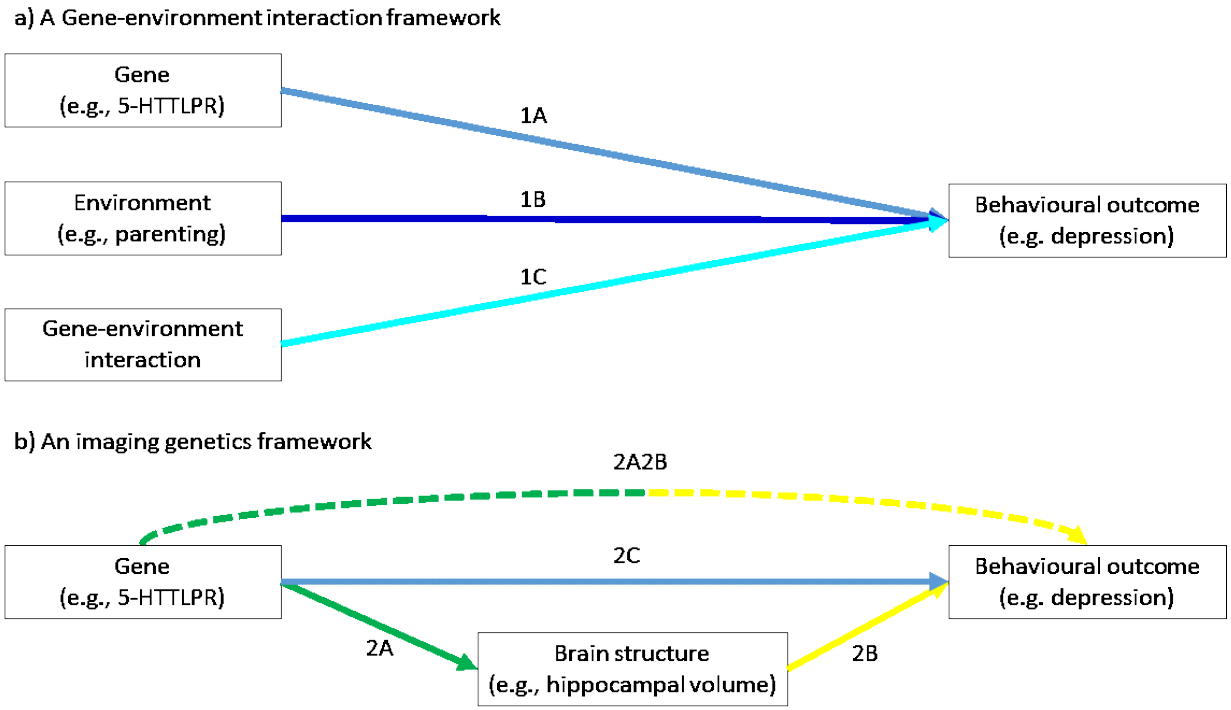

Figure 8-1. *A conceptual and statistical diagram of G x E and imaging genetics studies. (a) G x E framework.*
GxE studies assess whether an interaction term (Path 1 C) that is modelled as the product of genetic and environmental variables of interest significantly predicts the behavioural outcome. The genetic variable and the environmental factor may also have distinct direct ('main') effects on behavioural outcomes (paths 1A and 1B). (b) An imaging genetics framework. Genetic variation is associated with individual variability in brain structure (path 2A), individual variability in brain structure leads to differences in behavioural outcomes or psychopathology (path 2B). Genetic variation might or might not have a direct impact on distal complex behavioural phenotypes (path 2C). Genetic variation has an indirect or mediated effect on behaviour via its effect on brain structure (dotted arrow 2A2B: note that this path is not included as a path in the model but shown for conceptual clarity; this effect can be statistically modelled as the product of the 2A and 2B paths). Adapted from Hyde et al. (2011).

8.2 Moving to an imaging Gene-environment Framework

Whilst considerable advancement has been made by these two distinct lines of vulnerability research, there has not yet been significant progress in the capacity to make precise predictions about exactly who is likely to experience depression, the timing of first

onset of the disorder and the specific underlying mechanisms (Hankin, 2012). In response to these concerns, Hyde et al. (2011) have proposed that an integration of GxE and imaging genetics methods may lead to a more sophisticated understanding of the transactional mechanisms by which genes, environments and the brain might operate to predict behaviour and risk for conditions such as depression. This *imaging gene-environment interaction* (IGxE) approach aims to examine how potential effects of genetic and environmental variables on depression might be transmitted via brain structure.

IGxE interactions can be assessed statistically according to a number of different moderated mediation or mediated moderation models (also referred to as conditional indirect effects) (Hyde et al., 2011), whereby variation in one factor may alter the strength or direction of an indirect pathway (Preacher, Rucker, & Hayes, 2007). Each model evaluates slightly different relationships (Figure 8-2).

These IGxE frameworks can be evaluated using path analysis or structural equation modeling (SEM) and may involve moderation of any or all paths within a mediation framework (gene/environment → brain structure, brain structure → behaviour, or the entire indirect pathway from gene/environment → brain structure → behaviour). For instance, following from imaging genetics models, such as those tested in the previous chapter (Little et al., 2014) the environment could be conceptualised by IG x E studies as the moderator of a neurobiological pathway in which genes affect behavioural outcomes via their influence on brain structure (Figure 8-2A). This approach favours genetic factors as the 'direct' predictors of neuroanatomy. Evidence also suggests however that experience may directly alter the brain, and GxE studies predicting behavioural outcomes have often modelled genes as moderating the direct influence of environmental experiences. Another plausible

IGxE model is therefore one that identifies genetic background to be the moderator of a causal pathway that proceeds from environmental experiences through brain structure to behavioural outcomes Figure 8-2B.

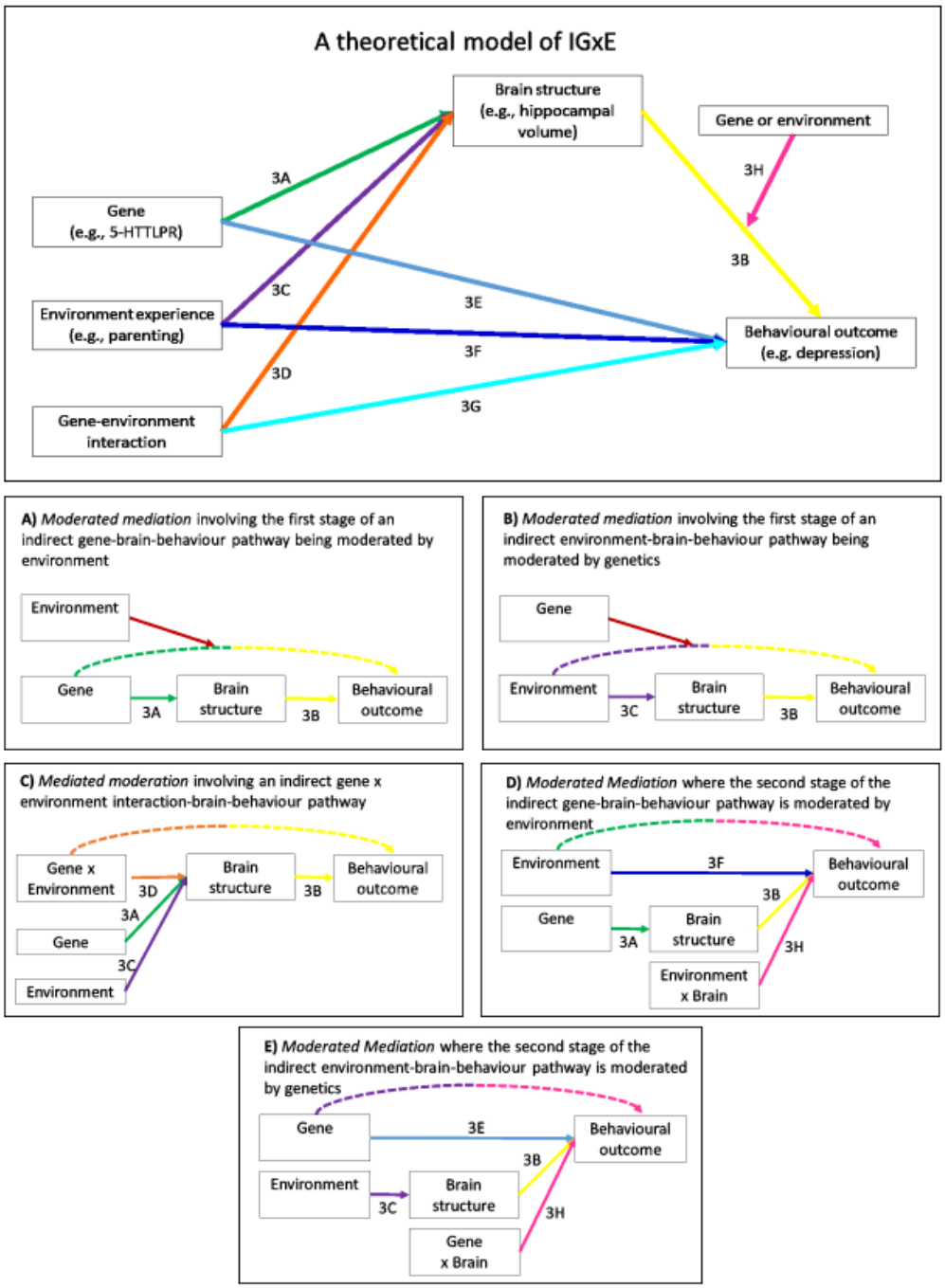

Figure 8-2. *A theoretical model of IG×E displaying how various IGxE frameworks can be conceived conceptually and statistically.*
Traditional GxE and imaging genetics paths as well as new paths of potential interest that can be considered within a IGxE framework are shown. Examples of plausible IGxE models are provided in (A-E). Paths 3E, 3F and 3G model traditional G×E relationships; paths 3A and 3B model traditional imaging genetics mediation links between gene and behaviour via brain structure; the 3C path show direct effects of the environment on brain structure; paths 3D and 3B together model gene–environment interactions predicting behaviour via brain structure; path 3H shows the potential for the brain-behaviour link (3B) to be moderated by either

genes or environment. (A) shows a first stage moderation mediation model from gene to behaviour via brain structure, where the indirect effect varies according to environment. The conditional indirect pathway is not included as a path in the model but can be statistically modelled as the product of the 3A and 3B paths (3A3B; shown as the green and yellow dotted arrow), which is calculated (rerun) at different levels of the environmental variable. (B) shows a first stage model from environment to behaviour via brain structure that varies according to genotype. The conditional indirect pathway is modelled as the product of the 3C and 3B paths (3C3B; purple and yellow dotted arrow) rerun at the values representing the different genotypes. (C) shows a mediated moderation model involving an interaction between genotype and environment that predicts brain structure, which in turn predicts behaviour, where the conditional indirect effect is assessed as the product of the 3D and 3B paths (3D3B; orange and yellow dotted arrow). (D) shows a second stage moderated mediation model where there is an indirect relationship between genotype and behaviour via brain structure, and the association between brain structure and behaviour is influenced by environment. This conditional indirect effect is assessed as the product of the 3A and 3H paths (3A3H; green and pink dotted arrow). (E) shows a second stage moderated mediation model where there is an indirect relationship between environment and behaviour via brain structure, and the association between brain structure is influenced by genotype. This conditional indirect effect is assessed as the product of the 3C and 3H paths (3C3H; purple and pink dotted arrow). Adapted from (Hyde et al., 2011).

Conceptually, both of these IGxE models are referred to first stage *moderated mediation* models because they consider whether an indirect effect varies across levels of the moderator – specifically whether the capacity of an indirect pathway (either from gene → brain → behaviour, or from environment → brain →behaviour) to account for variance in the behavioural outcome might differ across levels of another factor (genes or environment). In other words, the extent to which genotype's influence on depression may be transmitted via brain structure may be greater in adverse environments than non-adverse environments *or* the extent to which the environment's influence on depression may be transmitted via brain structure may be greater for individuals of one genotype compared to another genotype. For example, Glaser et al. (2014) identified an indirect pathway from corticotropin-releasing hormone receptor 1 (CRHR1) genotype to negative emotionality that was mediated by neural reactivity in the right ventrolateral prefrontal cortex (rVLPFC) that was moderated by childhood stress, consistent with a first stage moderated mediation pathway. Specifically, G-allele homozygous individuals exhibited greater rVLPFC

activation in response to negative emotional words, which in turn was predictive of lower levels of negative emotionality but only when lower levels of childhood stress, and not higher levels of childhood stress had been reported. This study thus identified an indirect gene→brain (reactivity)→behaviour pathway that was moderated by environment.

Further findings of a IGxE interaction comes from a study by Gard and colleagues (2017), which identified an indirect pathway from increased harsh parenting in early childhood to greater antisocial behaviour symptoms via lower amygdala reactivity to fearful facial expressions that was significant for individuals who were heterozygous or homozygous minor at two SNPs (rs7209436, rs110402) in the *CRHR1* gene but non-significant for those who were homozygous major at both SNPs. This study thus identified an indirect enironment→brain (reactivity)→behaviour pathway that was moderated by genotype.

A first stage moderated mediation IGxE is determined by assessing the significance of the particular indirect effect of interest (ie. the cross product of the gene→brain coefficient and the brain→behaviour coefficient (the *3A3B* cross product) or the environment→brain coefficient and the brain→behaviour coefficient (the *3C3B* cross product)) at different levels of the moderator. The index of moderated mediation (the cross product of the coefficients for the gene-environment interaction→brain path and the brain→behaviour path (i.e. the *3D3B* cross product) provides an indication of whether these estimated conditional indirect effects are significantly different from one another (Hayes, 2015).

Another IGxE model that has a slightly different focus is one that assesses whether genetics and environment together predicts behavioural outcomes via their combined

effects on neuroanatomy. Conceptually, this particular IGxE model can be referred to as a mediated moderation model because it is interpreted as a moderation effect that is at least partly mediated by or accounted for by a variable that occurs intermediate in the pathway (see discussions by Muller, Judd, & Yzerbyt, 2005). Here, as shown in Figure *8-2* (in detail in C), the direct effects of both genetic and environmental factors on brain structure are modelled (path *3A* and *3C* respectively) but their interaction (path *3D*) also accounts for non-additive, unique variance in brain structure which in turn accounts for variance in the behavioural outcome (path *3B*). A mediated moderation IGxE model therefore emphasises a significant gene-environment interaction predicting brain structure (path 3D) and a significant cross product of the coefficients of the gene-environment interaction→brain and brain→behaviour (ie., the *3D3B* cross product). Further analyses to enhance understanding of this mediated moderation IGxE typically involves post-hoc simple slope analyses of the gene-environment interaction predicting brain structure. There may also be a significant gene-environment interaction predicting the behavioural outcome (that would also be explored via post-hoc simple slope analyses) but this is not required for significant mediated moderation.

One study that has explored this particular IGxE model was conducted by van der Meer et al. (2015). This study found evidence of a gene-environment interaction between 5-HTTLPR genotype and stress predicting ADHD symptoms, such that stress was correlated with ADHD symptom count in S-allele carriers but not in L-allele homozygotes. This gene-environment interaction was mediated by a gene-environment interaction predicting frontal pole and anterior cingulate gyrus volume; stress exposure was associated with greater grey matter volume reductions in these regions in S-allele carriers compared to their L-

homozygous counterparts, and volume reductions were in turn associated with a higher ADHD symptom count.

First stage moderated mediation and mediated moderation are thus based upon the same SEM model but different emphases or interpretations are placed on the available statistics and slightly different post-hoc analyses may be conducted to understand the findings – this is discussed further in the 'data analysis' section of CHAPTER 9.

It is also possible for either genetics or environment to qualify an association between brain structure and behaviour (the *3B* path in an otherwise simple mediation model, as shown in Figure *8-2*, with the statistical models articulated in more detail in (D) and (E), showing the moderated path represented by *3H*) – this IGxE represents a second stage moderated mediation model. To my knowledge, a full second stage moderated mediation IGxE model has not been tested. One study has identified that a correlation between amygdala reactivity and trait anxiety may be moderated by variation in a gene thought to have a regulatory role in endocannabinoid signalling (the single nucleotide polymorphism (C385A) in the Fatty acid amide hydrolase (FAAH) gene) (Hariri et al., 2009). The same study identified this genetic variation to influence an association between ventral striatal reactivity and an index of impulsivity. This model could be extended to a full IGxE model by including a consideration of whether a particular environmental factor predicted amygdala or ventral striatal reactivity.

The models articulated here describe only some of the complex pathways that might exist between allelic variants, environmental factors and neuroanatomical vulnerabilities which could confer risk for psychiatric difficulties. Even greater complexity likely exists in the form of models involving multiple genes, environments and brain structures (Hyde,

2015; Hyde et al., 2011). Nonetheless, the current models provide an important starting point for characterising the intricate relationships that might be present between these factors.

The previous empirical chapters provided evidence of an interaction between the serotonin transporter gene and positive parenting (though not negative parenting) predicting depression (CHAPTER 4 and Chapter 5) as well as an indirect pathway from the serotonin transporter gene to depression via hippocampal volume (CHAPTER 7). These findings suggest that considering these variables in an IGxE approach may help us better understand how their complex interrelationships might contribute towards the emergence of depression. The final empirical chapter in this thesis (CHAPTER 9) will therefore investigate how the serotonin transporter gene and parenting might influence risk for depression through hippocampal volume. This study will specifically consider first-stage moderated mediation and mediated moderation models. Second-stage moderated mediation models will not be addressed in the current thesis.

CHAPTER 9: LINKING THE SEROTONIN TRANSPORTER GENE, FAMILY ENVIRONMENTS, BRAIN STRUCTURE AND DEPRESSION: A PROSPECTIVE, IMAGING GENE X ENVIRONMENT ANALYSIS

Chapter 9 comprises the prepublication copy of the manuscript

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Linking the Serotonin Transporter Gene, Family Environments, Hippocampal Volume and Depression Onset: A Prospective Imaging Gene x Environment Analysis

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Abstract

A single imaging gene-environment (IGxE) framework that is able to simultaneously model genetic, neurobiological and environmental influences on psychopathology outcomes is needed to improve understanding of how complex interrelationships between allelic variation, differences in neuroanatomy or neuroactivity and environmental experience affect risk for psychiatric disorder. In a longitudinal study of adolescent development we demonstrate the utility of such an IGxE framework by testing whether variation in parental behavior at age 12 altered the strength of an imaging genetics pathway, involving an indirect association between allelic variation in the serotonin transporter gene to variation in hippocampal volume and consequent onset of major depressive disorder by age 18. Results were consistent with the presence of an indirect effect of the serotonin transporter S-allele on depression onset via smaller left and right hippocampal volumes that was significant only in family environments involving either higher levels of parental aggression or lower levels of positive parenting. The previously reported finding of S-allele carriers' increased risk of depression in adverse environments may therefore be partly due to the effects of these environments on a neurobiological pathway from the serotonin transporter gene to depression onset that proceeds through variation in hippocampal volume.

Key Words: Imaging Gene x Environment Framework, Serotonin Transporter Gene, Major Depressive Disorder, Family Environment, Hippocampus

General Scientific Summary

The current study marks an important step in linking gene-environment interaction studies and imaging genetic studies into an overall framework that is able to address more nuanced questions regarding the complex contributions of genetic, neurobiological and environmental factors towards risk for psychopathology. In particular it demonstrates how parenting might "get under the skin" by influencing a neurobiological pathway involving the serotonin transporter gene, hippocampal volume and depression.

The prevalence of depression increases dramatically during adolescence. Depressive disorders are relatively rare in childhood, with a point prevalence estimate of 2.8% in children younger than 13 years (Costello, Erkanli, & Angold, 2006). However, the point prevalence doubles to 5.7% in adolescence, and by the age of 19 years, between a fifth and a quarter of individuals will have experienced a depressive disorder (Harrington & Dubicka, 2002; Lewinsohn, Hops, Roberts, Seeley, & Andrews, 1993; Lewinsohn, Rohde, & Seeley, 1998). Importantly, an earlier first onset of depression during childhood or adolescence is associated with greater risk of recurrence, more severe psychosocial impairment and suicide when compared to a later onset in adulthood (Zisook et al., 2007). These statistics underscore the importance of understanding vulnerability factors for depression that may be operating during adolescence, the peak developmental period for onset of the disorder.

Recent efforts to understand the mechanisms that underlie susceptibility to depression have focused on how interrelationships between genes, variation in neuroanatomy or neuroactivity and environmental experience affect risk for disorder. In particular, gene-environment interaction (GxE) studies have drawn attention to the conditional nature of relationships between genetics and environmental experiences in influencing vulnerability to depression (Caspi, Hariri, Holmes, Uher, & Moffitt, 2010; Hyde, 2015; Hyde, Bogdan, & Hariri, 2011). On the other hand imaging genetics studies have been important in identifying effects of specific genes on brain structure and function, suggesting plausible pathways by which genes may shape emotional and behavioral outcomes (Hyde et al., L. W. Hyde et al., 2011; 2010). As, discussed in detail below, these studies provide initial clues concerning how genetic and environmental factors might alter risk for psychopathology through their effects on neurobiological pathways.

Given the potential for both imaging genetics and GxE approaches to provide important information regarding mechanisms underlying the etiology of depressive disorders,

there have been calls for an integration of these two streams of enquiry into a single imaging gene-environment (IGxE) framework that is able to simultaneously consider genetic, neurobiological and environmental influences on depression (e.g., Bogdan, Hyde, & Hariri, 2012; Hyde, 2015; Hyde et al., 2011). This paper aims to address these calls by investigating an IGxE model that examines whether the imaging genetic pathway (i.e., the indirect effect of allelic variation on depression through variation in brain structure) depends on the degree of exposure to a particular environmental factor. Modelling such an IGxE will provide information about how genetically-based variation in brain structure might alter risk for depression across different contexts or experiences. We chose to focus our analyses on the influence of the serotonin transporter gene, the hippocampus and parenting on depression because, as we review in the following sections, a large body of research already suggests interplay between these factors in predicting this disorder, although the exact nature of these relationships is yet to be examined concurrently in a single study.

GxE interactions involving the Serotonin Transporter Gene

Allelic variation involving a 43 base pair insertion/deletion in the promoter region of the serotonin transporter gene (5-HTTLPR) is currently the most investigated genetic polymorphism in psychiatric genetics, and has been linked to depressive disorders in particular. A significant body of literature suggests that carriers of either one or two copies of the S-allele (which is associated with reduced serotonin uptake activity) appear to be at enhanced risk for depression when compared to individuals with two copies of an L-allele, but only in contexts involving adversity or stress - indicative of a gene-by-environment (GxE) interaction (Karg, Burmeister, Shedden, & Sen, 2011). Whilst positive replications of this pattern of results arguably predominate the literature, a number of inconsistent findings, involving null and opposite findings implicating the L-allele as a risk allele for depression in such environments have also been obtained. Furthermore, there are both meta-analyses that

support and negate the veracity of this effect (Risch et al., 2009; Sharpley, Palanisamy, Glyde, Dillingham, & Agnew, 2014), the findings of which appear to depend on their inclusion criteria, making this a highly controversial area of research. Intriguingly, there is some suggestion that the positive finding of the S-allele as a risk allele for depression is most reliably detected when considered in the context of adverse childhood experiences (e.g. child maltreatment rather than stressful life events encountered during adulthood) (Karg et al., 2011). Investigation of the action of the serotonin transporter gene during late childhood and adolescence, when this disorder tends to emerge, may therefore be particularly beneficial in resolving this debate.

Imaging Studies Implicating the Hippocampus in Depression

Structural abnormalities in the hippocampus have been strongly implicated in the neurobiology of depression (Scharinger, et al., 2010). The balance of the evidence, which has come almost exclusively from cross-sectional studies, has generally supported the presence of reduced volumes in adult patients with Major Depressive Disorder (MDD) compared to healthy controls (Campbell, Marriott, Nahmias, & MacQueen, 2004; McKinnon, Yucel, Nazarov & MacQueen, 2009), though there is some variability amongst individual studies with regard to whether reductions are evident bilaterally (Sheline, Sanghavi, Mintun, & Gado, 1999), or are limited to the left (e.g, Bremner et al., 2000; Mervaala et al., 2000) or right hemisphere (e.g., Janssen et al., 2004; Lange & Irle, 2004). Smaller hippocampal volumes have also been implicated in pediatric and adolescent depression (e.g., Caetano et al., 2007; MacMaster et al., 2008; Suzuki et al., 2013). The evidence base is smaller and somewhat less consistent than the adult literature however, with some studies documenting no volumetric differences (MacMillan et al., 2003; Rosso et al., 2005) as well as an association between larger volumes and greater illness duration (MacMaster & Kusumakar, 2004). Research however has tended to examine left and right hippocampal volumes

separately, making it difficult to know whether asymmetrical changes have occurred, or whether there are bilateral changes that happened to be significant for only one side. A further possibility is that volumetric differences in only one hemisphere, or more pronounced reductions in one hemisphere, reflect hemispheric asymmetries associated with depression. To our knowledge only one study has tested this possibility, identifying a left-right hippocampal grey matter asymmetry in first episode MDD patients, involving smaller left hippocampal grey matter volumes compared to right, that was not present in healthy controls (Frodl et al., 2002).

Findings implicating diminished hippocampal volumes have most commonly been discussed as reflecting stress-related neurotoxity, with changes potentially occurring during illness (Sapolsky, 2000), consistent with evidence suggesting a correlation between smaller volumes and longer depression duration (McKinnon et al., 2009). Diminishment in hippocampal volume may reflect neuronal death and cell loss as well as inhibition of neurogensis as a result of excessive exposure to glucocorticoids such as cortisol (McEwen, 1999; Sapolsky, 2000; Suri & Vaidya, 2013). High concentrations of glucocorticoid and mineralocorticoid steroid receptors make the hippocampus particularly vulnerable to the toxic effects of glucocorticoids (López, Chalmers, Little, & Watson, 1998). Damage to the hippocampus instigated by exposure to glucocorticoids may have the effect of reducing the inhibitory action of the hippocampus on the HPA axis, which results in further excessive glucocorticoid secretion, and a cascade of hippocampal damage; the "glucocorticoid cascade hypothesis" (Sapolsky, Krey, & McEwen, 1986).

Importantly however, there are studies that have observed reduced hippocampal volumes in healthy individuals at high risk for depression by virtue of their family history (e.g., Aminco, et al., 2011; Baaré et al., 2010; Chen, Hamilton, & Gotlib, 2010; Rao et al., 2010). These studies raise the possibility that an inherited (i.e. genetically based)

diminishment in hippocampal volume might be associated with an initial predisposition toward psychopathology that may in turn be triggered by stress. It may also be the case that individuals with smaller hippocampal volumes are more vulnerable to stress-induced atrophy or reductions in neurogenesis that further compound risk for ongoing depression. Longitudinal, prospective research is likely to be critical for a greater understanding of these issues.

Imaging Genetics Studies involving the Serotonin Transporter Gene and the Hippocampus

There is evidence, albeit inconsistent, that allelic variation in the promoter of the serotonin transporter gene is associated with variation in hippocampal volume (Scharinger et al., 2010). There are reports of smaller (Frodl, Koutsouleris, et al., 2008 Eker et al., 2011; Taylor et al., 2005), larger (Frodl et al., 2004; Frodl, Zill, et al., 2008) and equivalent (Cole et al., 2011; Dutt et al., 2009; Frodl et al., 2010; Pezawas et al., 2005) volumes in S-allele carriers compared to their L-allele homozygous counterparts in both psychiatrically healthy and MDD groups. Although their findings are somewhat conflicting, these imaging genetics studies suggest the possibility that the allelic variation in the serotonin transporter gene could account for differences in hippocampal volume associated with risk for depression. Indeed, in a previous study with the current cohort, we found that left hippocampal volume mediated an association between the serotonin transporter gene and depression during adolescence, such that an increasing number of S-allele copies was associated with smaller left hippocampal volume at 11-13 years of age, and smaller left hippocampal volume was in turn prospectively associated with increased risk of experiencing a first onset of MDD during a 6 year follow up period (Little et al., 2014). Allelic variation in 5-HTTLPR did not predict differences in right hippocampal volume, however larger right volumes were predictive of increased risk of MDD onset. We have speculated that the finding of an association between serotonin

transporter genotype with left but not right hippocampal volume may be related to greater concentrations of the serotonin transporter in the left hippocampus compared to the right hippocampus (Kranz et al., 2014). We also hypothesised that our findings of opposite effects of left and right hippocampal volumes on the onset of MDD may suggest a role of exaggerated hippocampal asymmetry in the disorder.

Critically, the hippocampus is also known to be highly plastic, and hippocampal volume has been found to be affected by environments that are regarded as often having an etiological role in the development of depression, including early life adversity, such as abuse or neglect, as well as more normative caregiving experiences (Belsky & De Haan, 2011). Smaller hippocampal volumes (particularly on the left side) have been consistently documented in adults with a history of childhood maltreatment (Bremner et al., 1997; Stein, Koverola, Hanna, Torchia, & McClarty, 1997; Vythilingam et al., 2002). Conversely, greater maternal support or higher-quality parental care in early life (i.e., 3-5 years) has been found to predict larger hippocampal volumes at school age, particularly for non-depressed (versus depressed) children (Luby et al., 2012). Other research with children exposed to cocaine *in utero* indicated that higher-quality parental care in early childhood (i.e., age 4) was predictive of smaller hippocampal volumes during early-mid adolescence (Rao, Betancourt, et al., 2010). Moreover, smaller hippocampus volumes have been found in both depressed (Frodl et al., 2010) and healthy individuals (Everaerd et al., 2012) carrying either one or two S-alleles of the serotonin transporter gene who have also experienced severe childhood adversity. Increased cortisol reactivity has also been documented in S-allele homozygous individuals compared with individuals of SL and LL genotype (Miller, Wankerl, Stalder, Kirschbaum, & Alexander, 2012). It seems conceivable that the serotonin transporter gene could interact with early-life stress to influence cortisol levels and in turn alter hippocampal structure. Intriguingly, there is a glucocorticoid response element within the promoter of the serotonin

transporter gene, which suggests the possibility that stress-induced glucocorticoid production may alter the expression of the serotonin transporter gene (Glatz, Mössner, Heils, & Lesch, 2003). This could also explain differences in hippocampal volume in individuals with different serotonin transporter genotypes with different environmental experiences.

The current study

In summary, separate strands of research suggests complex interrelationships between variation serotonin transporter gene, caregiving experiences and the hippocampus in predicting depression. Models of the onset of depression that link these individual strands of research by exploring the nature of these interrelationships within the one study are lacking. We therefore aimed to investigate how variation in the serotonin transporter gene and parental behavior experienced in early adolescence might influence left and right hippocampal volume and risk for a first onset of MDD during a six-year follow up period. Building upon our previous work (Little et al., 2014), we specifically hypothesized that an indirect effect demonstrating a relationship between serotonin transporter genotype and depression onset via left and/or right hippocampal volume would be stronger in contexts involving higher frequencies of adverse parenting (e.g., aggressive parenting behaviors or lower frequencies of positive parenting behaviors), thereby demonstrating an IGxE effect with respect to these variables.

Method

Participants and Procedures

Participants were from the longitudinal Orygen Adolescent Development Study (ADS), conducted in Melbourne Australia. A large group of final-year Grade 6 primary school students (N=2453, 53.5% of the total sampling population; 48% male; mean age 11.62 years) across metropolitan Melbourne, Australia, were recruited through their schools to take part in an initial screening assessment. The aim of the screening was to identify a smaller

sample representing the full spectrum of risk/resilience for psychopathology as a function of temperament (namely, Effortful Control and Negative Affectivity, as measured by the Early Adolescent Temperament Questionnaire—Revised; EATQ-R; Ellis and Rothbart 2001) to participate in further intensive longitudinal assessments. The selected sample of 415 students was comprised of equal numbers of male and female students who had EATQ-R scores that were 0–1, 1–2, 2–2.5, and greater than 2.5 standard deviations above and below the mean on the higher order factors of Negative Affectivity and Effortful Control. These 415 adolescents thus represented an oversampling of those with high and low temperamental risk for psychopathology, and an undersampling of those with an intermediate level of risk, resulting in a distribution that retained the variance associated with the larger screening sample but was still normally distributed. Of the 415 adolescents selected, 245 agreed to partake in further intensive research. Participants in the current research were the 174 adolescents (47.7% male), who had provided a DNA sample during the course of their participation in the intensive phases of the study (71% of the sample of 245 ADS participants). Adolescents with four grandparents who were born in Australia, New Zealand, Europe or United Kingdom were classified as 'Anglo-European' (86.2%) and participants with at least one grandparent born elsewhere (e.g. Asia, Africa, the Middle East, South America) were classified as 'Non-Anglo-European' (12.1%). This ethnicity classification was based upon findings by previous studies that different serotonin transporter gene allele frequencies have been reported in samples of different ancestries (e.g. African-American, Asian) compared with Anglo-European ancestry subjects (Gelernter, Kranzler, Coccaro, Siever, & New, 1998; Goldman, Glei, Lin, & Weinstein, 2010; Lotrich, Pollock, & Ferrell, 2003).

As shown in Table 1, the current study draws on all four waves of intensive ADS data collection: wave 1 (W1; *M* age 12.7 years, range 11.4 -13.7 years) included a structural magnetic resonance scan, observations of parent-child interactions and a diagnostic interview

that assessed for current and lifetime mood disorders to exclude participants with a history of an episode of major depression. The diagnostic interview was repeated at waves two, three and four (W2-W4), which were conducted approximately two-and-a-half, four and six years after W1, respectively. The W2-W4 diagnostic interviews assessed for current MDD and any new episodes since the date of the last assessment. Table 1 details the number of participants who completed the various components of assessment during the course of the longitudinal Adolescent Development Study for the current sample of 174 individuals who had provided a DNA sample.

[INSERT TABLE 1 HERE]

Measures

MDD Onset. At each of the four study waves, participants were administered the Kiddie-Schedule for Affective Disorders and Schizophrenia for School-Age Children, Present and Lifetime version (K-SADS-PL; Kaufman et al., 1997), a semi-structured diagnostic interview that assesses current and lifetime symptoms and diagnoses of Axis I disorders in youths aged 6 to 18 years. Diagnostic interview data from each of the time points was used to determine whether participants had experienced a first occurrence of an episode of MDD between the W1 and W4 time points. Due to variations in participation at the different waves, data that allowed a determination of whether or not MDD onset had occurred was available for 137 of the 174 participants in the current study, and there were no differences between these participants and the 37 participants with this missing data according to gender, $\chi^2(1) = .25$, $p > .05$, socio-economic status, $t[172] = -.99$, $p > .05$, and W1 depression symptoms (as measured by the Centre for Epidemiological Symptoms–Depression scale), $t[160] = .77$, $p > .05$. A total of 36 participants (26.2% of 137 participants) had experienced their first onset of MDD between W1 and W4. This rate of MDD onset during adolescence is similar to that of another study measuring first incidence of depression during the adolescent period (20%;

Rohde, Lewinsohn, Klein, Seeley, & Gau, 2013). Of the 36 participants who experienced an onset of MDD, 30 met criteria for one (or more) other lifetime psychiatric disorders compared to 34 of the 101 participants who did not experience an onset of MDD during adolescence (Supplementary Table 1).

Assessment of parenting. The frequency of aggressive and positive parenting behaviors displayed by mothers was assessed during two 20-minute parent-child interaction tasks at W1, which were videotaped for coding. An event-planning task was completed first, followed by a problem-solving task. The tasks were intended to differentially elicit positive and negative emotion and associated behavior, respectively. The ordering of tasks was fixed because of concern that negative affective states elicited by the problem-solving task had the potential to persist into the positive, event-planning task if the latter were conducted second (Gilboa & Revelle, 1994).

For the event-planning interaction (EPI), mothers and adolescents were instructed to plan one or more pleasant events to do together, with up to five events chosen based on items that both the mother and adolescent rated as being '*very pleasant*' on the Pleasant Events Schedule (MacPhillamy & Lewinsohn, 1976). For the problem-solving interaction (PSI), up to five issues for discussion were selected that both the mother and adolescent endorsed as occurring the most frequently and generating the highest intensity of anger on the Issues Checklist (Prinz, Foster, Kent, & O'Leary, 1979). Parenting behavior from the tasks was coded according to the Living in Family Environments (LIFE) coding system. The LIFE (Hops, Biglan, Tolman, Arthur, & Longoria, 1995) is an observational, microsocial coding system that enables a detailed analysis of individual family members' behaviors and interactive family behaviors.

In this study, the main constructs of interest were the frequency (rate per minute) of aggressive behaviors (whilst controlling for positive behaviors) in the PSI and positive

behaviors (controlling for aggressive behaviors) in the EPI that were displayed by mothers given that we had identified previously that these behaviors occurred at higher frequencies during these particular tasks (Schwartz et al., 2012). Aggressive behavior included all displays of contemptuous, angry, and belligerent affect, as well as disapproving, threatening, or argumentative verbal content with neutral affect. Positive behavior included displays of happy, pleasant, and caring affect as well as approving, validating, affectionate or humorous comments made with neutral affect. The frequency of positive behaviour in the EPI and the PSI and the frequency of aggressive behaviour in the PSI were normally distributed. The frequency of aggressive behaviour in the EPI was very slightly positively skewed. Approximately 20% of the interactions were coded by a second observer to provide an estimate of observer agreement. Kappa coefficients (a conservative index of interobserver reliability based on point-by-point agreement and corrected for chance) for the Positive and Aggressive behavior constructs were 0.89 and 0.77 respectively. The validity of the LIFE system as a measure of family processes has been established in numerous studies (Katz & Hunter, 2007; Sheeber, Davis, Leve, Hops & Tildesley, 2007).

Neuroimaging.

Image acquisition. One-hundred and twenty three participants from the current sample completed a Structural Magnetic resonance imaging (MRI) scan. MRI's were performed on a 3 Tesla GE scanner at the Brain Research Institute, Austin and Repatriation Medical Centre, Melbourne, Australia, using a gradient echo volumetric acquisition sequence (repetition time =36 ms; echo time =9 ms; flip angle =358, field of view=20cm², pixel matrix =410×410) to obtain 124 T1-weighted contiguous 1.5 mm-thick slices (voxel dimensions =0.4883×0.4883×1.5mm).

Image pre-processing. Images were transferred to a SGI/Linux workstation for morphometric analysis. Image pre-processing was carried out using tools from the FMRIB software library (http://www.frmib.ox.ac.uk/fsl). Each 3D scan was stripped of all non-brain tissue (Smith, 2002), and aligned to the MNI 152 average template (six-parameter rigid body transform with trilinear interpolation) using FLIRT (Jenkinson & Smith, 2001). This registration served to align each image axially along the anterior commissure–posterior commissure (AC–PC) plane and sagittally along the interhemispheric fissure without any deformation. Images were re-sampled to $1mm^3$.

Morphometric analysis. We manually traced the boundaries of the hippocampus using the software package ANALYZE (Mayo Clinic, Rochester, NY; http://www.mayo.edu/bir/). Hippocampal volumes included the hippocampus proper, the dendate gyrus, the subiculum, and part of the fimbria and alveus. Boundaries were defined as follows: posterior, section with the greatest length of continuous fornix; lateral, temporal horn; medial, open end of the hippocampal fissure posteriorly and the uncal fissure anteriorly; and superior, fimbria and alveus posteriorly and amygdala anteriorly. Hippocampal estimates were based on total voxels within the defined region.

Inter-rater and intra-rater reliabilities were assessed by means of the intraclass correlation coefficient (absolute agreement) using 15 brain images from a separate MRI database established for this purpose. Intraclass correlation coefficient values were acceptable (>.90) for both left and right hippocampal volumes. Hippocampal volume measures were corrected for whole-brain size separately by gender by means of a covariance adjustment method (Free et al., 1995) and converted from mm^3 to cm^3.

Genotyping. Saliva was collected from participants for genetic analysis using an ORAGENE saliva pot (www.dnagenotek.com). Methods used for PCR amplification and visualisation by gel electrophoresis were as described by Edenberg & Reynolds (1998). The

genotype distribution for 5-HTTLPR (*n* = 54, LL, *n* = 83SL, *n* = 37, SS) was in Hardy-Weinberg equilibrium (χ^2 (1, N = 174) = .24, *ns*).

Data Analytic Strategy

Exploration of a potential IGxE effect. The presence of a potential IGxE involving conditional indirect effects as outlined by Hyde and colleagues (2011) was investigated via path analysis (see below for further explanation). Path models were estimated in Mplus 7.0 (Muthén & Muthén, 1998-2012), using weighted least squares with a mean- and variance-adjusted chi-square test statistic (WLSMV). Separate models were estimated for the two different parenting variables of interest (Model 1: aggressive parental behavior in the PSI, Model 2: positive parental behavior in the EPI). Each model contained the subject's serotonin transporter genotype, the parenting variable of interest (aggressive parental behavior in the PSI or positive parental behavior in the EPI) and the specific serotonin transporter gene X parenting interaction of interest as independent variables, left and right hippocampal volume as mediating variables, and MDD onset as the dependent variable (see Figure 1). Adolescent gender, ethnicity and the other parenting variable recorded during the same task were also included in the model as covariates. We included left and right volumes in the same path model to better understand the relative contribution of each structure to risk for depression. Acceptable tolerance (>.2) and VIF (<5) values indicated no significant multicolinearity between left and right hippocampal volumes.

The interaction terms were computed with centered variables. The path model was saturated (i.e., all possible relationships between variables were estimated) thus, model fit indices are not available for this model. We did however rerun the model with one non-significant path (the covariance between gender and ethnicity) removed to obtain fit statistics. Model fit indices indicate that both models provided an acceptable fit to the data (see Supplementary Table 2).

[INSERT FIGURE 1 HERE]

We followed the procedures recommended by Preacher, Rucker and Hayes (2007) for testing moderated mediation with bootstrapped tests of the indirect effects. While both independent variable to mediator (serotonin transporter gene → hippocampal volume) and mediator to dependent variable (hippocampal volume to MDD onset) paths of the meditational chain can be moderated (Preacher et al. 2007), we only tested whether parenting behaviors moderated the path between serotonin transporter gene and hippocampal volume.

The current analyses were based upon 50,000 bootstrapped samples and bias-corrected bootstrapped parameter estimates were used to test the significance level of the indirect effects. If the 95% or the 90% confidence intervals for these estimates of an indirect effect do not contain zero, it can be concluded that the indirect effect is statistically significant at the .05 level or .01 respectively (Shrout & Bolger, 2002).

The IGxE of interest considered the possibility of the presence of conditional indirect effects in which left or right hippocampal volume served as mediators between serotonin transporter genotype and onset of depression during adolescence, and parenting influenced these meditational pathways. This model, which examines whether the indirect effect from an independent variable and dependent variable through mediating variables depends on the value of the moderator variable, allowed an examination of whether the strength of the indirect pathway from 5-HTTLPR to hippocampal volume to MDD onset varies across different caregiving environments.[1]

[1] The primary focus of this study was to explore whether the caregiving environment provided by parents would alter the strength of an indirect pathway from serotonin transporter genotype to MDD onset through hippocampal volume, based on previous findings indicating the presence of this indirect pathway. The current literature however would also support the possibility that serotonin transporter genotype might moderate a pathway from parenting to MDD onset through hippocampal volume. It may therefore be of interest to readers to know that we did test this model, and did not find evidence of an overall indirect pathway from parenting to hippocampal volume to MDD onset, nor did findings support the possibility that this pathway could be influenced by serotonin transporter genotype variation.

The presence of a conditional indirect effect was tested by identifying which indirect paths (i.e., the path through left or right hippocampal volume) were significant at different levels (i.e. mean, +1*SD* and −1*SD*) of aggressive parenting/positive parenting (Preacher et al. 2007). That is, the path model for each type of parenting behavior was fitted three times, first with the parenting variable of interest at a centered mean of 0, second with parenting variable of interest centered at +1*SD* and third with parenting variable of interest centered at −1*SD*, to estimate indirect effects between the serotonin transporter gene and depression onset at different levels of aggressive parenting or positive parenting. This conditional indirect effect can be represented as $f(\theta|parenting) = b(a + w[parenting])$, where *parenting* could take the values of each level described above.

It is often assumed that moderation of a component pathway of the indirect relationship is required for evidence of a conditional indirect effect (and that if one of the individual pathways comprising the indirect effect is moderated, then so too is the indirect effect). However, as discussed in detail by Fairchild and MacKinnon (2009) and by Hayes (2015), this is not necessarily the case – there are instances where (1) the significance of an indirect effect is conditional on the values of a particular variable, in the absence of moderation by this variable of one of the component pathways comprising the indirect effect, and (2) where a component pathway of the indirect effect might be moderated by a particular variable but this does not necessarily mean that the indirect effect is conditional on values of this variable. Whilst the conditional indirect effect depends on parenting to the extent that the interaction coefficient *w* departs from zero, determining variation in the $(a+w)b$ product term as a function of parenting is conceptually and statistically different from identifying a significant interaction predicting variation in the hippocampus (i.e. a significant path *w* predicting the mediator). Variation in the product estimate of the mediated effect from 5-

HTTLPR to hippocampal volume to MDD onset by parenting (i.e., variation in the significance of the (*a+w)b* product term) may suggest that the extent to which the serotonin transporter gene might alter risk for depression through hippocampal volume varies according to parenting environment (in other words, the extent to which differences in hippocampal volume specifically explained by serotonin transporter genotype can significantly account for variance in risk for MDD onset differs according to parenting) whilst variation in path *c* would suggest that parenting moderates the direct effect of the serotonin transporter gene on hippocampal volume.

Quantifying potential associations between indirect effects from 5-HTTLPR→ Hippocampus → MDD onset and parenting. A current challenge for researchers investigating the presence of conditional indirect effects is assessing whether indirect effects might be significantly different from each other at different levels of the potential moderator, which would arguably be required to claim "true" moderated mediation. Hayes (2015) has advocated for the use of an "index of moderated mediation," which quantifies the extent to which an indirect effect can be expressed as a *linear function* of a moderator, as a potential direct test of moderated mediation. He identifies the index of moderated mediation as the product of the coefficient of the interaction term between the independent variable and the putative moderator predicting the mediator (represented by $w_{L/R}$ in Figure 1) and the coefficient of the mediator predicting the dependent variable (represented by $b_{L/R}$ in Figure 1). Hayes (2015) proposes that there is significant moderation of an indirect effect if the index of moderated mediation (wb) is significantly different from zero according to bootstrapped confidence intervals. If the 95% or 90% confidence intervals for these estimates of the index of moderated mediation does not contain zero, it can be concluded that the indirect effect is statistically significant at the .05 or .10 level respectively. If the index of moderated mediation is found to be significantly different from zero, it means that any two

conditional effects estimated at different values of a moderator are significantly different from each other. Furthermore, if the index of moderated mediation is found not to be significantly different from zero, this would imply that no two conditional indirect effects are statistically different, regardless of the values of the moderator at which they are estimated.

Investigation of potential confounding effects of gender, ethnicity and the other parenting variable of interest. It has recently been suggested that to properly control for potential confounders in GxE research, all relevant covariates as well as all relevant gene x covariate and environment x covariate interaction terms must be included in analyses (Keller, 2014). For example, failure to include an ethnicity x environment interaction might result in a spurious gene x environment interaction in situations where people of different ethnic backgrounds might be differentially affected by a particular environmental experience, and the frequency of the particular genetic variation under investigation happens to differ amongst individuals of different ethnic backgrounds. In this case, ethnicity, rather than the gene might be the real moderator of an environmental effect, with the gene merely correlated with ethnic background.

In the current analyses, we considered the effects of three covariates, namely gender, ethnicity and the other dimension of parenting behavior (positive parenting in the model examining the effect of aversive parenting, and aversive parenting in the model examining the effect of positive parenting) on model findings. Given the size of the current sample, it was not feasible to include all gene x covariate and environment x interaction terms simultaneously in the same model. The effect of each interaction (parenting variable of interest x gender, parenting variable of interest x ethnicity, parenting variable of interest x other parenting variable, 5-HTTLPR x gender, 5-HTTLPR x ethnicity, 5-HTTLPR x other parenting variable) on results was therefore considered separately.

Treatment of missing data. Listwise deletion because of missing data would have resulted in only 73 cases remaining in the analysis due to non-participation in either the MRI or observational task measuring parenting at wave 1 or in the psychiatric interview at waves 2, 3 or 4. Little's MCAR test (Little, 1988) was non-significant, $\chi^2(60)=52.36$, p=.748. We therefore used pairwise deletion (which is the only option when using the WLSMV estimator and bootstrapping in *Mplus* other than listwise deletion) to account for missing data. Pairwise deletion has been shown to be unbiased when data is missing completely at random (Enders & Bandalos, 2001). Supplementary Table 3 documents the covariance coverage between variables, and Supplementary Table 4 indicates the number of participants that were included to calculate each statistic, as a result of the use of pairwise analysis. A simulation study by Preacher and colleagues (2007) documents the sample sizes that would likely be required to detect the individual paths of small, medium and large effect sizes in a simple moderated mediation analysis, and hence may provide some indication of the power of the current study to detect effects. The findings of this study suggested that the current sample may have adequate power to detect associations of medium effect size or above, though it is somewhat underpowered to detect findings of small effect size.

Results

Demographic features are outlined for the full sample, as well as for participants with and without an onset of MDD, and the three genotype participant groups in Table 2.

[INSERT TABLE 2]

Percentages or mean scores and standard deviations for all variables and their intercorrelations are presented in Table 3. Of particular relevance are the bivariate correlations between 5-HTTLPR genotype and the parenting variables, which are not significant. These non-significant correlations suggest that any GxE effect is not a function of an evocative gene-environment correlation (rGE) involving the adolescent's (heritable)

behavior evoking a particular parenting response. Gender and ethnic background were not significantly associated with serotonin transporter genotype, MDD onset, hippocampal volume or any of the parenting measures.

[INSERT TABLE 3 HERE]

Findings for the path models are displayed in Table 4. For parsimony, only key relationships of interest between the independent variables (5-HTTLPR, the specific parenting variable under consideration and the 5-HTTLPR x parenting interaction term), mediating variables (left and right hippocampus) and dependent variable (MDD onset) are shown here. Results of the complete models are provided in Supplementary Table 4.

[INSERT TABLE 4 HERE]

More frequent aggressive parenting in the PSI and less frequent positive parenting in the EPI was not associated with left or right hippocampal volume or increased risk for MDD onset. There was also no evidence of a significant interaction between 5-HTTLPR and aggressive parenting or positive parenting predicting MDD onset and none of the interaction terms between serotonin transporter genotype and parenting were predictive of left or right hippocampal volume (path $c_{L/R}$) in any of the path models. Rather, an increasing number of S-alleles at the 5-HTTLPR locus was associated with smaller left hippocampal volume as a main effect (path a_L) and smaller left hippocampal volume was also associated with increased risk for MDD onset (path b_L) in both path models. In contrast, 5-HTTLPR variation was not significantly associated with right hippocampal volume (path a_R), though larger right hippocampal volume was predictive of increased risk for MDD onset (path b_R) in both the path models. These relationships had previously been identified in this sample, when parenting was not taken into account (Little et al., 2014).

We went on to test for the presence of conditional indirect effects by testing whether pathways from the serotonin transporter gene to depression through either the left or the right hippocampus were significant at high (+1 SD), average/medium (M) and low (-1SD) frequencies of aggressive behaviors in the PSI task or positive parenting behaviors in the EPI task. Findings for these analyses are displayed in Table 5.

[INSERT TABLE 5 HERE]

Aggressive parenting in the PSI

As shown in table 5, the indirect pathway from 5-HTTLPR genotype to MDD onset through the left hippocampus was significant at high and average but not low levels of aggressive parenting, whilst the indirect pathway through the right hippocampus was significant at high frequencies of aggressive parenting only according to 95% CI (though the indirect pathway through the right hippocampus was also significant at average frequencies of aggressive parenting according to 90% CI). Both the 95% and 90% confidence intervals for the index of moderation for the indirect effects through both the left and right hippocampus contained zero however, meaning that there is no definitive support of moderation of these specific indirect effects by aggressive parenting.

Positive parenting in the EPI

The indirect pathway through the left hippocampus was significant at low levels but not at high or average levels of positive parenting, according to the 95% CI (though this pathway was also significant at average frequencies of aggressive parenting, according to the 90% CI) as can be seen table 5. The indirect pathway through the right hippocampus was also significant at low levels of positive parenting only. Both the 95% and the 90% confidence

intervals for the index of moderated mediation for the specific indirect effect through the left hippocampus are almost entirely negative but do contain zero (though only just for the 90% CI), thus it cannot be concluded that positive parenting definitely moderates this indirect effect. For the specific indirect effect through the right hippocampus, the index of moderated mediation was significantly positive according to the 90% but 95% CI, thus moderation of this indirect by positive parenting may be plausible.

Investigation of potential confounding effects of gender, ethnicity and the other parenting variable of interest.

Supplementary Tables 5 to 10 contain findings of the separate models controlling for the 5-HTTLPR x covariate or parenting x covariate interaction terms. They show that the pattern of results for the key paths of interest that were noted to be significant in the results presented above appeared largely unchanged, though in some of the analyses, they were marginally significant (where $p < .07$, with the exception of the 5-HTTLPR → left hippocampus path in the model controlling for an interaction between aggressive parenting and positive parenting, where positive parenting was the variable of interest, where $p = .086$). Controlling for particular interactions also resulted in two additional paths becoming significant. In the analysis controlling for an interaction involving 5-HTTLPR x gender when positive parenting was the parenting variable of interest, the 5-HTTLPR → right hippocampus path emerged as significant, such that increasing s-alleles predicted smaller volumes. Furthermore, in the analysis controlling for an interaction between positive parenting x ethnicity, the interaction between 5-HTTLPR x positive parenting significantly predicted MDD, however post-hoc simple slope analyses indicated that the relationships between positive parenting and MDD onset were non-significant for all three genotype

groups (LL: b = -.45 [95%CI: -0.97; 0.08], S.E. =.27, p=.098; SL: b=1.06 [95%CI: -0.51, 1.127], S.E.=.42, p=.46, SS: b=1.06 [95%CI: -.28;2.41], S.E.=.69, p=.12).[2]

Given that each of these paths were not observed consistently in the analyses, as was found for the other significant paths, we suggest that these findings be interpreted with caution until further replication occurs.

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[2] To further explore the influence that ethnicity might have on the current findings, we also ran the moderated mediation model separately for participants of Anglo-European background, the largest ethnic subsample (n=150), given evidence that 5-HTTLPR frequencies differ according to ancestry, which could result in differential association with psychiatric outcomes. These analyses, which are presented in Supplementary Document 3, should be interpreted with significant caution, as they are likely to be underpowered, according to the simulation study by Preacher and Hayes (2007), given the majority of the relationships were based upon samples of less than 90 participants. Nonetheless, the pattern of the findings for the individual paths were largely preserved (albeit mostly marginally significant), as shown in Supplementary Table 17, with similar effect sizes to those obtained for the overall sample. Left hippocampal volume no longer significantly predicted MDD onset however, though the effect size appeared only slightly attenuated. In the model examining the role of positive parenting, the interaction between 5-HTTLPR x positive parenting significantly predicted MDD onset. Post hoc simple slope investigations indicated that positive parenting and risk for MDD onset was unrelated in both the LL-homozygous (b=-.43 [95% CI:-1.47; .27], S.E.=.36, p=.233) and SL heterozygous (b=.43, [95 CI: -.15;1.01], S.E.=.30, β=.11, p=.15) groups (see Supplementary Figure 1). There was a relationship however in the SS homozygous group, such that higher frequencies of positive parenting was associated with an increased probability of a later MDD onset (b=1.29 [95 CI: .05; 2.53], S.E.=.51, p=.043).

The indirect effect through the right hippocampus was significant according to 90% CI at only at high levels of aggressive parenting and low levels of positive parenting. The index of moderated mediation for the model examining positive parenting was significant according to the 90% CI, thus moderation of this indirect effect is not inconceivable. All indirect effects were non-significant for the left hippocampus, but the pattern was similar to those obtained for the overall sample.

Supplementary Tables 11 to 16 show that the indirect pathways from 5-HTTLPR to MDD onset through the left and right hippocampus remained significant at high frequencies of aggressive parenting and low frequencies of positive parenting (though this finding was marginally significant, according to the 90% CI for the set of analyses that controlled for the interaction between 5-HTTLPR x ethnicity). These indirect pathways were not significant at low frequencies of aggressive parenting and high frequencies of positive parenting. The index of moderated mediation for the indirect pathway through the right hippocampus remained significant at the 90% CI level when positive parenting was examined as the moderator in all the analyses, and reached significance at the 95% CI level in the analyses controlling for an interaction between aggressive parenting x ethnicity, and in the analyses controlling for an interaction between 5-HTTLPR x gender. These findings therefore continue to suggest a trend for this indirect pathway to change linearly with changes in positive parenting.

Furthermore, the index of moderated mediation also reached significance at the 90% CI level for the specific indirect effect through the left hippocampus when positive parenting was examined as the putative moderator in the sets of analyses that controlled for an interaction between 5-HTTLPR x ethnicity, or an interaction between aggressive parenting x ethnicity. The index of moderated mediation for the indirect pathway through the right hippocampus was also significant according to the 90% CI's when aggressive parenting was examined as the moderator of interest and analyses controlled for the interaction between aggressive parenting x ethnicity.

Discussion

The current study provides some indication that the behavior of parents may influence an indirect risk relationship between the serotonin transporter gene and first onset of MDD during adolescence via individual differences in hippocampal volume. In particular, there

was tentative evidence that the action of an indirect pathway from the serotonin transporter gene to MDD onset through the right hippocampus may differ between environments involving different frequencies of positive parenting. Findings specifically suggested that increasing copies of S-alleles were predictive of smaller right hippocampal volume, and that the specific variation in hippocampal volume explained by serotonin transporter genotype was significantly associated with MDD onset only in family environments involving lower levels of positive parenting. The systematic impact of aggressive parenting on this indirect pathway was less clear – whilst the indirect pathway was significant only in family environments involving higher levels of parental aggression, it could not be established that the capacity of this indirect pathway to account for risk of MDD onset varied significantly across environments involving different levels of aggressive parenting. It was similarly difficult to determine the systematic influence of parenting on the indirect pathway from 5-HTTLPR to MDD onset through the left hippocampus – again, whilst the indirect pathway were significant in the presence of higher levels of parental aggression and lower levels of positive parenting, there was no definitive evidence that the action of this indirect pathway was significantly different across different parenting environments. It is possible that an indirect pathway from the 5-HTTLPR to MDD through the left hippocampus is less influenced by the environment than one that proceeds through the right hippocampus.

These findings demonstrate the potential importance of considering the influence of environmental experiences when attempting to understand neurobiological mechanisms associated with disorders like depression; indeed, the finding of an indirect pathway involving the right hippocampus had not been apparent in previous analyses which did not take parenting into account (Little et al., 2014). This study may provide some insight into the conditional mechanisms by which the S-allele of the serotonin transporter gene, adverse environments and brain morphological characteristics might together influence risk for MDD.

The current findings did not suggest that the serotonin transporter gene and the caregiving environment interact to directly alter hippocampal volume, but rather that the potential for a biological pathway from the serotonin transporter gene to explain or account for risk in MDD onset through hippocampal volume might vary according to the caregiving environment. Given that hippocampal volume was measured in early adolescence, prior to MDD onset, these results may be consistent with the notion of the hippocampus as an intermediate phenotype, or pre-existing vulnerability factor for stress-related disorders such as depression (rather than a change during illness) that forms an intermediary link in a biological pathway between genetic vulnerability and disorder (Meyer-Lindenberg & Weinberger, 2006; Savitz & Drevets, 2009). It is interesting to note that serotonin is known to have a role in neurodevelopment, including maturation of limbic circuit properties (Deepika Suri, Teixeira, Cagliostro, Mahadevia, & Ansorge, 2015). It is possible that the influence of allelic variation in the serotonin transporter gene on hippocampal volume (or other limbic structures) could be mediated by genetic influences on early neurodevelopmental processes that shape morphology and function, rather than by current *in vivo* serotonin transporter expression or serotonin availability (Kobiella et al., 2011). Critically, it is now recognised that an intermediate phenotype may require "a challenge" to become evident or turn pathogenic (Hasler & Northoff, 2011). Indeed, there is some suggestion that this may be the case for the hippocampus in stress-related illness more generally. For example, a study of hippocampal volumes in monozygotic twins discordant for trauma exposure documented smaller hippocampal volumes in trauma-unexposed co-twins of veterans with PTSD, compared to unexposed co-twins of veterans without PTSD (Gilbertson et al., 2002). We contend that more problematic caregiving behaviors or a maladaptive family environment may be one such challenge that can activate the pathogenic effects of hippocampal volume in a potential causal pathway from the serotonin transporter gene to MDD onset. Speculatively, it may be

that in adverse family environments, smaller hippocampal volumes associated with S-carrier status may affect negative feedback inhibition of the HPA axis, which could result in HPA axis hyperactivity and greater depression risk. We were not able to test these putative underlying mechanisms however in the current study, and this therefore remains an important avenue for future research.

The findings that parenting on its own was not associated with hippocampal volume, and that 5-HTTLPR and parenting behaviors did not significantly interact to predict hippocampal volume were somewhat inconsistent however with previous studies that documented direct effects of caregiving behavior at 3-5 years old on the hippocampus (Luby et al., 2012, Rao et al., 2010) as well as interactive effects involving smaller hippocampal volumes in carriers of an S-allele who had also experienced significant adversity (Frodl et al., 2010; Everaerd et al., 2012). It is possible that maladaptive parenting as measured in this study was not a severe enough form of adversity to produce neurotoxic effects on the hippocampus, either on its own or in interaction with the serotonin transporter genotype, or that a larger sample size is required to detect these effects. Alternatively, the lack of associations involving parenting and the hippocampus in the current study may relate to the age at which these were measured. Studies suggest that early adversity in infancy and early childhood may result in stronger diminishments in hippocampal volume than adversity experienced at other ages but that there are also delayed effects of stress or adversity on the hippocampus that do not manifest until adulthood (Lupien, McEwen, Gunnar, & Heim, 2009). The hippocampus undergoes significant structural change during adolescence and it is possible that the effects of maladaptive parenting behaviors experienced at 11-13 years on the hippocampus are less pronounced, or alternatively, not yet evident at this age, which was also when participants also underwent an MRI. It seems plausible that the serotonin transporter gene could have an important role in not only shaping brain networks during early development, and could also contribute also in more ongoing alterations to neural structure, function and connectivity in the adolescent adult brain in response to the environment, which may have impacts on cognitive and emotional responses. The finding that left and right volumes have opposite effects on the onset of MDD may initially seem somewhat surprising

and potentially inconsistent with previous studies which have found bilateral reductions in hippocampal volume that were predictive of depression. As far as we are aware, however, our study is unique in having considered the relative contribution of the left and right hippocampi to depression (i.e., controlling for hippocampal volume in one hemisphere while assessing the effect of the volume in the other hemisphere). It is also important to remember that associations between hippocampal volume and MDD onset are likely to be influenced by a range of factors beyond serotonin transporter genotype, including other genetic factors as well as other sources of stress or adversity beyond maladaptive parenting behaviour that were not contained in the current analyses. Thus, a conditional indirect pathway from increasing S-alleles to smaller right hippocampal volume that was predictive of MDD in adverse family contexts may still be compatible with a larger right hippocampal volume predicting MDD onset overall. This would occur if additional processes of risk were operating simultaneously to affect vulnerability to MDD. For example, whilst increasing S-alleles might have an effect of reducing right hippocampal volume, which in turn could have consequences for risk for MDD in adverse family environments, there might also be additional factors that could affect one hemisphere more than the other (either reducing the left hippocampus to a greater extent than the right, or increasing the right hippocampus to a greater extent than the left), or alter normal hippocampal development (for example attenuating growth of the hippocampus to a greater extent in the left hippocampus) during this developmental period that would account for the current result. In our previous paper (Little et al., 2014), we noted the possibility that the opposite effects of left and right hippocampal volume predicting depression as indicating a role of asymmetry in the disorder. In particular, the current findings may reflect a disruption to a normal developmental process that may see right>left structural asymmetry present in the general population decrease somewhat from childhood to adolescence (Isaacs et al., 2000; Szabo, Wyllie, Siavalas, Najm, & Kotagal, 1999; Thompson et al., 2009; Uematsu et al.,

2012). Disruption to normal hippocampal volume development may directly impact regulation of the HPA stress response system, and may also affect neural projections from the hippocampus to other brain regions involved in emotion processing, such as the amygdala, medial prefrontal cortex, posterior cingulate cortex and basal ganglia that have implications for depression risk (Price & Drevets, 2010; Small, Schobel, Buxton, Witter, & Barnes, 2011).

It should also be noted that there was suggestion in the analyses that particular findings might vary somewhat as a function of ethnicity. In particular, there was some indication that SS homozygous individuals of Anglo-European background were more vulnerable to depression in environments involving high parental warmth and positivity. Given this result was barely significant and family environments involving high levels of positive, nurturing parental behaviors have consistently been identified as protective against depression (Yap, Pilkington, Ryan, & Jorm, 2014), this particular finding should be interpreted with caution until further replications are documented, especially given that our sample size prohibited a systematic characterisation of the role of ethnicity on pathways between 5-HTTLPR, hippocampal volume, parenting and MDD onset.

There are a number of study limitations that must be kept in mind when considering these results. First, the number of participants might be considered preliminary for a study examining moderated mediation of genetic effects on MDD. In particular, it is possible that the smaller sample size of the current sample may have limited power to detect smaller effects. Equally, the possibility of results that are "false positives" is also a concern. It has been argued however that many of the best-designed studies for testing GxE hypotheses (and we suggest imaging IGxE hypotheses by extension) have smaller samples because these studies are significantly more likely to be prospective longitudinal and to utilize gold-standard measures (Caspi et al., 2010; Moffitt & Caspi, 2014; Uher & McGuffin, 2010). This is indeed true of the current study, which employed a longitudinal, prospective design and

conducted face-to-face diagnostic interviews to assess psychopathology, hand-traced hippocampal volume (with reliabilities exceeding .90) from structural MRIs conducted on a 3 Tesla GE scanner and obtained observational measures of parenting behavior. Moreover, our use of bias-corrected bootstrapping procedures, as recommended for conditional indirect effects analysis is likely to have improved our power to detect relationships of moderate effect sizes in the current sample (Preacher and Hayes, 2007). We believe that this statistical method, in combination with our study design, assists with mitigating concerns about power. Nonetheless, these results (particularly non-significant findings of small effect size), should be interpreted with caution until they are replicated. Future studies with larger samples will be particularly beneficial in this regard. We also note that these results would not survive Bonferroni adjustment. However, given the large effect sizes required to survive the loss of power associated with such a conservative test as the Bonferroni adjusted significance test (Jennions & Moller, 2003; Nakagawa, 2004), we suggest that the uncorrected estimates provide important information that might otherwise be lost to Type 2 error.

Second, the current analyses were conceived based on the premise that the hippocampus is a plastic structure that may be influenced by the environment, including parenting. It is possible however that adolescent hippocampal volume could influence parenting behaviour (indirectly, through the adolescent's own behaviour). The concurrent measurement of adolescent hippocampal volume and parenting at 11-13 years meant that causal relationships between these two factors could not be addressed within the current study. Third, as noted earlier, the timing of these measurements may also have affected our capacity to detect effects of parenting on hippocampal volume, either as a main effect or in interaction with the serotonin transporter gene, and in turn detection of significant mediated moderation effects. Fourth, examining hippocampal structure at only one time point during adolescence, a period of significant hippocampal development (e.g., Whittle et al., 2014),

means it is not possible to determine whether these findings reflect stable differences present prior to illness onset or abnormal developmental changes that emerge during early adolescence. A further point for consideration is the higher rates of other lifetime psychiatric conditions in the group of participants who experienced an onset of MDD compared with those who did not. Although depression is frequently co-morbid with other psychiatric conditions (e.g., Merikangas et al., 2010; Paul Rohde, Peter M. Lewinsohn, Daniel N. Klein, John R. Seeley, & Jeff M. Gau, 2013), it reduces our confidence in attributing the observed relationships to depression specifically as opposed to the presence of psychopathology more generally. Finally, while we were able to consider left and right hippocampal volume within the same analysis, we acknowledge that the hippocampus does not function independently and that other brain structures, particularly those known to be involved in emotion processing and the stress response will be important to consider. Equally, there are other polymorphisms, such as the BDNF Val66Met polymorphism, and other environmental experiences, including stressful life events, trauma and peer relationships, that may be associated with variation in the hippocampus and depression. We also acknowledge the potential role that epigenetic mechanisms, including DNA methylation, might play. Future work incorporating these factors is likely to be important in more thoroughly characterising the particular roles that genes, a network of brain structures and different environmental experiences might play in the development of depression or other psychopathologies.

Despite these limitations, the study has a number of strengths that warrant mention. This study is the first to examine the complex relationships between the serotonin transporter gene, hippocampal volume, family environment and MDD onset during adolescence, responding to calls to investigate these factors within one sample by employing an IGxE framework (e.g., Hyde et al., 2011; Hyde, 2015). Moreover, as noted above, the prospective longitudinal study design provides the opportunity for investigating a first onset of MDD and

permits greater confidence than does a cross-sectional design in making inferences of causality regarding the emergence of the disorder. Our observational measure of parenting may also offer some advantage over self-report questionnaire measures, as it may provide a more objective measure of the caregiving environment. Other noteworthy features of the current investigation include the community sample and the coverage of the adolescent period, one of the most critical periods of risk for onset of depression, in the study design.

Conclusions

In this investigation, we found evidence of complex pathways from the serotonin transporter gene to first onset of MDD via abnormalities in hippocampal volume that were conditional on the nature of parenting experienced during early adolescence. Specifically, in certain contexts involving either low frequencies of positive parenting or high frequencies of aggressive parental behaviour, increasing S-alleles were associated with smaller hippocampal volumes, and this specific variance in hippocampal volume accounted for increased risk in depression. Although understanding of these effects will benefit from further work elucidating more detailed mechanisms underlying these associations, the current study marks an important step in linking GxE interaction studies and imaging genetic studies into an overall IGxE framework that is able to address more nuanced questions regarding the complex contributions of genetic, neurobiological and environmental factors towards risk for psychopathology.

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Table 1

Participation across the four waves of the current sample of 174 individuals who had provided a DNA sample during the course of the longitudinal Adolescent Development Study

	Structural MRI	Parent-child interaction assessment	Diagnostic interview
Wave 1	123	124	174
Wave 2			168
Wave 3			156
Wave 4			141

Table 2

Demographic details for the full sample, participants with and without an onset of MDD, and the three genotype participant groups

	Full sample	MDD onset status		Serotonin Transporter Genotype		
		MDD onset	No MDD onset	SS	SL	LL
	Proportion or M (SD)	*Proportion or M (SD)*	*Proportion or M (SD)*	*Proportion or M (SD)*	*Proportion or M (SD)*	*Proportion or M (SD)*
Age	12.66 (.43)	12.66 (.37)	12.66 (.45)	12.63 (.42)	12.65 (.45)	12.68(.43)
Gender (male)	.48	.44	.48	.41	.47	.54
Ethnicity	.87	.92	.86	.81	.88	.93
Pubertal Status	2.17 (1.04)	2.64 (1.19)	2.05 (.94)	2.10 (.83)	2.11 (1.00)	2.31 (1.22)
Socioeconomic Status	56.60 (20.83)	56.77 (19.04)	57.64 (21.9)	56.14 (19.83)	55.51 (21.99)	58.59 (19.86)
Left hippocampus	2.77 (.33)	2.70 (.35)	2.77 (.33)	2.65 (.25)	2.80 (.35)	2.81 (.35)
Right hippocampus	2.95 (.34)	2.94 (.35)	2.91 (.33)	2.88 (.28)	2.96 (.36)	2.99 (.35)
Maternal aggressive behavior EPI	.57 (.41)	.75 (.54)	.49 (.33)	.63 (.44)	.51 (.37)	.63 (.44)
Maternal aggressive behavior PSI	1.26 (.61)	1.32 (.65)	1.22 (.61)	1.22 (.53)	1.23 (.65)	1.33 (.59)
Maternal positive behavior EPI	2.37 (.49)	2.30 (.52)	2.39 (.49)	2.40 (.43)	2.40 (.44)	2.30 (.59)
Maternal positive behavior PSI	1.76 (.68)	1.59 (.63)	1.87 (.62)	1.81 (.76)	1.85 (.64)	1.57 (.67)
Serotonin Transporter Genotype (LL/SL/SS)		.361/.472/.167	.277/.485/.238	-	-	-

Table 3

Descriptive statistics and intercorrelations

Variables	1	2	3	4	5	6	7	8	9	10
1. MDD onset (0=no onset, 1=MDD onset)	-									
2. Serotonin transporter genotype (0 =LL, 1=SL, 2=SS)	-0.14	-								
3. Gender (male = 0, female = 1)	0.05	0.13	-							
4. Ethnicity (0 = Aust-European descent, 1 = non-Aust-European descent)	-0.17	0.23	0.00	-						
5. Left hippocampal volume	-0.01	-0.20*	0.00	0.06	-					
6. Right hippocampal volume	0.23	-0.14	0.00	0.13	0.79***	-				
7. Aggressive parenting behavior - EPI	0.34**	-0.02	0.07	0.05	-0.02	-0.03	-			
8. Aggressive parenting behavior - PSI	0.09	-0.08	-0.07	0.12	-0.04	-0.05	0.52***	-		
9. Positive Parenting behavior -EPI	-0.11	0.10	-0.08	-0.26	-0.11	-0.08	-0.32***	-0.26**	-	
10. Positive parenting behavior - PSI	-0.28*	0.16	-0.05	-0.21	0.02	0.05	-0.43***	-0.44***	0.41***	-
Percentage of sample or *M* (SD)	MDD onset = 20.69%	SL=47.7% SS=21.3%	Male =47.70%	Aust-Europe descent = 86.20%	2.77 (.33)	2.95 (.34)	.57 (.41)	1.26 (.61)	2.368 (.64)	1.752 (.68)

$p \leq 05$ = *, $p \leq .01$ = **, $p \leq .001$ = ***

Table 4

IGxE path models testing associations between 5-HTTLPR genotype, hippocampal volume and parenting at 11-13 years and later MDD onset during a six year follow up period.

	b	SE	95% CI		β	p
			Upper	Lower		
Model 1: Aggressive Parenting in the PSI						
5-HTTLPR → MDD onset	-.12	.18	-.46	.23	-.08	.511
Aggressive parenting → MDD onset	.24	.38	-.49	1.00	.15	.522
5-HTTLPR X Aggressive Parenting → MDD onset	-.33	.36	-1.11	.33	-.22	.360
5-HTTLPR → Left hippocampus (a_L path)	**-.08**	**.04**	**-.16**	**-.01**	**-.20**	**.035**
Aggressive parenting → Left hippocampus	.02	.08	-.14	.17	.05	.767
5-HTTLPR X Aggressive Parenting → Left hippocampus (w_L path)	-.05	.07	-.18	.08	-.11	.448
Left hippocampus → MDD onset (b_L path)	**-1.78**	**.89**	**-3.62**	**-.12**	**-.53**	**.044**
5-HTTLPR → Right hippocampus (a_R path)	-.06	.04	-.15	.02	-.15	.126
Aggressive parenting → Right hippocampus	.08	.09	-.10	.26	.15	.411
5-HTTLPR X Aggressive Parenting → Right hippocampus (w_R path)	-.10	.08	-.26	.04	-.23	.169
Right hippocampus → MDD onset (b_R path)	**2.15**	**.81**	**.54**	**3.75**	**.65**	**.008**
Model 2: Positive Parenting in the EPI						
5-HTTLPR → MDD onset	-.18	.17	-.50	.16	-.13	.289
Positive parenting → MDD onset	-.50	.36	-1.19	.25	-.24	.168
5-HTTLPR X Positive Parenting → MDD onset	.66	.38	-.07	1.47	.32	.086
5-HTTLPR → Left hippocampus (a_L path)	**-.08**	**.04**	**-.15**	**.002**	**-.18**	**.048**
Positive parenting → Left hippocampus	-.14	.08	-.27	.05	-.22	.090
5-HTTLPR X Positive Parenting → Left hippocampus (w_L path)	.11	.08	-.04	.28	.17	.180
Left hippocampus → MDD onset (b_L path)	**-1.79**	**.89**	**-3.58**	**-.08**	**-.53**	**.044**
5-HTTLPR → Right hippocampus (a_R path)	-.06	.04	-.13	.03	-.13	.168
Positive parenting → Right hippocampus	-.15	.09	-.32	.04	-.24	.106
5-HTTLPR X Positive Parenting → Right hippocampus (w_R path)	.16	.09	-.01	.34	.25	.075
Right hippocampus → MDD onset (b_R path)	**2.03**	**.76**	**.49**	**3.51**	**.61**	**.008**

Table 5

Bootstrapping Estimates of the Indirect Effect (5-HTTLPR → Hippocampal Volume → MDD Onset) at Varying Levels of Parenting Behaviour and the Index of Moderated Mediation (the extent to which the indirect effect of 5-HTTLPR → Hippocampal Volume → MDD Onset varies as a linear function of parenting behaviour)

	Left Hippocampus						Right Hippocampus					
	Raw *M*	Raw *SE*	Bootstrapped 95% CI		Bootstrapped 90% CI		Raw *M*	Raw *SE*	Bootstrapped 95% CI		Bootstrapped 90% CI	
			Lower	Upper	Lower	Upper			Lower	Upper	Lower	Upper
Aggressive Parenting (PSI)												
High (+1SD)	**.20**	**.14**	**.01**	**.60**	**.04**	**.52**	**-.27**	**.19**	**-.79**	**-.01**	**-.69**	**-.04**
Average (M)	**.15**	**.11**	**.004**	**.47**	**.02**	**.41**	-.14	.12	-.45	.02	**-.39**	**-.003**
Low (-1SD)	.10	.14	-.07	.50	-.04	.41	-.001	.14	-.28	.28	-.22	.22
Index of Moderated Mediation	.09	0.13	-.11	.44	-.06	.37	-.223	0.20	-.74	.049	-.65	.005
Positive Parenting (EPI)												
High (+1SD)	.01	.13	-.24	.31	-.18	.24	.09	.14	-.15	.44	-.09	.38
Average (M)	.13	.11	-.002	.42	**.01**	**.37**	-.11	.10	-.37	.03	-.32	.009
Low (-1SD)	**.26**	**.20**	**.02**	**.82**	**.05**	**.70**	**-.31**	**.21**	**-.88**	**-.03**	**-.77**	**-.07**
Index of Moderated Mediation	-.19	.20	-.82	.03	-.69	.000	.317	.23	-.003	.93	**.04**	**.82**

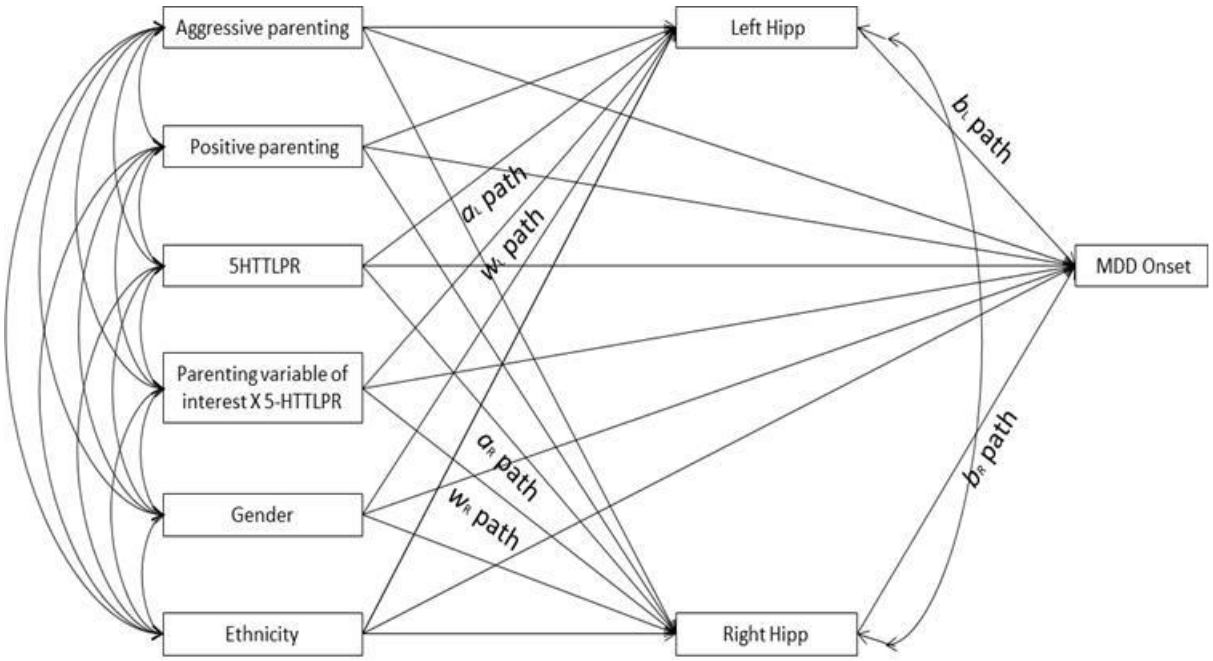


Figure 1. Path diagram of the SEM model to test mediated moderation and moderated mediation Path a ($_L$ or $_R$) is the total effect of serotonin transporter genotype on (left or right) hippocampal volume, path b ($_L$ or $_R$) is the total effect of (left or right) hippocampal volume on MDD onset and path w ($_L$ or $_R$) is the interactive effect between serotonin transporter genotype and parenting on MDD onset.

CHAPTER 10: DISCUSSION AND CONCLUSION – INTEGRATION, LIMTIATIONS AND OPPORTUNITIES

A substantial body of research has examined the role of the serotonin transporter gene in depressive disorders. Studies using *a candidate gene-environment (GxE)* approach suggest the possibility that in adverse contexts, particularly those involving a high degree threat or negative life stress, the short or *S*-allele of the serotonin transporter gene-linked polymorphic region (5-HTTLPR) may be associated with an enhanced risk for depression. However, as emphasised throughout this thesis, there is considerable contemporary debate about the status and interpretation of this gene by environment interaction. Moreover, the specific biological processes by which genetic or environmental variables may affect the aetiology of depression have not been resolved. Studies that have capacity to elucidate these mechanisms may be in a better position to verify the validity of interaction results. The first study of this thesis (CHAPTER 4 and CHAPTER 5) thus contributes to the current literature by focusing on developing a more nuanced understanding of the *environmental* contribution to a gene-environment interaction involving the serotonin transporter gene by considering how distinct aspects of the environment might influence findings. Specifically, the aim of **Study 1** was to examine whether allelic variations in the 5-HTTLPR moderate risk for depression in the context of (1) low levels of positive parenting (a form of deprivation), and (2) high levels of negative, hostile parenting (a form of threat). The second study of this thesis (CHAPTER 7) drew on a different framework, namely an *imaging genetics* approach, to further understanding of the potential neurobiological pathways by which the serotonin transporter gene might affect risk for depression. **Study 2** specifically investigated whether variation in hippocampus, amygdala, orbitofrontal cortex

(OFC) and anterior cingulate cortex volumes mediated the putative association between 5-HTTLPR genotype and first onset of MDD. Finally, the third study (CHAPTER 9) considered how gene-environment and imaging genetics approaches utilised in **Study 1** and **Study 2** and their separate findings might inform each other to build a more integrated *imaging gene-environment* (IGxE) model for understanding the development of depression. **Study 3** applied an IGxE framework that specifically tested whether variation in parenting moderated the strength of an imaging genetics pathway involving an indirect association between variation in serotonin transporter genotype to variation in hippocampal volume and consequent onset of MDD during adolescence.

This concluding chapter reviews and integrates the key findings and expounds on the discussion sections from each of the three studies in relation to the main aims presented in the Prologue. A critical discussion of key conceptual and methodological limitations is also presented. This chapter also considers potential theoretical and clinical implications of these findings, and suggests further areas of exploration for future research.

10.1 Key Findings and Further Opportunities

10.1.1 **Gene-environment models predicting depression**

Study 1 identified a significant interaction between the serotonin transporter gene and positive parenting that predicted depression in two independent longitudinal cohorts. Findings suggested that adolescents carrying at least one copy of the S-allele showed little variation in their risk for depression as a function of the positive parenting they received, whilst the risk of depression appeared to increase as levels of positive parenting decreased amongst adolescents homozygous for the L-allele. In contrast, negative parenting

behaviours were not found to interact with the serotonin transporter gene to influence depression in either cohort.

For many years, the dominant model of psychopathology was the diathesis-stress model, which theorised that some individuals have a latent (e.g., genetic) predisposition toward particular illnesses, which could be activated under certain conditions (e.g., high stress). In this framework, the S-allele of the serotonin transporter gene had been the designated risk allele, which, in the context of adversity, increased propensity for depression. More recently the differential susceptibility theory was proposed and this framework is gaining increasing traction. The differential susceptibility theory argues individuals vary in their sensitivity to the environment more broadly, with some, including those carrying an S-allele, being sensitive to a range of both positive and negative environmental experiences, whilst others, namely LL-homozygotes, are relatively unaffected by their contexts (Belsky et al., 2009; Belsky & Pluess, 2009). Some though not all meta-analyses suggest that the S-allele may be associated with greater vulnerability to depression in adverse environments (Culverhouse et al., 2017; Karg et al., 2011; Risch et al., 2009; Sharpley et al., 2014). The only meta-analysis that has tested the DSH identified the 'for better and for worse' pattern consistent with this framework, involving the S-allele appearing to increase risk for negative outcomes in negative environments as well being associated with more positive outcomes in propitious environments (van Ijzendoorn et al., 2012). However, individual studies in these meta-analyses show substantial variability in their findings. Indeed, the meta-analysis by Sharpley and colleagues (2014) emphasised that approximately one quarter of studies have failed to identify any significant interactions involving the serotonin transporter gene, whilst a smaller but not insubstantial number of

studies (nearly 10%) have documented a link between the L-allele and depression in certain environments.

The finding in **Study 1** that low levels of positive parenting represented a risk for depression amongst L-homozygous individuals conflicts somewhat with the differential susceptibility hypothesis and adds to the body of research which suggests that the L-allele may confer vulnerability to psychopathology in certain environments. Review of the current available literature (CHAPTER 2) suggested an alternative conceptual framework into which these findings might be placed, which may enhance understanding of this interaction. This framework, referred to as the *differential capability theory*, suggests that it may not be only the S-allele that determines differential sensitivity to varying environments. Rather, both alleles might confer sensitivity to a maladaptive outcome such as depression (as well as potentially positive outcomes), dependent on the match or mismatch of the phenotypic characteristics of the individual and the challenges posed by the particular environment in which they are developing. Specifically, the more emotionally reactive, stress-sensitive S-carriers may be particularly vulnerable to depression in the context of highly threatening environments. In contrast, the putatively emotionally hyporesponsive LL-homozygous individuals who may also possess some relative deficits in executive function relative to their SS or SL counterparts may be at greater risk of depression in more deprived contexts.

Given that this appears to be the first study to explicitly test a differential interaction of this nature, and it was only possible to demonstrate a moderating effect of deprivation but not threat in the same two independent samples, this theory remains speculative. Indeed, CHAPTER 3's review of studies that have considered gene-environment

interactions involving aspects of the family context suggested that interactions involving parenting specifically have not been easily detectable, which is somewhat surprising given parenting is regarded as a particularly proximal and potent influence on child wellbeing, a characteristic that increases its plausibility as a candidate environmental exposure for gene-environment interactions (Moffitt et al., 2005). Moreover, whilst the review in CHAPTER 2 suggested that different patterns of interaction between different combinations of 5-HTTLPR genotypes and dimensions of the environment may be at least partially mediated by variation in emotional reactivity, stress responsivity and executive functioning, this model has not been systematically tested – i.e. the conditional indirect links between the serotonin transporter gene, these traits and onset of depression in different environmental contexts remain inferential as the majority of studies to date have tended to focus their efforts on establishing associations between 5-HTTLPR variation and these characteristics without linking these variables directly to depression (e.g., Borg et al., 2009; Brocke et al., 2006; Gyurak et al., 2013; Papousek et al., 2013). The conceptual and quantitative approaches utilised in **Study 3** (e.g., moderated mediation and path analysis/structural equation modelling) may be helpful in clarifying such a model in future studies.

10.1.2 **Biological pathways from the serotonin transporter gene to depression**

The field of imaging genetics is ultimately concerned with establishing how genetically based variability in the brain affects behaviour. However, to date imaging genetics studies have tended to focus their efforts on establishing associations between genetic polymorphisms and brain structure or activity but have neglected to then also link these variables directly to meaningful differences in behavioural outcomes (as evident in reviews such as the one by Scharinger et al., 2010). Building on previous research

described in CHAPTER 6, **Study 2** found that increasing copies of the 5-HTTLPR S-allele were associated with smaller left hippocampal volume in early adolescence, and that smaller left hippocampal volume in turn prospectively predicted the emergence of a first onset of MDD in later adolescence. A significant indirect (mediation) pathway from 5-HTTLPR genotype to MDD onset via left hippocampal volume was also obtained, indicating that the variance explained by serotonin transporter genotype significantly accounted for variance in the depression outcome. Given that none of the participants had experienced clinical depression at the time of MRI, a smaller left hippocampal volume may represent a pre-existing vulnerability factor rather than a change correlated with active illness.

Extending on the findings of **Study 2**, **Study 3** provided evidence of greater complexity in the pathways from the serotonin transporter gene to first onset of MDD via alterations in hippocampal volume. Specifically, in certain contexts involving either low frequencies of positive parenting or high frequencies of aggressive parental behaviour, increasing S-alleles were associated with smaller hippocampal volumes, and this specific variance in hippocampal volume accounted for increased risk in depression.

Importantly, whilst there had been no evidence in **Study 1** of a statistically significant interaction between the serotonin transporter gene and negative, aggressive parenting on depression, analyses in **Study 3** revealed a significant conditional indirect effect from the genetic polymorphism to depression via its effect on hippocampal volume that was dependent on higher levels of negative parenting. This IGxE finding implicating the S-allele with increased risk for depression in these more threatening contexts would be expected within a diathesis stress paradigm, the differential susceptibility theory and the

differential capability hypothesis, the new conceptual framework proposed by the current thesis. Clarification of the roles that the serotonin transporter gene and negative parenting might play in the emergence of depression thus only became possible once the putative intermediate phenotype of hippocampal volume was included in the model. Given that this study was conducted with a community sample and is based on an observational measure of more normative parenting behaviours (rather than behaviours occurring at the more extreme range, such as those that would be consistent with child maltreatment), it is conceivable that a greater level of threat may be required to reveal the interaction at the behavioural level without the incorporation of such intermediate phenotypes.

Interestingly, whilst in **Study 1** it was the L-allele that was implicated in depression in the contexts of lower positive parenting, in **Study 3** the S-allele was implicated in the conditional indirect pathway from the serotonin transporter gene to depression via hippocampal volume that was dependent on lower levels of positive parenting. It is noteworthy that both the L-allele and the S-allele of the 5-HTTLPR polymorphism have been found by prior research to be associated with hippocampal volume reduction in major depression (Frodl, Meisenzahl, Zill, et al., 2004; Taylor et al., 2005). Moreover, previous studies have also indicated that hippocampal volume may vary according to both reduced maternal positivity, warmth or support (Luby et al., 2012; Schneider et al., 2012) and threatening, aggressive behaviour (Hanson et al., 2015; Teicher, Anderson, & Polcari, 2012). Considering the finding of **Study 1** and the results of these previous studies, a finding that a biological pathway from the S-allele to increased risk for depression via hippocampal volume might be sensitive to the effects of low positive parenting (deprivation) might initially seem to contradict the differential capability theory, which

would rather predict that neurobiological pathways implicating the L-allele should be more vulnerable to the depressogenic effects of low positive parenting whilst neurobiological pathways implicating the S-allele would be relatively unaffected by such environments. One possible explanation for the current finding is that lower levels of positive parenting has capacity to alter risk for depression via different mechanisms. It may be that when no intermediate neural phenotypes are included, the risk relationship that is most clearly apparent is the one that affects L-homozygous individuals via the deprivation mechanism described in detail in CHAPTER 2. In contrast, it is possible that the experience on the continuum of deprivation, including low positive parenting, may also be a stressful experience that is capable of provoking distress and activating the stress response system to some extent in a similar way that threatening experiences, such as high levels of negative, aggressive parenting might do (De Bellis, 2005; Gunnar & Quevedo, 2007). This specific effect of less nurturant care may have particular consequences for S-carriers and may be more easily apparent when the hippocampus, a structure important in regulating the HPA axis, is included as an intermediate phenotype.

By probing the conditional nature of indirect gene-brain-behaviour pathways, **Study 3** thus illustrates how IGxE studies may be able to illuminate complex interrelationships between genes, environment and the brain on behaviour when no direct gene–behaviour or gene x environment interaction–behaviour links are apparent, or when there are seemingly contradictory associations. Indeed, including an objectively measured neural intermediate phenotype such as brain structure in an IGxE framework may increase power to uncover otherwise undetected effects. These IGxE findings thus emphasize the importance of using statistical approaches that can model conditional indirect pathways (Preacher et al., 2007).

Indeed, to further verify the differential capability hypothesis, there is a need for future IGxE research that explores whether environmental deprivation and threat exert clearly distinct influences on neurobiological pathways involving the serotonin transporter gene that predict psychopathology. One possibility is that the specific brain regions that mediate a serotonin transporter gene→depression link might differ depending on whether individuals are exposed to more deprived environments or threatening environments. Alternatively, a deprivation (or threat) exposure→depression link might be accounted for by different brain structures for S carriers versus L-homozygous individuals.

McLaughlin and colleagues (2014; 2017) suggested that early exposure to cognitive and social deprivation may produce neural structures are equipped to deal predominantly with low complexity environments. They anticipate that the impact of such deprivation would be seen in reductions in thickness and volume of the association cortex – namely the areas of cortex that do not have a primary role in processing sensory stimuli or motoric responding but instead become activated during higher-level cognitive processing across different sensory modalities (Goldman-Rakic, 1988; Mountcastle, Lynch, Georgopoulos, Sakata, & Acuna, 1975). In particular, they hypothesised changes in regions of association cortex such as the prefrontal cortex, superior and inferior parietal cortex, and superior temporal cortex that have been implicated in processes such as executive function, social cognition, language and spatial navigation. McLaughlin and colleagues (McLaughlin et al., 2014; McLaughlin et al., 2017) also noted that reduced performance on tasks that rely on these regions would be expected. Given some indication that LL-homozygous individuals may show weaknesses in executive function and social cognition (Borg et al., 2009; Glenn, 2011; Homberg & Lesch, 2011; Tukel et al., 2016), it is interesting to posit a

potentially increased role for a neurobiological pathway involving increasing L-alleles to depression via volume alterations in these specific regions in more deprived contexts. Alternatively, deprivation driven changes in these regions may be more predictive of depression for LL-homozygous individuals than S-allele carriers.

In contrast, McLaughlin and colleagues (2014) have hypothesised that effects of early trauma exposure may be particularly evident in the hippocampus, amygdala and ventromedial prefrontal cortex. It might therefore be anticipated that S-carriers could be particularly vulnerable to depressogenic effects of threat-induced changes in these brain regions. It is interesting to speculate that a particular psychopathology might be underpinned by different brain regions that could share the same genetic correlate. Certainly, the brain does appear to have distinct circuits involved in different functions including the stress-response, fear conditioning, executive functioning, emotion regulation and reward-processing that might be potentially more vulnerable to deprivation or threat experiences. However, many brain regions also form part of multiple circuits, and therefore may be vulnerable to exposure to both deprivation and threat.

Study 2 also considered the potential for other brain regions to act as intermediate phenotypes in a biological pathway from the serotonin transporter gene to depression onset. This study suggested that an increasing number of S-alleles was associated with smaller volumes of the left rostral limbic ACC, and that, in turn, smaller rostral limbic ACC volumes were associated with decreased risk for depression onset (or alternatively that an increasing number of L-alleles was associated with larger volumes, and that, in turn, larger volumes were associated with increased risk for depression onset) at trend level. The indirect mediating pathway from 5-HTTLPR genotype to the left rostral limbic ACC

volume to MDD onset was also marginally significant, indicating that the specific variance in the left rostral ACC explained by serotonin transporter genotype may account for variance in depression onset. Possession of a greater number of S-allele copies also predicted smaller medial OFC volumes bilaterally though neither the left or the right volume showed a prospective association with MDD onset. **Study 2** also did not provide evidence to suggest that the lateral OFC, amygdala, dorsal and ventral regions of the limbic ACC or right rostal region of the limbic AAC might represent intermediate phenotypes between serotonin transporter gene and depression onset. An IGxE approach that includes environmental factors however holds great promise with further clarifying both positive and null findings of imaging genetics research. Indeed, it is possible that effects of the 5-HTTLPR polymorphism on depression via the OFC, ACC and amygdala, the other brain regions considered in **Study 2**, may become apparent with an IGxE framework such as the ones considered in **Study 3**.

10.2 Strengths, Limitations and Opportunities for Future Research

The methodological strengths of the current thesis are its longitudinal nature, the replication of findings in Study 1 across two independent samples, and the assessment of independent variables at an age when depressive symptomatology is relatively low in community samples. Furthermore, in the ADS, the sample considered was restricted to participants who had no history of case-level depression, and all subjects in the ADS underwent careful clinical assessment at multiple time points. MRI data were hand traced using validated methods. In both the ATP and ADS, all of the key variables of interest relied on different informants or assessors, and, in the ADS were assessed according to different methods (DNA sequencing, MRI, observational methods, semi-structured clinical

interview and questionnaire), reducing the likelihood that method invariance might be contributing to significant findings.

Another strength was the focus on specific family environment risk variables, with analyses drawing on theory suggesting their potentially differential effects could reflect different neurodevelopmental consequences of distinct experiences of threat and deprivation. Examining specific, measured environmental risk factors is important given the variation in the current serotonin transporter gene x environment interaction literature, which suggests a need to move away from research that simply aims to identify whether an "overall" GxE interaction effect exists, and to rather identify the sources of variation in findings, including the different environments which confer risk, the mechanisms that underlie effects and the particular sub groups that may show the greatest vulnerability.

There are however also a number of theoretical and methodological limitations in the current thesis, many of which may pose opportunities for future research. These are discussed below.

10.2.1 **Developmental considerations**

Although much of the current thesis focused on the unfolding of depression during the developmental period of adolescence and certain analyses had a longitudinal, prospective design, investigations were not able to address questions regarding the impact of development. Both brain structure (in the ADS) and the family environment (in the ATP and ADS) were measured at one timepoint only, in early adolescence. Depressive symptomatology was also considered at one timepoint, in late adolescence (in the ATP and ADS). The emergence of a first onset of MDD during adolescence in the ADS was

determined based on multiple assessments of depression but analyses did not consider effects of the timing of this emergence (e.g. during early versus late adolescence).

Development is likely to have a critical role in the materialisation of gene–environment–brain–behaviour relationships (Hyde, 2015). Serotonin has a role in basic structural brain development and is also thought to influence the adaptive capacity of the brain throughout the lifespan (e.g., Daubert & Condron, 2010; Gaspar et al., 2003; Lesch & Waider, 2012). Serotonin may also have different effects on different areas of the brain and associated behaviour at different times (Yu et al., 2014), therefore it seems plausible that variation in the serotonin transporter gene may influence outcomes differently depending on developmental stage.

The change in role that variation in brain structure might play across development must also be appreciated. Neuroimaging studies that capture normative brain development show changes in brain structure across the lifespan, including particularly steep growth and then neural pruning during early childhood and adolescence (Giedd et al., 1999; Gogtay et al., 2004; Shaw et al., 2008). Developmental trajectories vary for the different brain regions, and there is also marked heterogeneity in individual developmental trajectories that is not yet well understood. Whilst this thesis did show that there may be differences in hippocampal volume that are predictive of MDD onset, examining brain structure at only one time point during adolescence, a period of significant brain development, means it is not possible to determine whether significant findings in **Study 2** and **Study 3** reflect stable differences present before illness onset or abnormal changes that emerged during early adolescence. Given that further maturation occurs in adolescence, it is also possible that associations between serotonin transporter genotype and specific brain regions or between

these brain regions and depression onset that were not apparent in **Study 2** may become evident later in adolescence or adulthood. Extending the current findings by considering the volume of these brain structures at different timepoints as well as their developmental trajectories would be instructive.

The influence of particular environmental experiences on outcomes may also vary as a function of developmental stage (Sroufe & Rutter, 1984), including whether these experiences occurred during particular "sensitive periods" of development, a stage of amplified brain plasticity where there may be particularly pronounced environmental influences on the shaping of brain structure and function, that in turn affects behaviour across the lifespan (Meaney, 2010). For example, parent-child interactions during early childhood are thought to be of particular importance to the development of the amygdala–medial prefrontal cortex (mPFC) network and to the emergence of cognitive, social, and emotional competencies (Callaghan & Tottenham, 2016). Consideration of parenting in **Study 1** and **Study 3** at an alternative developmental stage to early adolescence, such as early childhood may therefore have produced a different pattern of results.

Issues regarding the timing of measurements is further complicated by the potential presence of "sleeper effects," where the impact of a particular experience on neural systems or behaviour may not be immediately apparent, rather only becoming evident at later developmental time points (Humphreys, Lee, et al., 2014; Zeanah, Gunnar, McCall, Kreppner,&Fox, 2011). The implication is that behavioural or neurological outcomes assessed in earlier stages of development will reveal deficits that unfold in later years. Certainly, in **Study 3**, it is possible that effects of the environment on hippocampal volume, either direct or in interaction with the serotonin transporter gene may not yet have

manifested. Longitudinal applications of imaging genetics studies and investigations examining the role of environmental experiences on the brain will be important in resolving these matters as well as potentially assisting in revealing whether variation in brain structure reflects abnormal neurodevelopmental processes or environment- or experience-induced atrophy.

It is also important to consider the role of development on the clinical or behavioural phenotype of interest; for example, interactions predicting depression may also be more readily detected during adolescence than in childhood, given rates of symptoms and clinical disorder become higher from childhood to adolescence (e.g., Hankin, 1998).

There is therefore a need for studies of longitudinal design that are able to assess IGxE interactions at multiple timepoints across development to reveal further potential nuance in these relationships (i.e., IGxExDevelopment – or "IGxExD"; Hyde, 2015).

10.2.2 **Consideration of gender and ethnicity and related power and methodological issues**

Given suggestions by a small number of studies that findings involving the serotonin transporter gene may differ as a function of sex and/or ethnicity (e.g., Gressier et al., 2016; Perry, Goldstein-Piekarski, & Williams, 2017; van Ijzendoorn et al., 2012), it has been recommended that the influence of these factors should be examined more explicitly in research by including these factors as covariates, testing three-way interactions or conducting stratified grouped analyses (Dunn et al., 2011; Keller, 2014). The current samples however are quite small for genetic analyses and whilst rigorous consideration of potential confounding influences is important, it is also critical to consider whether such analyses might increase the risk of type I or type II errors as a function of decreased power

or model instability. Moreover, assignment of ethnicity based on parents' country of birth in the ATP and grandparents' country of birth in the ADS meant that it was possible that a number of participants' ethnic background may have been incorrectly classified (e.g., in the ATP, a participant who had parents born in Australia, but grandparents born in Asia may have been identified by this system as of 'Anglo/European-Australian' descent). Based on migration patterns to Australia (Coughlan & McNamara, 1997), this number is likely to be very small and it therefore seems very unlikely that coding inaccuracies would have any effect on the current results. Nonetheless, this consideration of ethnicity might be considered less optimal compared to other forms of analyses (such as a SNP panel to identify ancient geographic ancestry).

Given the concerns about sample size and the methodological issue pertaining to the measurement of ethnicity, the current samples were assessed as unsuitable to systematically address questions around differential effects of gender or ethnicity. A decision was therefore use a covariate adjustment approach to manage potential effects of population stratification by gender and ethnicity, which has been maintained in each of the studies. Through the process of editorial review however, there have been some different additional analysis requests across the three studies to assess the role of ethnicity in particular. In **study 1**, an additional set of analyses for both the ATP and ADS samples which limit the samples to those participants of presumed Anglo/European-Australian descent was completed. Only key differences were noted in the manuscript, with full findings contained in supplementary material. In **study 2**, the primary set of analyses presented in the manuscript contained only the independent, mediator and dependent variables, and additional analyses were conducted that included the covariates of adolescent gender and

ethnicity, as well as IQ and age at time of the MRI scan (two additional factors that have been to show relationships with brain development and hence are often adjusted for (Giedd et al., 1999; Gogtay et al., 2004; Shaw et al., 2006). As the pattern of findings were unaltered, these results were not reported in the manuscript. In **study 3,** reviewers requested the use of a particularly stringent method proposed by Keller (2012) to assess the specific possibility that detected interactions might be driven by sex and ethnicity confounding effects rather than by the specified genetic or environmental variables. This method involves entering covariate-by-environment and the covariate-by-gene interaction terms in the same model that tests the G×E term. Given the size of the current sample, it was not feasible to include all gene x covariate and environment x covariate interaction terms simultaneously in the same model (this would have introduced more than 54 new paths, not accounting for correlations with one another). The effect of each interaction (parenting x gender, parenting x ethnicity, 5-HTTLPR x gender, 5-HTTLPR x ethnicity) on results was therefore considered separately. We were also requested to repeat the analyses in the subgroup of participants of presumed Anglo-European background. All of these analyses were presented in supplementary material. The findings of these different analyses have been discussed in the relevant manuscripts and hence will not be repeated here. It seems important however to reiterate concerns about the assessment of the impact of ethnicity and gender in samples that may not be appropriate for addressing such questions (such as the ones in the current thesis) given concerns that psychiatric genetics research may be particularly affected by problems of insufficient power to detect effects and a high false discovery rate. Research employing much larger sample sizes with male and female participants from diverse ethnic backgrounds will be required to provide the power

necessary to elucidate any potential gender and ethnicity differences in the gene-brain-environment-depression relationships tested here. As noted above, ethnicity would ideally be assessed according to a SNP panel to identify ancient geographic ancestry.

It also seems important to note that whilst there have been some suggestions that findings may differ according to ethnic background (specifically that that the interaction may be reversed in African Americans versus Caucasians, such that the L-allele acts as the 'risk' or 'susceptibility' allele in the former group whilst the 'S-allele acts as the 'risk/susceptibility' allele in the latter group, (Anderson & Mayes, 2010; Davies & Cicchetti, 2014; van Ijzendoorn et al., 2012; Williams et al., 2003; Williams et al., 2008), this possibility has not been systematically tested and, perhaps more importantly, no explanation for why this might occur has been proposed. There was no indication of an effect of ethnicity on the significance of findings in the systematic review of studies involving an interaction between 5-HTTLPR and family environment predicting depression outcomes, presented in CHAPTER 3. Although there are certainly differences in the frequency of alleles between ethnicities that may affect the capacity of different studies with different numbers of participants from different ethnic backgrounds to detect the association, there is no clear biological reason as to why a *reversal* in the association might be expected, particularly given the range of genetic differences among individuals *within* ethnic populations is typically far greater than that exists *between* groups (National Research Council Panel on Race, Ethnicity, and Health in Later Life, 2004).

If this phenomenon does in fact exist, one possibility might be that ethnic background may be serving as a proxy for different environments (including different rates of deprivation and threat) between groups rather than reflecting biological differences

(Keller, 2014; Mersha & Abebe, 2015). Certainly, African American children are significantly more likely to live in poverty and attend high-poverty, poorly resourced schools than Caucasian children in the United States of America (Costello, Keeler, & Angold, 2001; National Center for Education Statistics, 2007; Williams & Jackson, 2005). African-Americans also report higher frequencies of experiences of violence, child maltreatment, and crime victimization as well as greater polyvictimization than their Caucasian counterparts (Andrews, Jobe-Shields, et al., 2015; Buka, Stichick, Birdthistle, & Earls, 2001; Roberts, Gilman, Breslau, Breslau, & Koenen, 2011; Williams & Jackson, 2005). It may therefore be instructive for further research that specifically aims to uncover whether ethnicity might moderate the direction of serotonin transporter gene-environment interactions to consider the potential for contamination or confounding from other elements of the environment beyond the environment that is the focus of the particular GxE study.

10.2.3 **Considerations of other genes, brain structures, dimensions of experience and psychiatric outcomes**

The current thesis has aimed to provide an illustration of how gene-environment, imaging genetics and (ultimately) imaging gene-environment studies might provide a more nuanced and complex understanding of psychopathology by uncovering specific underlying mechanisms through which the environment and genetics might get ‘under the skin’ to influence behaviour at the level of the brain. The serotonin transporter gene, parenting, brain (hippocampus, amygdala, OFC and ACC) volume and the outcome of depressive symptomatology and depression onset over adolescence were specifically selected to exemplify how these studies might deepen understanding because there was already a large

body of research suggesting complex interrelationships between these factors but clarification of the particular nature of these associations were still required. As acknowledged in Study 3, it will be important to expand our understanding by applying these models to other genes and other brain structures (as well as brain activity and connectivity) that may play a role in depression, and to also consider the influence of epigenetics.

Moreover, this current thesis has focused on understanding the influence that two particular dimensions of experience, namely threat and deprivation, might have on biological pathways predicting depression, and have attempted to illustrate their potentially unique effects by considering negative parenting and positive parenting specifically. Clearly there are other factors that could have been selected to investigate these two dimensions. There are also almost certainly other dimensions along which experiences can be conceptualised. For example, McLaughlin and colleagues (2014) identify two other dimensions of experience that are worthy of future consideration, namely the extent of *environmental predictability* (the degree to which environments might change from one condition to another) and the *loss of attachment figure* due to occurrences such as parental separations or death (distinguishable from the complete absence of a preferential attachment figure, as is often experienced during institutionalism). These two environmental dimensions are likely to have consequences for neural development that cannot be fully accounted for by either deprivation or threat, and sensitivity to variation in these experiences may be underpinned by different genetic dispositions. Future studies may wish to focus on the identification of other key dimensions of experience and the mechanisms by which they may affect behaviour. Research that expands understanding of

the contribution of different genes, brain regions and environments and their interrelationships will hopefully assist with the identification of specific risk/resilience profiles that represent the cumulative impact of multiple functional polymorphisms, neurobiological circuits and experiences and the ways that they operate together to influence risk for psychopathology.

10.3 Clinical Implications and Opportunities

An important issue for psychiatric research is the extent to which findings might be incorporated into and even transform prevention and clinical treatment of psychiatric disorders. Whilst there are a number of evidence-based treatments available for depression, treatment efficacy arguably remains inadequate as a sizeable minority of patients fail to achieve remission. Approximately 40% of patients continue to experience clinically significant symptoms following a trial of pharmacology, psychological intervention or a combination of the two (Gaynes et al., 2009; Kennard et al., 2006; March et al., 2004; Trivedi et al., 2006).

One of the ultimate goals of psychiatry is the achievement of *precision medicine*, which can obtain a precise diagnosis and identify the most accurate, favourable and arguably cost-effective treatment for an individual, based on their specific unique characteristics, including clinical, genetic, neurobiological and environmental factors (Ozomaro, Wahlestedt, & Nemeroff, 2013). Increasingly, it has been argued that achievement of this goal will require a new approach that moves away from DSM-defined disorders to biologically homogenous treatment-relevant subtypes that may cut across current behavioural diagnoses (Kapur, Phillips, & Insel, 2012). To realise this ambition, an initiative called the Research Domain Criteria (RDoC) project has been developed by the

National Institute of Mental Health (NIMH) in the United States of America (Insel et al., 2010). The RDoC project aims to encourage research that focuses on transdiagnostic dimensional constructs associated with psychiatric conditions but also related to human behaviour more broadly, such as cognitive systems, executive functioning, social processes, positive and negative valence systems and arousal/modulatory systems. The use of multiple methodologies to investigate these constructs as well as a focus on the role of development and environmental influences has been emphasised. It is hoped that such research will ultimately translate into a framework for understanding psychiatric disorders that incorporates both a biological (e.g., genetics, neuroscience) and psychosocial basis (Insel, 2014).

Research that investigates and draws on the differential capability paradigm is arguably consistent with the philosophy of RDoC and is potentially able to contribute to the goal of precision medicine. Proponents of the differential susceptibility theory have argued that in order to more effectively allocate limited resources, it may be appropriate to identify and disproportionately target individuals with more susceptible genotypes (such as S-carriers) for services and intervention as they are likely to be more effective for these individuals relative to others (such as LL-homozygous individuals) (Belsky, 2014). This implies the possibility of discriminatory selective interventions reserved for individuals with certain genotypes. In contrast, a differential capability framework would suggest the need for a plurality of interventions that may be tailored to an individual's needs based on their combination of genes, neurobiology and environmental experiences. It would be expected that certain treatments would vary in effectiveness for individuals depending on the permutation of such factors. In particular, **Study 1** draws attention to the potential

importance of parental warmth and positivity for the subgroup of L-homozygous adolescents and children. It points to the possibility that prevention and intervention efforts that focus on enhancing positive parenting may be of value to LL-homozygous individuals who have experienced less nurturant care. Importantly, this perspective does not suggest, for example, that S carriers do not require positive parenting or that L-homozygous individuals are immune to aggressive, critical parenting, but rather that there may be more homogenous subgroups of individuals within the same diagnosis who possess combinations of genotypes and environments that may be particularly responsive to certain interventions relative to other combinations.

This possibility is important to consider as the field of "therapygenetics," a new line of research focused on the prediction of psychological therapy outcomes based on genetic markers (gene-intervention interactions), gains momentum (Eley, 2014). The field of "therapygenetics" has frequently drawn on a differential susceptibility framework (Bakermans-Kranenburg & IJzendoorn, 2015; Eley, 2014), however, a meta-analysis of seven studies did not provide evidence that the serotonin transporter gene might be a marker of susceptibility to intervention, with no significant differences in response to intervention evident between genotype groups (Bakermans-Kranenburg & IJzendoorn, 2015), suggesting either that responses are similar in S-allele versus L-allele carriers or that a differential effect might be present for both alleles depending on the nature of the intervention, outcomes being considered or the sample at hand.

Indeed, is interesting to note that several of the studies that have identified increased susceptibility of S-allele carriers to interventions have focused on therapies targeted at reducing anxiety (a condition associated with increased vigilance for and over-estimation of

threat) or anxiety-related phenotypes. For example, Eley et al. (2012) identified that children homozygous for the S-allele showed a greater reduction in anxiety response following cognitive behavioural therapy than children of SL or LL genotypes. Knuts et al. (2014) identified a similar finding for cognitive behavioural therapy for agoraphobia. Fox, Zougkou, Ridgewell, and Garner (2011) documented increased response to attention bias modification (a brief intervention that may be effective in reducing anxiety) in functional S-carriers. Cognitive behavioural therapy for depression has been found to be more efficacious for depression in S-allele carriers amongst a sample of participants who had experienced a stroke (Kohen et al., 2011) but not wider sample of depressed adults (Bockting, Mocking, Lok, Koeter, & Schene, 2013), raising questions about whether this differential effect for depression may occur specifically when significant stress or threat (such as that associated with a major medical event) directly precedes the onset of psychological illness.

In contrast, a recent study with pre-school children from lower socioeconomic backgrounds, identified a gene-intervention interaction, which suggested that a family-based training program (which included an emphasis on increasing positive parenting practices and parental responsiveness) could modify neural mechanisms of selective attention (one component of executive functioning) in LL-homozygous children (Isbell et al., 2017). Prior to the intervention, LL-homozygous children had been observed to show attenuated neural responses (event-related brain potentials) for selective attention compared to S-carriers but this difference was eliminated after the intervention. This research group has also reported enhancements in this neural mechanisms selective attention in response to the parent training to co-occur with improvements on standardized measures of nonverbal

intelligence and language and parent reports of child behaviour (Neville et al., 2013), raising questions about whether these improvements might be more pronounced in LL-homozygous children.

Imaging genetics and IGxE research may also be important in revealing underlying neurobiological pathways or mechanisms that explain variation in presentation and response to particular treatments. For example, **Study 2** and **Study 3** suggested that smaller left but larger right hippocampal volume (or asymmetry in hippocampal volume) may represent an endophenotype or premorbid risk factor for depression. Moreover, at least some of variation in hippocampal volume that predicted the emergence of the disorder was accounted for by variation in the serotonin transporter gene but only in adverse environments. Whilst there is a clear need for further verification and clarification of these findings (for example, whether there are deviations in hippocampal volume and/or asymmetry above a certain threshold that are reliably predictive of increased risk, most likely in combination with other variables), they arguably suggest the possibility that with future research, it may be possible to compile a profile of a specific individual's risk for a condition such as depression based on a multi-level analysis of relevant factors, such as their genetic background, brain structure and environmental experiences.

A further important aspect of the current thesis is that whilst variations in serotonin transporter genotype and hippocampal volume were identified as potential risk factors for depression, the effects of these variables were revealed to be dependent on the environment, as was shown specifically in **Study 1** and **Study 3**. These findings are consistent with the proposition that genes may provide a "blueprint" that guides or directs brain development, but that environmental context can influence the implementation of the

blueprint (Macdonald, Goines, Novacek, & Walker, 2016). Heritability therefore does not necessarily equate to determinism. Moreover, the parenting factors that comprised the environment of interest in the current thesis constitutes a potentially modifiable factor (in contrast to experiences such as medical illnesses, natural disasters and even some stressful life events, which arguably are less controllable).

10.4 Conclusion

Debates surrounding the presence of a true GxE effect involving the serotonin transporter gene are ongoing (Caspi et al., 2010; Culverhouse et al., 2017; Karg et al., 2011; Moffitt & Caspi, 2014; Risch et al., 2009; Sharpley et al., 2014). The current thesis suggested that greater clarity regarding the presence of this interaction might be gained from consideration of how different dimensions of environmental experience might interact with the serotonin transporter gene, within a *Differential Capability Framework* that emphasises the importance of a match/mismatch between traits associated with allelic variation and particular environmental challenges. Imaging genetics and IGxE approaches were also found to be informative, and the latter approach in particular revealed further complexity in the relationships between 5-HTTLPR genotype, environmental contexts, brain structure and depression. The prospective longitudinal design of these studies also meant that this thesis could make some contribution towards debate regarding the extent to which abnormalities in certain brain structures implicated in emotional processing and the stress response might represent endophenotypes for depression. Reduced left and larger right hippocampal volume (or possibly asymmetry in hippocampal volume) may constitute premorbid markers for vulnerability to MDD that are present prior to disorder onset during the adolescent period. Importantly, hippocampal volume differences associated with

variation in serotonin transporter genotype were only found to be predictive of MDD onset in adverse family environments, suggesting that if these neuroanatomical variations do represent depression endophenotypes, their 'relevance' to depression may depend on environmental factors.

Although this thesis had a number of limitations, it was strengthened by replication of findings across two independent samples where this was possible (Study 1), the use of a prospective longitudinal design, and a multi-method approach to measurement. The findings offer a number of avenues for further research, including systematic testing of the Differential Capability Hypothesis, exploring the influence of gender and ethnicity on the relationships explored here, as well as considering the role of development and an expanded range of relationships that would incorporate additional genes, brain structures and environments to enhance understanding the emergence of depression, a condition that is widely acknowledged to be multifactorial in its etiology.

APPENDICES

Appendix A: Supplementary Material for Chapter 4

(Paper: Little, K., Olsson, C. A., Whittle, S., MacDonald, J., Sheeber, L. B., Youssef, G. J., . . . Allen, N. B. (accepted). Sometimes it's good to be short: The serotonin transporter gene, positive parenting and adolescent depression. *Child Development*.)

Missing data analysis for Study 1 based on participants from the Australian Temperament Project (ATP)

An investigation of missing data was conducted by comparing (1) those who did (n= 574) and did not (n = 107) provide data on depressive symptoms at 17-18 years, and (2) those whose parents did (n = 612) and did not (n = 69) report on their parenting behavior when their child was 13-14 years, on key variables of interest. There were no differences between these groups on ethnic background, parental ratings of child temperament dimensions of approach-withdrawal, irritability, rhythmicity and activity, nor on a behavior problems composite index assessed in infancy (4-8 months) at the commencement of the study. However, participants for whom data were missing on parenting behavior at 13-14 years were more likely to report higher levels of depressive symptoms at age 17-18 years (effect size: r=.19). Participants for whom data were missing on depressive symptoms also had parents who reported higher use of physical punishment (effect size: r=.27) and lower levels of warmth (effect size: r=.17). These participants were also more likely to be male (effect size: r=.095) and of lower socioeconomic background (effect size: r=.14), which is consistent with the overall pattern of attrition that had occurred in the sample by this age. Critically, these findings suggested that data were missing at random (MAR), such that the missing values on particular variables appeared to be systematically related to variance on other variables (Schlomer, Bauman, & Card, 2010). The relations of these variables to missingness all constituted small effects and did not reach previously cited thresholds for introducing substantial bias (e.g., r>0.40; see Collins, Schafer, & Kam, 2001) suggesting little bias had been introduced to missing data. Nonetheless, missing data that related to gender, parenting and depression were accounted for by the Full Information Maximum Likelihood (FIML) method, to increase statistical power and to make optimal use of the data.

Study 1 Primary Analyses

Supplementary Table 1

Fit Statistics of Path Models for Study 1 and Study 2 Primary Analyses

	χ^2	df	p-value	RMSEA	CFI	SRMR
Study 1						
Dominant	.43	1	.513	.00	1.00	.005
Additive	.43	1	.513	.00	1.00	.004
Study 2						
Dominant						
EPI Task	.00	1	.982	.00	1.00	.00
PSI Task	.00	1	.984	.00	1.00	.00
Additive						
EPI Task	.00	1	.993	.00	1.00	.00
PSI Task	.00	1	.996	.00	1.00	.00

df = degrees of freedom, RMSEA= Root Mean Square Error of Approximation, CFI= Comparative Fit Index SRMR= Standardized Root Mean Square Residual

Supplementary Table 2

Complete findings for the path model testing the interaction between 5-HTTLPR genotype x parental warmth at 13-14 years on depressive symptomatology at 17-18 years in Study 1.

Pathway	b	SE	Lower 95% CI	Upper 95% CI	β	p
5-HTTLPR S-allele Dominant						
5-HTTLPR → Depressive symptoms	-.06	.05	-.16	.04	-.05	.245
Parental warmth → Depressive symptoms	**-.29**	**.07**	**-.43**	**-.14**	**-.29**	**.000**
Physical punishment → Depressive symptoms	.10	.06	-.01	.21	.08	.080
Ethnicity → Depressive symptoms	.16	.15	-.11	.47	.05	.268
Gender → Depressive symptoms	**.34**	**.05**	**.25**	**.44**	**.29**	**.000**
5-HTTLPR X Parental warmth → Depressive symptoms	**.20**	**.09**	**.02**	**.39**	**.16**	**.028**
5-HTTLPR ↔ Parental warmth	.00	.01	-.02	.03	.01	.777
5-HTTLPR ↔ Physical punishment	-.01	.01	-.02	.01	-.02	.551
5-HTTLPR ↔ Gender	-.01	.01	-.03	.01	-.04	.274
5-HTTLPR ↔ Ethnicity	.00	.00	-.01	.00	-.03	.410
5-HTTLPR ↔ 5-HTTLPR X Parental warmth	.00	.01	-.01	.01	.01	.841
Parental warmth ↔ Physical punishment	**-.04**	**.01**	**-.06**	**-.01**	**-.13**	**.008**
Parental warmth ↔ Gender	**.04**	**.01**	**.01**	**.06**	**.12**	**.003**
Parental warmth ↔ Ethnicity	.00	.00	-.01	.00	-.04	.342
Parental warmth ↔ 5-HTTLPR X Parental warmth	**.22**	**.02**	**.19**	**.26**	**.78**	**.000**
Physical Punishment ↔ Gender	**-.03**	**.01**	**-.05**	**-.01**	**-.11**	**.005**
Physical punishment ↔ Ethnicity	.00	.00	-.01	.00	-.04	.068
Physical punishment ↔ 5-HTTLPR X Parental warmth	-.01	.01	-.03	.00	-.06	.104
5-HTTLPR X Parental warmth ↔ Gender	**.03**	**.01**	**.01**	**.05**	**.12**	**.003**
5-HTTLPR X Parental warmth ↔ Ethnicity	.00	.00	-.01	.00	-.05	.243
Simple Slopes:						
LL-homozygous	.29	.07	-.43	-.15	-	.000
S carrier	.08	.05	-.19	.02	-	.126
R^2 = .12						
5-HTTLPR Additive						
5-HTTLPR → Depressive symptoms	-.01	.03	-.08	.06	-.01	.755
Parental warmth → Depressive symptoms	**-.25**	**.07**	**-.38**	**-.11**	**-.25**	**.000**
Physical punishment → Depressive symptoms	.10	.06	-.01	.21	.08	.077
Ethnicity → Depressive symptoms	.16	.15	-.12	.47	.05	.284
Gender → Depressive symptoms	**.35**	**.05**	**.25**	**.44**	**.29**	**.000**
5-HTTLPR X Parental warmth → Depressive symptoms	.11	.07	-.02	.24	.12	.083
5-HTTLPR ↔ Parental warmth	.00	.02	-.03	.04	.00	.939
5-HTTLPR ↔ Physical punishment	-.01	.01	-.03	.02	-.02	.660
5-HTTLPR ↔ Gender	-.01	.01	-.03	.02	-.03	.517
5-HTTLPR ↔ Ethnicity	.00	.01	-.01	.01	-.03	.428
5-HTTLPR ↔ 5-HTTLPR X Parental warmth	-.01	.02	-.05	.04	-.01	.823
Parental warmth ↔ Physical punishment	**-.04**	**.01**	**-.06**	**-.01**	**-.13**	**.008**
Parental warmth ↔ Gender	**.04**	**.01**	**.01**	**.06**	**.12**	**.003**
Parental warmth ↔ Ethnicity	.00	.00	-.01	.00	-.04	.342
Parental warmth ↔ 5-HTTLPR X Parental warmth	**.28**	**.02**	**.23**	**.32**	**.74**	**.000**
Physical Punishment ↔ Gender	**-.03**	**.01**	**-.05**	**-.01**	**-.11**	**.005**
Physical punishment ↔ Ethnicity	.00	.00	-.01	.00	-.04	.068
Physical punishment ↔ 5-HTTLPR X Parental warmth	-.01	.01	-.04	.01	-.04	.302
5-HTTLPR X Parental warmth ↔ Gender	**.03**	**.01**	**.01**	**.06**	**.11**	**.007**
5-HTTLPR X Parental warmth ↔ Ethnicity	-.01	.00	-.01	.00	-.04	.255
R^2 = .11						

Follow-up Analyses

Supplementary Table 3

Path model testing the interaction between 5-HTTLPR genotype x parental use of physical punishment at 13-14 years on depressive symptomatology at 17-18 years in Study 1.

Specified Paths	b	SE	Lower 95% CI	Upper 95% CI	β	*p*
5-HTTLPR S-allele Dominant						
5-HTTLPR → Depressive symptoms	-.05	.05	-.15	.05	-.04	.326
Physical punishment → Depressive symptoms	.12	.11	-.10	.34	.09	.298
Parental warmth → Depressive symptoms	**-.16**	**.05**	**-.25**	**-.07**	**-.16**	**.001**
Ethnicity → Depressive symptoms	.16	.15	-.14	.46	.05	.291
Gender → Depressive symptoms	**.35**	**.05**	**.25**	**.44**	**.29**	**.000**
5-HTTLPR X Physical punishment → Depressive symptoms	-.02	.13	-.28	.24	-.01	.877
5-HTTLPR ↔ Physical punishment	-.01	.01	-.02	.01	-.02	.551
5-HTTLPR ↔ Parental warmth	.00	.01	-.02	.03	.01	.772
5-HTTLPR ↔ Gender	-.01	.01	-.03	.01	-.04	.273
5-HTTLPR ↔ Ethnicity	.00	.00	-.01	.00	-.03	.410
5-HTTLPR ↔ 5-HTTLPR X Physical punishment	.00	.01	-.01	.01	-.01	.731
Physical punishment ↔ Parental warmth	**-.04**	**.01**	**-.06**	**-.01**	**-.13**	**.008**
Physical punishment ↔ Gender	**-.03**	**.01**	**-.05**	**-.01**	**-.11**	**.005**
Physical punishment ↔ Ethnicity	.00	.00	-.01	.00	-.04	.069
Physical punishment ↔ 5-HTTLPR X Physical punishment	.00	.01	-.01	.01	-.01	.731
Parental warmth ↔ Gender	**.04**	**.01**	**.01**	**.06**	**.12**	**.003**
Parental warmth ↔ Ethnicity	.00	.00	-.01	.00	-.04	.344
Parental warmth ↔ 5-HTTLPR X Physical punishment	-.01	.01	-.03	.00	-.06	.104
5-HTTLPR X Physical punishment ↔ Gender	**-.02**	**.01**	**-.04**	**-.01**	**-.12**	**.002**
5-HTTLPR X Physical punishment ↔ Ethnicity	.00	.00	.00	.00	-.02	.234
R^2 = .11						
5-HTTLPR Additive						
5-HTTLPR → Depressive symptoms	-.01	.03	-.08	.06	-.01	.805
Physical punishment → Depressive symptoms	.17	.10	-.03	.36	.13	.098
Parental warmth → Depressive symptoms	**-.15**	**.05**	**-.24**	**-.06**	**-.15**	**.001**
Ethnicity → Depressive symptoms	.16	.15	-.12	.47	.05	.275
Gender → Depressive symptoms	**.35**	**.05**	**.25**	**.45**	**.29**	**.000**
5-HTTLPR X Physical punishment → Depressive symptoms	-.06	.09	-.23	.11	-.06	.456
5-HTTLPR ↔ Physical punishment	-.01	.01	-.03	.02	-.02	.659
5-HTTLPR ↔ Parental warmth	.00	.02	-.03	.04	.00	.938
5-HTTLPR ↔ Gender	-.01	.01	-.03	.02	-.03	.518
5-HTTLPR ↔ Ethnicity	.00	.01	-.01	.01	-.03	.427
5-HTTLPR ↔ 5-HTTLPR X Physical punishment	.00	.02	-.04	.04	-.01	.902
Physical punishment ↔ Parental warmth	**-.04**	**.01**	**-.06**	**-.01**	**-.13**	**.008**
Physical punishment ↔ Gender	**-.03**	**.01**	**-.05**	**-.01**	**-.11**	**.005**
Physical punishment ↔ Ethnicity	.00	.00	-.01	.00	-.04	.068
Physical punishment ↔ 5-HTTLPR X Physical punishment	**.20**	**.03**	**.14**	**.28**	**.78**	**.000**
Parental warmth ↔ Gender	**.04**	**.01**	**.01**	**.06**	**.12**	**.003**
Parental warmth ↔ Ethnicity	.00	.00	-.01	.00	-.04	.342
Parental warmth ↔ 5-HTTLPR X Physical punishment	-.01	.01	-.04	.01	-.04	.303
5-HTTLPR X Physical punishment ↔ Gender	**-.03**	**.01**	**-.05**	**-.01**	**-.12**	**.003**
5-HTTLPR X Physical punishment ↔ Ethnicity	.00	.00	-.01	.00	-.02	.357
R^2 = .11						

Supplementary Table 4

Path model testing the interaction between 5-HTTLPR genotype x parental warmth at 13-14 years on depressive symptomatology at 17-18 years in individuals of Anglo-European background only (n=656) in Study 1.

Pathway	b	SE	Lower 95% CI	Upper 95% CI	β	p
5-HTTLPR S-allele Dominant						
5-HTTLPR → Depressive symptoms	-0.05	0.05	-0.16	0.04	-0.04	0.297
Parental warmth → Depressive symptoms	-0.29	0.08	-0.43	-0.14	-0.29	0.000
Parental punishment → Depressive symptoms	0.09	0.06	-0.01	0.20	0.07	0.090
Gender → Depressive symptoms	0.32	0.05	0.22	0.42	0.27	0.000
5-HTTLPR X Parental warmth → Depressive symptoms	0.19	0.09	0.00	0.37	0.15	0.049
5-HTTLPR ↔Positive parenting	0.01	0.01	-0.02	0.03	0.02	0.707
5-HTTLPR ↔ Parental punishment	-0.01	0.01	-0.03	0.01	-0.03	0.487
5-HTTLPR ↔ Gender	-0.01	0.01	-0.03	0.01	-0.05	0.228
5-HTTLPR ↔ 5-HTTLPR X Parental warmth	0.00	0.01	-0.01	0.01	0.01	0.671
Parental warmth ↔ Parental punishment	-0.04	0.01	-0.07	-0.01	-0.13	0.005
Parental warmth ↔ Gender	0.04	0.01	0.01	0.06	0.12	0.004
Parental warmth ↔ 5-HTTLPR X Parental warmth	0.22	0.02	0.19	0.25	0.78	0.000
Parental punishment ↔ Gender	-0.03	0.01	-0.05	-0.01	-0.11	0.005
Parental punishment ↔ 5-HTTLPR X Parental warmth	-0.02	0.01	-0.03	0.00	-0.07	0.072
5-HTTLPR X Parental warmth ↔ Gender	0.03	0.01	0.01	0.04	0.11	0.010
Simple Slopes:						
LL-homozygous	-0.29	0.08	-0.43	-0.14	-	0.000
S carrier	-0.10	0.06	-0.21	0.01	-	0.085
5-HTTLPR Additive						
5-HTTLPR → Depressive symptoms	-0.01	0.04	-0.08	0.06	-0.01	0.812
Parental warmth → Depressive symptoms	-0.25	0.07	-0.39	-0.11	-0.25	0.000
Parental punishment → Depressive symptoms	0.09	0.06	-0.01	0.21	0.08	0.089
Gender → Depressive symptoms	0.32	0.05	0.23	0.42	0.27	0.000
5-HTTLPR X Parental warmth → Depressive symptoms	0.11	0.07	-0.02	0.23	0.11	0.108
5-HTTLPR ↔Positive parenting	0.00	0.02	-0.03	0.04	0.01	0.890
5-HTTLPR ↔ Parental punishment	-0.01	0.02	-0.04	0.02	-0.03	0.587
5-HTTLPR ↔ Gender	-0.01	0.01	-0.04	0.02	-0.03	0.418
5-HTTLPR ↔ 5-HTTLPR X Parental warmth	0.00	0.02	-0.05	0.04	-0.01	0.896
Parental warmth ↔ Parental punishment	-0.04	0.01	-0.07	-0.01	-0.13	0.005
Parental warmth ↔ Gender	0.04	0.01	0.01	0.06	0.12	0.004
Parental warmth ↔ 5-HTTLPR X Parental warmth	0.28	0.02	0.23	0.32	0.74	0.000
Parental punishment ↔ Gender	-0.03	0.01	-0.05	-0.01	-0.11	0.005
Parental punishment ↔ 5-HTTLPR X Parental warmth	-0.02	0.01	-0.04	0.01	-0.05	0.254
5-HTTLPR X Parental warmth ↔ Gender	0.03	0.01	0.01	0.06	0.10	0.018
R^2 = .10						

Supplementary Table 5

Path model testing the interaction between 5-HTTLPR genotype x parental physical punishment at 13-14 years on depressive symptomatology at 17-18 years in individuals of Anglo-European background only (n=656) in Study 1.

Pathway	b	SE	Lower 95% CI	Upper 95% CI	β	p
5-HTTLPR S-allele Dominant						
5-HTTLPR → Depressive symptoms	-0.04	0.05	-0.15	0.06	-0.03	0.400
Parental warmth → Depressive symptoms	-0.17	0.05	-0.26	-0.08	-0.17	0.000
Parental punishment → Depressive symptoms	0.12	0.11	-0.11	0.33	0.09	0.299
Gender → Depressive symptoms	0.32	0.05	0.23	0.42	0.27	0.000
5-HTTLPR X Physical punishment → Depressive symptoms	-0.02	0.13	-0.28	0.25	-0.02	0.855
5-HTTLPR ↔Positive parenting	0.01	0.01	-0.02	0.03	0.02	0.704
5-HTTLPR ↔ Parental punishment	-0.01	0.01	-0.03	0.01	-0.03	0.487
5-HTTLPR ↔ Gender	-0.01	0.01	-0.03	0.01	-0.05	0.228
5-HTTLPR ↔ 5-HTTLPR X Physical punishment	0.00	0.01	-0.01	0.01	-0.01	0.801
Parental warmth ↔ Parental punishment	-0.04	0.01	-0.07	-0.01	-0.13	0.005
Parental warmth ↔ Gender	0.04	0.01	0.01	0.06	0.12	0.004
Parental warmth ↔ 5-HTTLPR X Physical punishment	-0.02	0.01	-0.03	0.00	-0.07	0.072
Parental punishment ↔ Gender	-0.03	0.01	-0.05	-0.01	-0.11	0.005
Parental punishment ↔ 5-HTTLPR X Physical punishment	0.15	0.02	0.11	0.21	0.82	0.000
5-HTTLPR X Physical punishment ↔ Gender	-0.03	0.01	-0.04	-0.01	-0.13	0.002
r^2=.10						
5-HTTLPR Additive						
5-HTTLPR → Depressive symptoms	-0.01	0.04	-0.07	0.06	-0.01	0.879
Parental warmth → Depressive symptoms	-0.16	0.05	-0.26	-0.07	-0.16	0.001
Parental punishment → Depressive symptoms	0.16	0.10	-0.04	0.34	0.13	0.112
Gender → Depressive symptoms	0.32	0.05	0.23	0.42	0.27	0.000
5-HTTLPR X Physical punishment → Depressive symptoms	-0.06	0.09	-0.23	0.12	-0.06	0.500
5-HTTLPR ↔Positive parenting	0.00	0.02	-0.03	0.04	0.01	0.889
5-HTTLPR ↔ Parental punishment	-0.01	0.02	-0.04	0.02	-0.03	0.586
5-HTTLPR ↔ Gender	-0.01	0.01	-0.04	0.02	-0.03	0.418
5-HTTLPR ↔ 5-HTTLPR X Physical punishment	0.00	0.02	-0.04	0.05	-0.01	0.912
Parental warmth ↔ Parental punishment	-0.04	0.01	-0.07	-0.01	-0.13	0.005
Parental warmth ↔ Gender	0.04	0.01	0.01	0.06	0.12	0.004
Parental warmth ↔ 5-HTTLPR X Physical punishment	-0.02	0.01	-0.04	0.01	-0.04	0.255
Parental punishment ↔ Gender	-0.03	0.01	-0.05	-0.01	-0.11	0.005
Parental punishment ↔ 5-HTTLPR X Physical punishment	0.21	0.04	0.15	0.29	0.78	0.000
5-HTTLPR X Physical punishment ↔ Gender	-0.03	0.01	-0.06	-0.01	-0.12	0.004
r^2=.10						

Supplementary Table 6

Path model testing the interaction between 5-HTTLPR genotype x parental warmth at 13-14 years on depressive symptomatology at 17-18 years, controlling for gender, ethnicity, physical punishment and baseline depressive symptomatology at 13-14 in Study 1.

Pathway	B	SE	Lower 95% CI	Upper 95% CI	β	*p*
5-HTTLPR S-allele Dominant						
5-HTTLPR → Depressive symptoms	-0.03	0.05	-0.12	0.07	-0.02	0.559
Parental warmth → Depressive symptoms	-0.18	0.08	-0.33	-0.03	-0.18	0.015
Physical punishment → Depressive symptoms	0.05	0.05	-0.05	0.16	0.04	0.306
Ethnicity → Depressive symptoms	0.07	0.16	-0.24	0.37	0.02	0.642
Gender → Depressive symptoms	0.28	0.04	0.19	0.37	0.23	0.000
Baseline depressive symptoms → Depressive symptoms	0.07	0.01	0.06	0.09	0.41	0.000
5-HTTLPR X Parental warmth → Depressive symptoms	0.15	0.09	-0.04	0.32	0.11	0.104
5-HTTLPR ↔ Parental warmth	0.00	0.01	-0.02	0.03	0.01	0.786
5-HTTLPR ↔ Physical punishment	-0.01	0.01	-0.02	0.01	-0.02	0.561
5-HTTLPR ↔ Gender	-0.01	0.01	-0.03	0.01	-0.04	0.271
5-HTTLPR ↔ Ethnicity	0.00	0.00	-0.01	0.00	-0.03	0.407
5-HTTLPR ↔ Baseline depressive symptoms	-0.12	0.07	-0.26	0.01	-0.08	0.074
5-HTTLPR ↔ 5-HTTLPR X Parental warmth	0.00	0.01	-0.01	0.01	0.01	0.849
Parental warmth ↔ Physical punishment	-0.04	0.01	-0.06	-0.01	-0.13	0.007
Parental warmth ↔ Gender	0.04	0.01	0.01	0.06	0.12	0.003
Parental warmth ↔ Ethnicity	0.00	0.00	-0.01	0.00	-0.04	0.346
Parental warmth ↔ Baseline depressive symptoms	-0.31	0.10	-0.52	-0.13	-0.15	0.002
Parental warmth ↔ 5-HTTLPR X Parental warmth	0.22	0.02	0.19	0.26	0.78	0.000
Physical Punishment ↔ Gender	-0.03	0.01	-0.05	-0.01	-0.11	0.005
Physical punishment ↔ Ethnicity	0.00	0.00	-0.01	0.00	-0.04	0.068
Physical punishment ↔ Baseline depressive symptoms	0.17	0.08	0.03	0.35	0.11	0.037
Physical punishment ↔ 5-HTTLPR X Parental warmth	-0.01	0.01	-0.03	0.00	-0.06	0.103
5-HTTLPR X Parental warmth ↔ Gender	0.19	0.07	0.05	0.32	0.11	0.005
5-HTTLPR X Parental warmth ↔ Ethnicity	0.05	0.02	0.01	0.11	0.09	0.024
5-HTTLPR X Parental warmth ↔ Baseline depressive symptoms	0.03	0.01	0.01	0.05	0.12	0.003
r^2 = .28						
5-HTTLPR Additive						
5-HTTLPR → Depressive symptoms	-0.01	0.03	-0.07	0.06	-0.01	0.821
Parental warmth → Depressive symptoms	-0.15	0.07	-0.28	-0.01	-0.15	0.028
Physical punishment → Depressive symptoms	0.06	0.05	-0.04	0.16	0.04	0.301
Ethnicity → Depressive symptoms	0.28	0.04	0.20	0.37	0.23	0.000
Gender → Depressive symptoms	0.07	0.16	-0.24	0.37	0.02	0.658
Baseline depressive symptoms Depressive symptoms	0.07	0.01	0.06	0.09	0.41	0.000
5-HTTLPR X Parental warmth → Depressive symptoms	0.07	0.06	-0.04	0.19	0.08	0.200
5-HTTLPR ↔ Parental warmth	0.00	0.02	-0.03	0.04	0.00	0.940
5-HTTLPR ↔ Physical punishment	-0.01	0.01	-0.03	0.02	-0.02	0.664
5-HTTLPR ↔ Gender	-0.01	0.01	-0.04	0.02	-0.03	0.518
5-HTTLPR ↔ Ethnicity	0.00	0.01	-0.01	0.01	-0.03	0.422
5-HTTLPR ↔ Baseline depressive symptoms	-0.06	0.10	-0.25	0.14	-0.02	0.577
5-HTTLPR ↔ 5-HTTLPR X Parental warmth	-0.01	0.02	-0.05	0.04	-0.01	0.822
Parental warmth ↔ Physical punishment	-0.04	0.01	-0.06	-0.01	-0.13	0.007
Parental warmth ↔ Gender	0.04	0.01	0.01	0.06	0.12	0.003
Parental warmth ↔ Ethnicity	0.00	0.00	-0.01	0.00	-0.04	0.346
Parental warmth ↔ Baseline depressive symptoms	-0.31	0.10	-0.52	-0.13	-0.15	0.002
Parental warmth ↔ 5-HTTLPR X Parental warmth	0.28	0.02	0.23	0.32	0.74	0.000
Physical Punishment ↔ Gender	-0.03	0.01	-0.05	-0.01	-0.11	0.005
Physical punishment ↔ Ethnicity	0.00	0.00	-0.01	0.00	-0.04	0.068
Physical punishment ↔ Baseline depressive symptoms	0.17	0.08	0.03	0.35	0.11	0.035
Physical punishment ↔ 5-HTTLPR X Parental warmth	-0.01	0.01	-0.04	0.01	-0.04	0.303
Baseline depressive symptoms ↔ Gender	0.19	0.07	0.05	0.32	0.11	0.005

Baseline depressive symptoms ↔ Ethnicity	0.05	0.02	0.01	0.11	0.09	0.024
5-HTTLPR X Parental warmth ↔ Gender	0.03	0.01	0.01	0.06	0.11	0.007
5-HTTLPR X Parental warmth ↔ Ethnicity	-0.01	0.00	-0.01	0.00	-0.04	0.260
5-HTTLPR X Parental warmth ↔ Baseline depressive symptoms	-0.12	0.09	-0.31	0.04	-0.06	0.168
r^2=.28						

Supplementary Table 7

Path model testing the interaction between 5-HTTLPR genotype x parental physical punishment at 13-14 years on depressive symptomatology at 17-18 years, controlling for gender, ethnicity, physical warmth and baseline depressive symptomatology at 13-14

Pathway	B	SE	Lower 95% CI	Upper 95% CI	β	*P*
5-HTTLPR S-allele Dominant						
5-HTTLPR → Depressive symptoms	-0.02	0.05	-0.11	0.07	-0.02	0.671
Parental warmth → Depressive symptoms	-0.09	0.04	-0.18	-0.01	-0.09	0.034
Physical punishment → Depressive symptoms	0.05	0.10	-0.14	0.27	0.04	0.624
Ethnicity → Depressive symptoms	0.28	0.04	0.20	0.37	0.23	0.000
Gender → Depressive symptoms	0.07	0.16	-0.25	0.37	0.02	0.661
Baseline depressive symptoms Depressive symptoms	0.07	0.01	0.06	0.09	0.42	0.000
5-HTTLPR X Physical punishment → Depressive symptoms	0.02	0.12	-0.23	0.25	0.01	0.901
5-HTTLPR ↔ Parental warmth	0.00	0.01	-0.02	0.03	0.01	0.783
5-HTTLPR ↔ Physical punishment	-0.01	0.01	-0.02	0.01	-0.02	0.560
5-HTTLPR ↔ Gender	-0.01	0.01	-0.03	0.01	-0.04	0.273
5-HTTLPR ↔ Ethnicity	0.00	0.00	-0.01	0.00	-0.03	0.406
5-HTTLPR ↔ Baseline depressive symptoms	-0.12	0.07	-0.25	0.01	-0.08	0.074
5-HTTLPR ↔ 5-HTTLPR X Physical punishment	0.00	0.01	-0.01	0.01	-0.01	0.740
Parental warmth ↔ Physical punishment	-0.04	0.01	-0.06	-0.01	-0.13	0.007
Parental warmth ↔ Gender	0.04	0.01	0.01	0.06	0.12	0.003
Parental warmth ↔ Ethnicity	0.00	0.00	-0.01	0.00	-0.04	0.346
Parental warmth ↔ Baseline depressive symptoms	-0.31	0.10	-0.51	-0.12	-0.15	0.002
Parental warmth ↔ 5-HTTLPR X Physical punishment	-0.01	0.01	-0.03	0.00	-0.06	0.102
Physical Punishment ↔ Gender	-0.03	0.01	-0.05	-0.01	-0.11	0.005
Physical punishment ↔ Ethnicity	0.00	0.00	-0.01	0.00	-0.04	0.068
Physical punishment ↔ Baseline depressive symptoms	0.17	0.08	0.03	0.35	0.11	0.038
Physical punishment ↔ 5-HTTLPR X Physical punishment	0.15	0.02	0.11	0.20	0.82	0.000
Baseline depressive symptoms ↔ Gender	0.19	0.07	0.05	0.32	0.11	0.005
Baseline depressive symptoms ↔ Ethnicity	0.05	0.02	0.01	0.11	0.09	0.024
5-HTTLPR X Physical punishment ↔ Gender	0.07	0.05	-0.03	0.18	0.05	0.201
5-HTTLPR X Physical punishment ↔ Ethnicity	-0.02	0.01	-0.04	-0.01	-0.12	0.002
5-HTTLPR X Physical punishment ↔ Baseline depressive symptoms	0.00	0.00	0.00	0.00	-0.02	0.234
r^2 = .27						
5-HTTLPR Additive						
5-HTTLPR → Depressive symptoms	-0.01	0.03	-0.07	0.06	-0.01	0.858
Parental warmth → Depressive symptoms	-0.09	0.04	-0.17	0.00	-0.09	0.042
Physical punishment → Depressive symptoms	0.11	0.09	-0.07	0.29	0.08	0.253
Ethnicity → Depressive symptoms	0.07	0.16	-0.25	0.37	0.02	0.650
Gender → Depressive symptoms	0.28	0.04	0.20	0.37	0.23	0.000
Baseline depressive symptoms → Depressive symptoms	0.07	0.01	0.06	0.09	0.42	0.000
5-HTTLPR X Physical punishment → Depressive symptoms	-0.05	0.08	-0.20	0.11	-0.05	0.524
5-HTTLPR ↔ Parental warmth	0.00	0.02	-0.03	0.04	0.00	0.939
5-HTTLPR ↔ Physical punishment	-0.01	0.01	-0.03	0.02	-0.02	0.664
5-HTTLPR ↔ Gender	-0.01	0.01	-0.04	0.02	-0.03	0.519
5-HTTLPR ↔ Ethnicity	0.00	0.01	-0.01	0.01	-0.03	0.422
5-HTTLPR ↔ Baseline depressive symptoms	-0.06	0.10	-0.25	0.14	-0.02	0.574
5-HTTLPR ↔ 5-HTTLPR X Physical punishment	0.00	0.02	-0.04	0.05	-0.01	0.905
Parental warmth ↔ Physical punishment	-0.04	0.01	-0.06	-0.01	-0.13	0.007
Parental warmth ↔ Gender	0.04	0.01	0.01	0.06	0.12	0.003
Parental warmth ↔ Ethnicity	0.00	0.00	-0.01	0.00	-0.04	0.345
Parental warmth ↔ Baseline depressive symptoms	-0.31	0.10	-0.52	-0.12	-0.15	0.002
Parental warmth ↔ 5-HTTLPR X Physical punishment	-0.01	0.01	-0.04	0.01	-0.04	0.301
Physical Punishment ↔ Gender	-0.03	0.01	-0.05	-0.01	-0.11	0.005
Physical punishment ↔ Ethnicity	0.00	0.00	-0.01	0.00	-0.04	0.068
Physical punishment ↔ Baseline depressive symptoms	0.17	0.08	0.03	0.35	0.11	0.034
Physical punishment ↔ 5-HTTLPR X Physical punishment	0.20	0.04	0.14	0.28	0.78	0.000

Baseline depressive symptoms ↔ Gender	0.19	0.07	0.05	0.32	0.11	0.005
Baseline depressive symptoms ↔ Ethnicity	0.05	0.02	0.01	0.11	0.09	0.024
5-HTTLPR X Physical punishment ↔ Gender	-0.03	0.01	-0.06	-0.01	-0.12	0.004
5-HTTLPR X Physical punishment ↔ Ethnicity	0.00	0.00	-0.01	0.00	-0.02	0.358
5-HTTLPR X Physical punishment ↔ Baseline depressive symptoms	0.12	0.09	-0.03	0.30	0.06	0.167
r^2=.27						

Study 2 Primary Analyses

Supplementary Table 8

Path model testing the interaction between 5-HTTLPR genotype x positive parenting behavior at 11-13 years on depressive symptomatology at 18-19 years in Study 2.

	EPI Task						PSI Task					
	b	**SE**	**95% CI**		**β**	***p***	**b**	**SE**	**95% CI**		**β**	***p***
			Lower	**Upper**					**Lower**	**Upper**		
5-HTTLPR S-allele Dominant												
5-HTTLPR → Depressive symptoms	1.04	1.61	-2.18	4.19	.05	.520	1.02	1.58	-2.04	4.16	.05	.517
Positive parenting → Depressive symptoms	-5.19	2.39	-10.20	-.68	-.27	.030	-6.28	2.32	-11.36	-2.19	-.46	.007
Aversive parenting → Depressive symptoms	5.31	2.14	1.09	9.52	.23	.013	.80	1.88	-2.96	4.41	.05	.672
Ethnicity → Depressive symptoms	1.28	2.36	-3.33	5.92	.05	.587	1.19	2.35	-3.62	5.67	.04	.614
Gender → Depressive symptoms	1.82	1.54	-1.05	5.01	.10	.238	1.81	1.57	-1.15	5.00	.10	.248
5-HTTLPR X Positive parenting → Depressive symptoms	5.86	3.50	-.93	12.79	.23	.094	6.18	2.87	.58	11.94	.37	.031
5-HTTLPR ↔Positive parenting	.02	.02	-.03	.06	.09	.375	.05	.03	.00	.11	.17	.054
5-HTTLPR ↔ Aversive parenting	-.02	.02	-.05	.02	-.09	.366	-.02	.03	-.07	.03	-.08	.394
5-HTTLPR ↔ Gender	.02	.02	-.02	.05	.08	.285	.02	.02	-.02	.05	.08	.286
5-HTTLPR ↔ Ethnicity	.02	.01	-.01	.04	.10	.161	.02	.01	-.01	.04	.10	.166
5-HTTLPR ↔ 5-HTTLPR X Positive parenting	.01	.01	-.01	.03	.04	.564	.02	.02	-.02	.05	.06	.304
Positive parenting ↔ Aversive parenting	-.06	.02	-.11	-.02	-.31	.006	-.18	.03	-.25	-.12	-.44	.000
Positive parenting ↔ Gender	-.02	.02	-.06	.03	-.07	.421	-.02	.03	-.08	.04	-.05	.560
Positive parenting ↔ Ethnicity	-.02	.02	-.06	.00	-.15	.092	-.03	.03	-.08	.02	-.13	.272
Positive parenting ↔ 5-HTTLPR X Positive parenting	.13	.02	.09	.18	.75	.000	.32	.05	.23	.43	.83	.000
Aversive parenting ↔ Gender	.01	.02	-.02	.05	.06	.488	-.01	.03	-.07	.04	-.05	.601
Aversive parenting ↔ Ethnicity	.00	.02	-.03	.04	.03	.845	.01	.02	-.03	.06	.06	.580
Aversive parenting ↔ 5-HTTLPR X Positive parenting	-.04	.02	-.08	-.01	-.25	.026	-.10	.03	-.17	-.06	-.31	.000
5-HTTLPR X Positive parenting ↔ Gender	-.01	.02	-.04	.03	-.04	.659	-.01	.03	-.05	.05	-.02	.846
5-HTTLPR X Positive parenting ↔ Ethnicity	-.02	.01	-.05	.00	-.18	.120	-.02	.03	-.07	.03	-.12	.390
Simple Slopes:												
LL-homozygous	-	-	-	-	-	-	-6.28	2.54	-11.26	-1.30	-	.014
S carrier	-	-	-	-	-	-	-0.10	1.70	-3.42	3.22	-	.953
			r^2 for EPI = 0.12						r^2 for PSI = 0.09			
5-HTTLPR Additive												
5-HTTLPR → Depressive symptoms	.51	1.12	-1.59	2.81	.04	.647	.56	1.10	-1.51	2.83	.04	.613
Positive parenting → Depressive symptoms	-5.29	2.30	-10.10	-.95	-.28	.022	-5.02	2.24	-9.63	-0.84	-.37	.025
Aversive parenting → Depressive symptoms	5.36	2.16	1.11	9.62	.24	.013	.72	1.84	-3.01	4.21	.05	.694

Ethnicity → Depressive symptoms	1.60	2.40	-3.07	6.34	.06	.506	1.55	2.49	-3.73	6.14	.06	.534
Gender → Depressive symptoms	1.81	1.53	-1.05	4.96	.10	.237	1.91	1.59	-1.09	5.16	.10	.228
5-HTTLPR X Positive parenting → Depressive symptoms	4.68	2.90	-.77	10.66	.25	.106	3.16	2.26	-1.67	7.24	.27	.162
5-HTTLPR ↔Positive parenting	.03	.03	-.04	.09	.08	.405	.07	.05	-.02	.16	.14	.149
5-HTTLPR ↔ Aversive parenting	-.01	.03	-.06	.05	-.03	.806	-.03	.04	-.10	.04	-.07	.392
5-HTTLPR ↔ Gender	.03	.03	-.02	.09	.10	.206	.03	.03	-.02	.09	.10	.206
5-HTTLPR ↔ Ethnicity	.03	.02	.00	.07	.13	.108	.03	.02	.00	.07	.13	.110
5-HTTLPR ↔ 5-HTTLPR X Positive parenting	.01	.04	-.07	.09	.03	.757	.03	.07	-.11	.16	.04	.721
Positive parenting ↔ Aversive parenting	-.06	.02	-.11	-.02	-.32	.006	-.18	.03	-.25	-.12	-.44	.000
Positive parenting ↔ Gender	-.02	.02	-.06	.03	-.07	.427	-.02	.03	-.08	.04	-.05	.567
Positive parenting ↔ Ethnicity	-.02	.02	-.06	.00	-.15	.107	-.03	.03	-.08	.02	-.12	.303
Positive parenting ↔ 5-HTTLPR X Positive parenting	.17	.03	.12	.23	.70	.000	.43	.08	.30	.60	.78	.000
Aversive parenting ↔ Gender	.01	.02	-.02	.05	.07	.480	-.02	.03	-.07	.04	-.05	.597
Aversive parenting ↔ Ethnicity	.00	.02	-.03	.04	.02	.858	.01	.02	-.03	.06	.06	.598
Aversive parenting ↔ 5-HTTLPR X Positive parenting	-.05	.02	-.10	-.01	-.24	.034	-.12	.04	-.20	-.06	-.26	.001
5-HTTLPR X Positive parenting ↔ Gender	-.01	.02	-.05	.04	-.03	.723	-.01	.04	-.08	.06	-.03	.766
5-HTTLPR X Positive parenting ↔ Ethnicity	-.03	.02	-.08	.00	-.21	.072	-.04	.04	-.12	.02	-.17	.219
			r^2 for EPI = 0.13						r^2 for PSI = 0.08			

Supplementary Table 9

Path model testing the interaction between 5-HTTLPR genotype x aversive parenting behavior at 11-13 years on depressive symptomatology at 18-19 years in Study 2.

Specified Paths	EPI Task						PSI Task					
	b	SE	95% CI		β	*p*	b	SE	95% CI		β	*p*
			Lower	Upper					Lower	Upper		
5-HTTLPR S-allele Dominant												
5-HTTLPR → Depressive symptoms	.90	1.65	-2.36	4.15	.05	.584	0.76	1.67	-2.53	4.02	.04	.648
Positive parenting → Depressive symptoms	-1.98	1.93	-6.02	1.63	-.10	.306	-1.49	1.86	-5.21	2.07	-.11	.423
Aversive parenting → Depressive symptoms	4.94	3.81	-2.10	12.92	.22	.196	4.29	3.39	-1.36	12.10	.28	.205
Ethnicity → Depressive symptoms	.77	2.41	-3.95	5.55	.03	.751	1.20	2.46	-3.80	5.90	.04	.626
Gender → Depressive symptoms	1.78	1.55	-1.10	5.02	.10	.251	2.16	1.62	-.86	5.48	.12	.182
5-HTTLPR X Aversive parenting → Depressive symptoms	.78	4.51	-8.40	9.35	.03	.863	-4.02	3.52	-11.38	2.44	-.22	.253
5-HTTLPR ↔Positive parenting	.02	.02	-.03	.06	.09	.374	.05	.03	.00	.11	.17	.053
5-HTTLPR ↔ Aversive parenting	-.02	.02	-.05	.02	-.08	.367	-.02	.03	-.07	.03	-.08	.390
5-HTTLPR ↔ Gender	.02	.02	-.02	.05	.08	.285	.02	.02	-.02	.05	.08	.286
5-HTTLPR ↔ Ethnicity	.02	.01	-.01	.04	.10	.157	.02	.01	-.01	.04	.10	.169
5-HTTLPR ↔ 5-HTTLPR X Aversive parenting	.00	.01	-.02	.02	-.03	.662	-.01	.01	-.04	.02	-.03	.661
Positive parenting ↔ Aversive parenting	-.06	.02	-.11	-.02	-.31	.006	-.18	.03	-.25	-.12	-.44	.000
Positive parenting ↔ Gender	-.02	.02	-.06	.03	-.07	.418	-.02	.03	-.08	.04	-.05	.558
Positive parenting ↔ Ethnicity	-.02	.02	-.06	.00	-.15	.093	-.03	.03	-.08	.02	-.13	.270
Positive parenting ↔ 5-HTTLPR X Aversive parenting	-.04	.02	-.08	-.01	-.24	.023	-.10	.03	-.17	-.06	-.30	.000
Aversive parenting ↔ Gender	.01	.02	-.02	.05	.07	.480	-.01	.03	-.07	.04	-.05	.604
Aversive parenting ↔ Ethnicity	.00	.02	-.03	.04	.03	.847	.01	.02	-.03	.06	.06	.573
Aversive parenting ↔ 5-HTTLPR X Aversive parenting	.11	.02	.08	.16	.81	.000	.26	.04	.19	.34	.85	.000
5-HTTLPR X Aversive parenting ↔ Gender	.00	.02	-.03	.03	.01	.885	.00	.02	-.05	.05	.00	.995
5-HTTLPR X Aversive parenting ↔ Ethnicity	.01	.02	-.02	.05	.10	.545	.01	.02	-.03	.05	.04	.724
			r^2 for EPI =.10						r^2 for PSI =.06			
5-HTTLPR Additive												
5-HTTLPR → Depressive symptoms	.41	1.13	-1.75	2.71	.03	.715	0.34	1.16	-1.85	2.69	.03	.768
Positive parenting → Depressive symptoms	-1.91	1.86	-5.73	1.62	-.10	.306	-1.55	1.79	-5.12	1.91	-.11	.386
Aversive parenting → Depressive symptoms	6.68	3.45	.53	13.93	.29	.053	4.14	2.88	-1.17	10.24	.27	.150
Ethnicity → Depressive symptoms	1.16	2.47	-3.62	6.07	.04	.639	1.49	2.52	-3.47	6.46	.05	.554
Gender → Depressive symptoms	1.78	1.55	-1.11	5.01	.10	.252	2.21	1.62	-0.86	5.55	.12	.173
5-HTTLPR X Positive parenting → Depressive symptoms	-1.40	2.81	-7.26	3.74	-.07	.618	-3.16	2.42	-8.11	1.35	-.22	.193
5-HTTLPR ↔Positive parenting	.03	.03	-.04	.09	.08	.410	.07	.05	-.02	.16	.14	.149
5-HTTLPR ↔ Aversive parenting	-.01	.03	-.06	.05	-.02	.812	-.03	.04	-.10	.04	-.07	.390
5-HTTLPR ↔ Gender	.03	.03	-.02	.09	.10	.206	.03	.03	-.02	.09	.10	.206
5-HTTLPR ↔ Ethnicity	.03	.02	.00	.07	.13	.106	.03	.02	.00	.07	.13	.110
5-HTTLPR ↔ 5-HTTLPR X Positive parenting	.03	.04	-.05	.12	.08	.532	-.02	.05	-.11	.08	-.04	.736

Positive parenting ↔ Aversive parenting	-.06	.02	-.11	-.02	-.32	.006	-.18	.03	-.25	-.12	-.44	.000
Positive parenting ↔ Gender	-.02	.02	-.06	.03	-.07	.426	-.02	.03	-.08	.04	-.05	.567
Positive parenting ↔ Ethnicity	-.02	.02	-.06	.00	-.15	.107	-.03	.03	-.08	.02	-.12	.306
Positive parenting ↔ 5-HTTLPR X Positive parenting	-.05	.02	-.10	-.01	-.21	.029	-.13	.04	-.20	-.06	-.28	.001
Aversive parenting ↔ Gender	.01	.02	-.02	.05	.07	.476	-.02	.03	-.07	.04	-.05	.599
Aversive parenting ↔ Ethnicity	.00	.02	-.03	.04	.02	.860	.01	.02	-.03	.05	.06	.614
Aversive parenting ↔ 5-HTTLPR X Positive parenting	.15	.03	.10	.24	.76	.000	.32	.05	.24	.41	.80	.000
5-HTTLPR X Aversive parenting ↔ Gender	.01	.02	-.03	.05	.03	.786	.00	.03	-.05	.06	.01	.906
5-HTTLPR X Aversive parenting ↔ Ethnicity	.03	.03	-.02	.10	.17	.377	.02	.03	-.03	.07	.08	.510
			r^2 for EPI =.10						r^2 for PSI =.07			

Supplementary Table 10

Path model testing the interaction between 5-HTTLPR genotype x positive parenting behaviour at 11-13 years on depressive symptomatology at 18-19 years in individuals of Anglo-European backgrounds (n=150) in Study 2.

	EPI Task						PSI Task					
	b	**SE**	**95% CI**		**β**	***p***	**b**	**SE**	**95% CI**		**β**	***p***
			Lower	**Upper**					**Lower**	**Upper**		
5-HTTLPR S-allele Dominant												
5-HTTLPR → Depressive symptoms	1.70	1.63	-1.46	4.98	0.08	0.297	1.49	1.68	-1.75	4.88	0.07	0.374
Positive parenting → Depressive symptoms	-4.51	2.46	-9.56	0.16	-0.24	0.066	-6.31	2.51	-12.03	-2.01	-0.45	0.012
Aversive parenting → Depressive symptoms	5.70	2.47	1.20	10.93	0.24	0.021	0.32	2.27	-4.11	4.69	0.02	0.889
Gender → Depressive symptoms	2.31	1.70	-0.90	5.75	0.12	0.175	1.93	1.83	-1.63	5.50	0.10	0.292
5-HTTLPR X Positive parenting → Depressive symptoms	4.99	3.81	-2.70	12.31	0.19	0.190	5.42	3.19	-1.15	11.43	0.31	0.089
5-HTTLPR ↔Positive parenting	0.02	0.03	-0.03	0.07	0.10	0.369	0.05	0.03	-0.01	0.11	0.16	0.095
5-HTTLPR ↔ Aversive parenting	-0.02	0.02	-0.06	0.01	-0.12	0.229	-0.01	0.03	-0.07	0.04	-0.05	0.611
5-HTTLPR ↔ Gender	0.02	0.02	-0.02	0.06	0.08	0.327	0.02	0.02	-0.02	0.06	0.08	0.327
5-HTTLPR ↔ 5-HTTLPR X Positive parenting	0.01	0.01	-0.01	0.03	0.07	0.273	0.02	0.02	-0.01	0.06	0.08	0.222
Positive parenting ↔ Aversive parenting	-0.07	0.02	-0.12	-0.02	-0.34	0.006	-0.18	0.04	-0.26	-0.12	-0.46	0.000
Positive parenting ↔ Gender	-0.02	0.02	-0.07	0.02	-0.10	0.313	-0.04	0.03	-0.11	0.02	-0.13	0.170
Positive parenting ↔ 5-HTTLPR X Positive parenting	0.13	0.02	0.09	0.19	0.73	0.000	0.30	0.05	0.21	0.42	0.82	0.000
Aversive parenting ↔ Gender	0.01	0.02	-0.03	0.04	0.03	0.743	-0.02	0.03	-0.08	0.04	-0.07	0.498
Aversive parenting ↔ 5-HTTLPR X Positive parenting	-0.04	0.02	-0.08	-0.01	-0.27	0.023	-0.11	0.03	-0.18	-0.06	-0.33	0.001
5-HTTLPR X Positive parenting ↔ Gender	-0.01	0.02	-0.04	0.03	-0.05	0.622	-0.02	0.03	-0.07	0.03	-0.08	0.389
			r^2 in EPI =						r^2 in PSI =			
5-HTTLPR S-allele Additive												
5-HTTLPR → Depressive symptoms	1.06	1.19	-1.21	3.47	0.08	0.374	0.95	1.29	-1.42	3.67	0.07	0.460
Positive parenting → Depressive symptoms	-4.98	2.35	-9.89	-0.62	-0.26	0.034	-5.10	2.47	-10.36	-0.58	-0.36	0.039
Aversive parenting → Depressive symptoms	5.63	2.47	1.06	10.67	0.24	0.023	0.27	2.22	-4.23	4.39	0.02	0.905
Gender → Depressive symptoms	2.27	1.67	-0.91	5.63	0.12	0.176	1.98	1.85	-1.59	5.58	0.11	0.286
5-HTTLPR X Positive parenting → Depressive symptoms	4.45	3.16	-1.59	10.84	0.22	0.159	2.50	2.67	-3.34	7.20	0.21	0.349
5-HTTLPR ↔Positive parenting	0.04	0.04	-0.03	0.11	0.11	0.267	0.08	0.05	-0.01	0.18	0.17	0.095
5-HTTLPR ↔ Aversive parenting	-0.03	0.03	-0.08	0.03	-0.09	0.337	-0.03	0.04	-0.10	0.04	-0.07	0.444
5-HTTLPR ↔ Gender	0.03	0.03	-0.03	0.09	0.09	0.289	0.03	0.03	-0.03	0.09	0.09	0.289
5-HTTLPR ↔ 5-HTTLPR X Positive parenting	0.04	0.04	-0.03	0.12	0.13	0.274	0.07	0.07	-0.06	0.21	0.13	0.302
Positive parenting ↔ Aversive parenting	-0.07	0.02	-0.12	-0.02	-0.35	0.006	-0.18	0.04	-0.26	-0.12	-0.46	0.000

Positive parenting ↔ Gender	-0.02	0.02	-0.07	0.02	-0.10	0.303	-0.05	0.03	-0.11	0.02	-0.14	0.160
Positive parenting ↔ 5-HTTLPR X Positive parenting	0.16	0.03	0.11	0.23	0.69	0.000	0.40	0.08	0.28	0.59	0.77	0.000
Aversive parenting ↔ Gender	0.01	0.02	-0.03	0.04	0.04	0.708	-0.02	0.03	-0.08	0.04	-0.07	0.501
Aversive parenting ↔ 5-HTTLPR X Positive parenting	-0.04	0.02	-0.08	-0.01	-0.22	0.025	-0.13	0.04	-0.22	-0.06	-0.27	0.002
5-HTTLPR X Positive parenting ↔ Gender	-0.01	0.02	-0.05	0.04	-0.03	0.722	-0.04	0.04	-0.11	0.04	-0.09	0.330
			r^2 in EPI =						r^2 in PSI =			

Supplementary Table 11

Path model testing the interaction between 5-HTTLPR genotype x aversive parenting behaviour at 11-13 years on depressive symptomatology at 18-19 years in individuals of Anglo-European backgrounds (n=150) in Study 2.

	EPI Task						PSI Task					
	b	**SE**	**95% CI**		**β**	***p***	**b**	**SE**	**95% CI**		**β**	***p***
			Lower	**Upper**					**Lower**	**Upper**		
5-HTTLPR S-allele Dominant												
5-HTTLPR → Depressive symptoms	1.70	1.65	-1.49	5.05	0.08	0.302	1.42	1.70	-1.86	4.80	0.07	0.403
Positive parenting → Depressive symptoms	-1.82	2.13	-6.11	2.33	-0.10	0.393	-2.21	2.24	-6.70	2.10	-0.16	0.323
Aversive parenting → Depressive symptoms	6.62	3.92	-0.17	15.05	0.28	0.091	4.16	3.78	-2.46	12.18	0.26	0.271
Gender → Depressive symptoms	2.26	1.72	-1.04	5.67	0.12	0.190	2.16	1.87	-1.37	5.87	0.11	0.248
5-HTTLPR X Aversive parenting → Depressive symptoms	-1.23	5.23	-11.76	8.24	-0.04	0.814	-4.69	3.80	-12.36	2.47	-0.25	0.217
5-HTTLPR ↔Positive parenting	0.02	0.03	-0.03	0.07	0.10	0.369	0.05	0.03	-0.01	0.11	0.16	0.093
5-HTTLPR ↔ Aversive parenting	-0.02	0.02	-0.06	0.01	-0.12	0.231	-0.01	0.03	-0.07	0.04	-0.05	0.607
5-HTTLPR ↔ Gender	0.02	0.02	-0.02	0.06	0.08	0.327	0.02	0.02	-0.02	0.06	0.08	0.327
5-HTTLPR ↔ 5-HTTLPR X Aversive parenting	-0.01	0.01	-0.03	0.01	-0.06	0.348	-0.01	0.02	-0.04	0.02	-0.04	0.591
Positive parenting ↔ Aversive parenting	-0.07	0.02	-0.12	-0.02	-0.34	0.006	-0.18	0.04	-0.26	-0.12	-0.46	0.000
Positive parenting ↔ Gender	-0.02	0.02	-0.07	0.02	-0.10	0.311	-0.04	0.03	-0.11	0.02	-0.13	0.169
Positive parenting ↔ 5-HTTLPR X Aversive parenting	-0.04	0.02	-0.08	-0.01	-0.25	0.024	-0.11	0.03	-0.17	-0.05	-0.31	0.001
Aversive parenting ↔ Gender	0.01	0.02	-0.03	0.04	0.03	0.734	-0.02	0.03	-0.08	0.04	-0.07	0.499
Aversive parenting ↔ 5-HTTLPR X Aversive parenting	0.09	0.02	0.07	0.13	0.77	0.000	0.25	0.04	0.19	0.35	0.84	0.000
5-HTTLPR X Aversive parenting ↔ Gender	-0.01	0.02	-0.03	0.02	-0.03	0.734	-0.01	0.02	-0.06	0.04	-0.05	0.603
			r^2 in EPI = .11						r^2 in PSI =.08			
5-HTTLPR S-allele Additive												
5-HTTLPR → Depressive symptoms	1.08	1.22	-1.10	3.68	0.08	0.377	0.95	1.29	-1.42	3.67	0.07	0.460
Positive parenting → Depressive symptoms	-1.77	2.05	-5.91	2.19	-0.09	0.389	-5.10	2.47	-10.36	-0.58	-0.36	0.039
Aversive parenting → Depressive symptoms	8.17	3.72	1.81	16.57	0.34	0.028	0.27	2.22	-4.23	4.39	0.02	0.905
Gender → Depressive symptoms	2.18	1.71	-1.11	5.63	0.12	0.202	1.98	1.85	-1.59	5.58	0.11	0.286
5-HTTLPR X Aversive parenting → Depressive symptoms	-3.02	3.61	-10.50	4.00	-0.13	0.404	2.50	2.67	-3.34	7.20	0.21	0.349
5-HTTLPR ↔Positive parenting	0.04	0.04	-0.03	0.11	0.11	0.272	0.08	0.05	-0.01	0.18	0.17	0.095
5-HTTLPR ↔ Aversive parenting	-0.03	0.03	-0.08	0.03	-0.09	0.343	-0.03	0.04	-0.10	0.04	-0.07	0.444
5-HTTLPR ↔ Gender	0.03	0.03	-0.03	0.09	0.09	0.289	0.03	0.03	-0.03	0.09	0.09	0.289
5-HTTLPR ↔ 5-HTTLPR X Aversive parenting	-0.01	0.03	-0.07	0.06	-0.03	0.818	0.07	0.07	-0.06	0.21	0.13	0.302
Positive parenting ↔ Aversive parenting	-0.07	0.02	-0.12	-0.02	-0.35	0.006	-0.18	0.04	-0.26	-0.12	-0.46	0.000

Positive parenting ↔ Gender	-0.02	0.02	-0.07	0.02	-0.10	0.303	-0.05	0.03	-0.11	0.02	-0.14	0.160
Positive parenting ↔ 5-HTTLPR X Aversive parenting	-0.04	0.02	-0.08	-0.01	-0.21	0.025	0.40	0.08	0.28	0.59	0.77	0.000
Aversive parenting ↔ Gender	0.01	0.02	-0.03	0.04	0.04	0.703	-0.02	0.03	-0.08	0.04	-0.07	0.501
Aversive parenting ↔ 5-HTTLPR X Aversive parenting	0.12	0.02	0.08	0.16	0.73	0.000	-0.13	0.04	-0.22	-0.06	-0.27	0.002
5-HTTLPR X Aversive parenting ↔ Gender	-0.01	0.02	-0.05	0.03	-0.03	0.723	-0.04	0.04	-0.11	0.04	-0.09	0.330
			r^2 in EPI =.12						r^2 in PSI =.09			

Supplementary Table 12

Path model testing the interaction between 5-HTTLPR genotype x positive parenting behavior at 11-13 years on depressive symptomatology at 18-19 years, controlling for gender, ethnicity, aversive parenting behavior and baseline depressive symptomatology at 11-13 years in Study 2.

Specified Paths	EPI Task						PSI Task					
	b	**SE**	**95% CI**		**β**	***p***	**b**	**SE**	**95% CI**		**β**	***p***
			Lower	**Upper**					**Lower**	**Upper**		
5-HTTLPR S-allele Dominant												
5-HTTLPR → Depressive symptoms T4	1.04	1.63	-2.16	4.34	0.05	0.523	0.84	1.66	-2.30	4.29	0.04	0.614
Positive parenting → Depressive symptoms T4	-9.21	2.96	-16.00	-3.96	-0.48	0.002	-6.46	2.66	-12.14	-1.73	-0.47	0.015
Aversive parenting → Depressive symptoms T4	3.08	2.08	-0.91	7.17	0.14	0.139	0.12	1.67	-3.09	3.35	0.01	0.943
Gender → Depressive symptoms T4	2.42	1.56	-0.49	5.64	0.13	0.121	2.28	1.58	-0.78	5.39	0.12	0.147
Ethnicity → Depressive symptoms T4	2.02	2.20	-2.34	6.30	0.07	0.358	1.72	2.21	-2.77	5.88	0.06	0.437
Depressive Symptoms T1 → Depressive symptoms T4	0.42	0.10	0.24	0.61	0.43	0.000	0.34	0.10	0.14	0.54	0.34	0.001
5-HTTLPR X Positive parenting → Depressive symptoms T4	11.22	3.75	4.25	19.48	0.44	0.003	6.95	3.10	1.10	13.26	0.42	0.025
5-HTTLPR ↔Positive parenting	0.02	0.02	-0.03	0.07	0.10	0.349	0.05	0.03	0.00	0.11	0.17	0.062
5-HTTLPR ↔ Aversive parenting	-0.02	0.02	-0.05	0.02	-0.08	0.400	-0.02	0.03	-0.07	0.03	-0.07	0.436
5-HTTLPR ↔ Gender	0.02	0.02	-0.02	0.05	0.08	0.284	0.02	0.02	-0.01	0.05	0.08	0.284
5-HTTLPR ↔ Ethnicity	0.02	0.01	-0.01	0.04	0.10	0.159	0.02	0.01	-0.01	0.03	0.10	0.165
5-HTTLPR ↔ Depressive Symptoms T1	-0.06	0.37	-0.79	0.62	-0.01	0.871	-0.11	0.38	-0.90	0.59	-0.03	0.768
5-HTTLPR ↔ 5-HTTLPR X Positive parenting	0.00	0.01	-0.02	0.03	0.03	0.664	0.02	0.02	-0.02	0.05	0.06	0.350
Positive parenting ↔ Aversive parenting	-0.06	0.02	-0.11	-0.02	-0.31	0.006	-0.18	0.03	-0.25	-0.12	-0.44	0.000
Positive parenting ↔ Gender	-0.02	0.02	-0.06	0.02	-0.08	0.351	-0.02	0.03	-0.07	0.05	-0.04	0.626
Positive parenting ↔ Ethnicity	-0.03	0.01	-0.06	0.00	-0.15	0.086	-0.03	0.03	-0.08	0.02	-0.13	0.272
Positive parenting ↔ Depressive Symptoms T1	0.43	0.61	-0.68	1.72	0.09	0.485	-0.75	0.63	-1.96	0.52	-0.12	0.232
Positive parenting ↔ 5-HTTLPR X Positive parenting	0.13	0.02	0.09	0.18	0.74	0.000	0.32	0.05	0.23	0.43	0.83	0.000
Aversive parenting ↔ Gender	0.01	0.02	-0.03	0.05	0.05	0.557	-0.02	0.03	-0.07	0.04	-0.06	0.523
Aversive parenting ↔ Ethnicity	0.00	0.02	-0.03	0.04	0.03	0.840	0.01	0.02	-0.03	0.05	0.06	0.586
Aversive parenting ↔ Depressive symptoms T1	0.57	0.45	-0.32	1.46	0.15	0.209	0.78	0.88	-0.84	2.61	0.14	0.375
Aversive parenting ↔ 5-HTTLPR X Positive parenting	-0.04	0.02	-0.08	-0.01	-0.25	0.028	-0.10	0.03	-0.17	-0.06	-0.31	0.000
Depressive symptoms T1 ↔ Gender	-0.56	0.38	-1.30	0.21	-0.12	0.145	-0.52	0.39	-1.27	0.25	-0.11	0.181
Depressive symptoms T1 ↔ Ethnicity	-0.16	0.17	-0.49	0.18	-0.05	0.346	-0.16	0.18	-0.50	0.19	-0.05	0.366
Depressive symptoms T1 ↔ 5-HTTLPR X Positive parenting	-0.50	0.36	-1.34	0.08	-0.14	0.166	-0.52	0.40	-1.29	0.30	-0.10	0.189
5-HTTLPR X Positive parenting ↔ Gender	0.00	0.02	-0.04	0.03	-0.02	0.785	0.00	0.03	-0.05	0.05	-0.01	0.945
5-HTTLPR X Positive parenting ↔ Ethnicity	-0.02	0.01	-0.05	0.00	-0.18	0.116	-0.02	0.02	-0.07	0.02	-0.12	0.387

Simple Slopes												
LL	-9.21	2.96	-16.00	-3.96	-	0.002	-6.46	2.66	-12.14	-1.73	-	0.015
SL and SS	2.01	2.31	-2.31	6.79	-	0.384	0.48	2.00	-3.39	4.48	-	0.810
			r^2 for the EPI =.26						r^2 for the PSI =.21			
5-HTTLPR Additive												
5-HTTLPR → Depressive symptoms T4	0.57	1.08	-1.38	2.94	0.04	0.597	0.45	1.11	-1.59	2.80	0.03	0.686
Positive parenting → Depressive symptoms T4	-8.41	2.65	-13.99	-3.49	-0.44	0.002	-4.98	2.28	-9.51	-0.57	-0.36	0.029
Aversive parenting → Depressive symptoms T4	3.24	2.11	-0.88	7.36	0.14	0.125	0.05	1.65	-3.28	3.16	0.00	0.975
Gender → Depressive symptoms T4	2.40	1.53	-0.59	5.46	0.13	0.116	2.41	1.60	-0.74	5.51	0.13	0.131
Ethnicity → Depressive symptoms T4	2.40	2.24	-2.04	6.80	0.09	0.284	2.15	2.35	-2.81	6.56	0.08	0.361
Depressive Symptoms T1 → Depressive symptoms T4	0.40	0.09	0.23	0.60	0.41	0.000	0.33	0.11	0.13	0.55	0.34	0.002
5-HTTLPR X Positive parenting → Depressive symptoms T4	7.81	2.88	2.55	13.86	0.41	0.007	3.54	2.18	-0.94	7.57	0.31	0.104
5-HTTLPR ↔Positive parenting	0.03	0.03	-0.04	0.09	0.08	0.397	0.07	0.05	-0.02	0.16	0.14	0.154
5-HTTLPR ↔ Aversive parenting	-0.01	0.03	-0.06	0.05	-0.02	0.815	-0.03	0.04	-0.11	0.04	-0.07	0.408
5-HTTLPR ↔ Gender	0.03	0.03	-0.02	0.09	0.10	0.200	0.03	0.03	-0.02	0.09	0.10	0.201
5-HTTLPR ↔ Ethnicity	0.03	0.02	0.00	0.07	0.13	0.108	0.03	0.02	0.00	0.07	0.13	0.111
5-HTTLPR ↔ Depressive Symptoms T1	-0.24	0.52	-1.30	0.73	-0.03	0.651	-0.33	0.53	-1.43	0.66	-0.05	0.541
5-HTTLPR ↔ 5-HTTLPR X Positive parenting	0.01	0.04	-0.07	0.08	0.03	0.773	0.02	0.07	-0.11	0.16	0.04	0.735
Positive parenting ↔ Aversive parenting	-0.06	0.02	-0.11	-0.02	-0.32	0.006	-0.18	0.03	-0.25	-0.12	-0.44	0.000
Positive parenting ↔ Gender	-0.02	0.02	-0.06	0.02	-0.08	0.362	-0.01	0.03	-0.07	0.05	-0.04	0.643
Positive parenting ↔ Ethnicity	-0.02	0.02	-0.06	0.00	-0.15	0.101	-0.03	0.03	-0.08	0.03	-0.12	0.303
Positive parenting ↔ Depressive Symptoms T1	0.35	0.61	-0.77	1.60	0.07	0.570	-0.86	0.61	-2.05	0.36	-0.13	0.157
Positive parenting ↔ 5-HTTLPR X Positive parenting	0.17	0.03	0.12	0.23	0.70	0.000	0.43	0.08	0.29	0.60	0.78	0.000
Aversive parenting ↔ Gender	0.01	0.02	-0.03	0.05	0.05	0.551	-0.02	0.03	-0.07	0.04	-0.06	0.516
Aversive parenting ↔ Ethnicity	0.00	0.02	-0.03	0.04	0.02	0.854	0.01	0.02	-0.03	0.05	0.06	0.604
Aversive parenting ↔ Depressive symptoms T1	0.61	0.45	-0.22	1.54	0.16	0.171	0.82	0.88	-0.80	2.63	0.14	0.350
Aversive parenting ↔ 5-HTTLPR X Positive parenting	-0.05	0.02	-0.10	-0.01	-0.24	0.036	-0.12	0.04	-0.20	-0.06	-0.26	0.001
Depressive symptoms T1 ↔ Gender	-0.58	0.39	-1.33	0.20	-0.12	0.135	-0.53	0.39	-1.29	0.24	-0.11	0.179
Depressive symptoms T1 ↔ Ethnicity	-0.15	0.17	-0.48	0.18	-0.05	0.356	-0.16	0.17	-0.50	0.19	-0.05	0.358
Depressive symptoms T1 ↔ 5-HTTLPR X Positive parenting	-0.71	0.44	-1.71	0.05	-0.15	0.111	-0.68	0.53	-1.79	0.30	-0.09	0.199
5-HTTLPR X Positive parenting ↔ Gender	0.00	0.02	-0.05	0.04	-0.02	0.860	-0.01	0.04	-0.07	0.07	-0.02	0.854
5-HTTLPR X Positive parenting ↔ Ethnicity	-0.03	0.02	-0.07	0.00	-0.21	0.068	-0.04	0.04	-0.12	0.02	-0.16	0.215
Simple Slopes												
LL	-8.41	2.66	-13.99	-3.49	-	0.002	-	-	-	-	-	-
SL	-0.60	2.00	-4.69	3.34	-	0.763	-	-	-	-	-	-
SS	7.21	4.19	-0.27	15.82	-	0.085	-	-	-	-	-	-
			r^2 for the EPI =.27						r^2 for the PSI =.19			

Supplementary Table 13

Path model testing the interaction between 5-HTTLPR genotype x aversive parenting behavior at 11-13 years on depressive symptomatology at 18-19 years (T4), controlling for gender, ethnicity, positive parenting behavior and baseline depressive symptomatology at 11-13 years (T1) in Study 2.

Specified Paths	EPI Task						PSI Task					
	b	**SE**	**95% CI**		**β**	***p***	**b**	**SE**	**95% CI**		**β**	***p***
			Lower	**Upper**					**Lower**	**Upper**		
5-HTTLPR S-allele Dominant												
5-HTTLPR → Depressive symptoms T4	0.81	1.67	-2.38	4.21	0.04	0.626	0.58	1.72	-2.64	4.08	0.03	0.737
Positive parenting → Depressive symptoms T4	-2.70	1.98	-6.74	1.03	-0.14	0.174	-0.93	1.72	-4.32	2.34	-0.07	0.589
Aversive parenting → Depressive symptoms T4	5.05	4.07	-2.15	13.96	0.22	0.214	5.14	4.62	-3.39	15.29	0.33	0.265
Gender → Depressive symptoms T4	2.17	1.58	-0.86	5.33	0.12	0.169	2.74	1.61	-0.32	6.02	0.15	0.088
Ethnicity → Depressive symptoms T4	1.33	2.32	-3.19	5.93	0.05	0.567	1.80	2.37	-3.01	6.24	0.06	0.447
Depressive Symptoms T1 → Depressive symptoms T4	0.32	0.10	0.14	0.53	0.32	0.002	0.35	0.10	0.14	0.55	0.36	0.001
5-HTTLPR X Aversive parenting → Depressive symptoms T4	-1.95	4.69	-11.69	6.82	-0.07	0.677	-5.94	4.56	-15.75	2.40	-0.33	0.192
5-HTTLPR ↔Positive parenting	0.02	0.02	-0.03	0.07	0.10	0.343	0.05	0.03	0.00	0.11	0.17	0.061
5-HTTLPR ↔ Aversive parenting	-0.02	0.02	-0.05	0.02	-0.08	0.407	-0.02	0.03	-0.07	0.03	-0.07	0.431
5-HTTLPR ↔ Gender	0.02	0.02	-0.01	0.05	0.08	0.284	0.02	0.02	-0.02	0.05	0.08	0.284
5-HTTLPR ↔ Ethnicity	0.02	0.01	-0.01	0.03	0.10	0.156	0.02	0.01	-0.01	0.03	0.10	0.169
5-HTTLPR ↔ Depressive Symptoms T1	-0.10	0.38	-0.86	0.61	-0.02	0.784	-0.10	0.38	-0.87	0.60	-0.02	0.794
5-HTTLPR ↔ 5-HTTLPR X Aversive parenting	0.00	0.01	-0.02	0.02	-0.02	0.794	0.00	0.02	-0.03	0.03	-0.02	0.786
Positive parenting ↔ Aversive parenting	-0.06	0.02	-0.11	-0.02	-0.31	0.006	-0.18	0.03	-0.25	-0.12	-0.44	0.000
Positive parenting ↔ Gender	-0.02	0.02	-0.07	0.02	-0.08	0.339	-0.02	0.03	-0.07	0.05	-0.04	0.628
Positive parenting ↔ Ethnicity	-0.02	0.01	-0.06	0.00	-0.15	0.089	-0.03	0.03	-0.08	0.02	-0.13	0.271
Positive parenting ↔ Depressive Symptoms T1	0.45	0.64	-0.68	1.80	0.10	0.484	-0.77	0.60	-1.92	0.45	-0.12	0.199
Positive parenting ↔ 5-HTTLPR X Aversive parenting	-0.04	0.02	-0.08	-0.01	-0.23	0.027	-0.10	0.03	-0.17	-0.05	-0.30	0.000
Aversive parenting ↔ Gender	0.01	0.02	-0.03	0.05	0.05	0.551	-0.02	0.03	-0.07	0.04	-0.06	0.529
Aversive parenting ↔ Ethnicity	0.00	0.02	-0.03	0.04	0.03	0.842	0.01	0.02	-0.03	0.05	0.06	0.579
Aversive parenting ↔ Depressive symptoms T1	0.60	0.47	-0.34	1.56	0.15	0.208	0.77	0.88	-0.87	2.59	0.13	0.385
Aversive parenting ↔ 5-HTTLPR X Aversive parenting	0.11	0.02	0.07	0.15	0.81	0.000	0.26	0.04	0.19	0.34	0.85	0.000
Depressive symptoms T1 ↔ Gender	-0.47	0.39	-1.22	0.29	-0.10	0.227	-0.51	0.40	-1.27	0.28	-0.11	0.201
Depressive symptoms T1 ↔ Ethnicity	-0.19	0.17	-0.54	0.15	-0.06	0.286	-0.16	0.18	-0.50	0.19	-0.05	0.363
Depressive symptoms T1 ↔ 5-HTTLPR X Aversive parenting	0.66	0.36	0.02	1.45	0.21	0.065	0.89	0.71	-0.28	2.51	0.18	0.210
5-HTTLPR X Aversive parenting ↔ Gender	0.00	0.02	-0.03	0.03	-0.01	0.959	-0.01	0.02	-0.05	0.04	-0.02	0.835
5-HTTLPR X Aversive parenting ↔ Ethnicity	0.01	0.02	-0.02	0.05	0.10	0.539	0.01	0.02	-0.03	0.05	0.04	0.733

			r^2 for the EPI =.19						r^2 for the PSI =.18			
5-HTTLPR S-allele Additive												
5-HTTLPR → Depressive symptoms T4	0.43	1.10	-1.60	2.77	0.03	0.693	0.21	1.15	-1.97	2.63	0.02	0.854
Positive parenting → Depressive symptoms T4	-2.65	1.92	-6.67	0.94	-0.14	0.168	-1.04	1.65	-4.32	2.08	-0.08	0.529
Aversive parenting → Depressive symptoms T4	5.91	3.48	-0.41	13.37	0.26	0.089	4.18	3.49	-2.68	11.05	0.27	0.231
Gender → Depressive symptoms T4	2.17	1.57	-0.90	5.31	0.12	0.168	2.75	1.63	-0.45	5.99	0.15	0.091
Ethnicity → Depressive symptoms T4	1.79	2.37	-2.80	6.55	0.06	0.451	2.15	2.43	-2.76	6.86	0.08	0.375
Depressive Symptoms T1 → Depressive symptoms T4	0.32	0.10	0.14	0.53	0.33	0.001	0.34	0.10	0.13	0.54	0.34	0.001
5-HTTLPR X Aversive parenting → Depressive symptoms T4	-2.49	2.68	-7.92	2.55	-0.13	0.353	-3.84	2.78	-9.66	1.25	-0.27	0.167
5-HTTLPR ↔Positive parenting	0.03	0.03	-0.04	0.09	0.08	0.400	0.07	0.05	-0.02	0.16	0.14	0.155
5-HTTLPR ↔ Aversive parenting	-0.01	0.03	-0.06	0.05	-0.02	0.826	-0.03	0.04	-0.11	0.04	-0.07	0.406
5-HTTLPR ↔ Gender	0.03	0.03	-0.02	0.09	0.10	0.201	0.03	0.03	-0.02	0.09	0.10	0.201
5-HTTLPR ↔ Ethnicity	0.03	0.02	0.00	0.07	0.13	0.106	0.03	0.02	-0.01	0.07	0.13	0.110
5-HTTLPR ↔ Depressive Symptoms T1	-0.31	0.53	-1.38	0.68	-0.05	0.558	-0.34	0.53	-1.43	0.64	-0.05	0.528
5-HTTLPR ↔ 5-HTTLPR X Aversive parenting	0.03	0.04	-0.05	0.12	0.08	0.519	-0.02	0.05	-0.11	0.08	-0.03	0.757
Positive parenting ↔ Aversive parenting	-0.06	0.02	-0.11	-0.02	-0.32	0.006	-0.18	0.03	-0.25	-0.12	-0.44	0.000
Positive parenting ↔ Gender	-0.02	0.02	-0.06	0.02	-0.08	0.350	-0.01	0.03	-0.07	0.05	-0.04	0.645
Positive parenting ↔ Ethnicity	-0.02	0.02	-0.06	0.00	-0.15	0.104	-0.03	0.03	-0.08	0.03	-0.12	0.307
Positive parenting ↔ Depressive Symptoms T1	0.38	0.64	-0.78	1.72	0.08	0.548	-0.87	0.60	-2.06	0.32	-0.14	0.147
Positive parenting ↔ 5-HTTLPR X Aversive parenting	-0.05	0.02	-0.10	-0.01	-0.21	0.033	-0.12	0.04	-0.20	-0.06	-0.28	0.001
Aversive parenting ↔ Gender	0.01	0.02	-0.03	0.05	0.05	0.554	-0.02	0.03	-0.07	0.04	-0.06	0.521
Aversive parenting ↔ Ethnicity	0.00	0.02	-0.03	0.04	0.03	0.853	0.01	0.02	-0.03	0.05	0.06	0.620
Aversive parenting ↔ Depressive symptoms T1	0.65	0.47	-0.24	1.64	0.17	0.168	0.80	0.89	-0.83	2.64	0.14	0.367
Aversive parenting ↔ 5-HTTLPR X Aversive parenting	0.15	0.04	0.09	0.23	0.76	0.000	0.32	0.05	0.24	0.41	0.80	0.000
Depressive symptoms T1 ↔ Gender	-0.47	0.39	-1.22	0.31	-0.10	0.231	-0.51	0.40	-1.26	0.29	-0.11	0.199
Depressive symptoms T1 ↔ Ethnicity	-0.20	0.17	-0.56	0.13	-0.06	0.254	-0.17	0.18	-0.51	0.18	-0.05	0.341
Depressive symptoms T1 ↔ 5-HTTLPR X Aversive parenting	0.84	0.48	0.00	1.87	0.19	0.077	0.87	0.78	-0.51	2.56	0.14	0.267
5-HTTLPR X Aversive parenting ↔ Gender	0.00	0.02	-0.04	0.04	0.01	0.928	0.00	0.03	-0.06	0.06	0.00	0.965
5-HTTLPR X Aversive parenting ↔ Ethnicity	0.03	0.03	-0.02	0.10	0.17	0.372	0.02	0.03	-0.03	0.07	0.08	0.508
			r^2 for the EPI =.19						r^2 for the PSI =.18			

Appendix B: Supplementary Material for Chapter 7

(Paper: Little, K., Olsson, C. A., Whittle, S., Youssef, G. J., Byrne, M. L., Simmons, J. G., . . . Allen, N. B. (2014). Association between serotonin transporter genotype, brain structure and adolescent-onset major depressive disorder: A longitudinal prospective study. *Translational Psychiatry, 4*, e445. doi:10.1038/tp.2014.85)

Little *et al.* Association between Serotonin Transporter Genotype, Brain Structure and Adolescent Onset Major Depressive Disorder: A Longitudinal Prospective Study

Supplementary Table 1. Specific lifetime psychopathology recorded at completed assessments

	Overall (N=174)	**MDD onset (n=36)**	**No MDD onset (n=101)**	**MDD onset undetermined (missing) (n=37)**
No other diagnosis given*	87	6	67	20
Depression NOS	7	4	3	0
Adjustment disorder	11	6	3	2
PTSD/ASD	6	5	0	1
GAD	7	4	2	1
Social Phobia	20	7	9	4
Specific Phobia	17	10	7	0
Separation Anxiety	3	1	1	1
OCD	5	1	2	2
Panic Disorder	1	1	0	0
ODD	9	2	5	2
Conduct Disorder	12	4	2	6
Attention Deficit Disorders	6	1	0	5
Alcohol Use Disorder	23	8	12	3
Substance Use Disorder	12	8	3	1
Enuresis	5	1	2	2
Eating Disorder	5	2	3	0

GAD, generalized anxiety disorder; NOS, not otherwise specified; OCD, obsessive- compulsive disorder; ODD, oppositional defiant disorder; PTSD, posttraumatic stress disorder; ASD, Acute Stress Disorder

* Except for Major Depressive Disorder in the MDD onset group

Supplementary Table 2. Intra- and Inter-Class Correlation Coefficients for all ROIs

	Intra		Inter	
	Left	**Right**	**Left**	**Right**
Hippocampus	.95	.98	.91	.92
Amygdala	.97	.93	.88	.85
Medial OFC	.77	.78	.76	.77
Lateral OFC	.95	.95	.98	.98
Limbic ACC	.98	.96	.96	.93
Rostral Limbic	.99	.99	.97	.99
Ventral Limbic	.97	.94	.96	.98
Dorsal Limbic	.98	.91	.95	.83
Paralimbic ACC	.89	.92	.90	.92
Rostral Paralimbic	.82	.89	.94	.95
Ventral Paralimbic	.90	.91	.82	.91
Dorsal Paralimbic	.97	.95	.94	.91

Little *et al*. Association between Serotonin Transporter Genotype, Brain Structure and Adolescent Onset Major Depressive Disorder: A Longitudinal Prospective Study

Supplementary Methods: Details regarding image acquisition, image pre-processing and tracing protocols for morphometric analysis

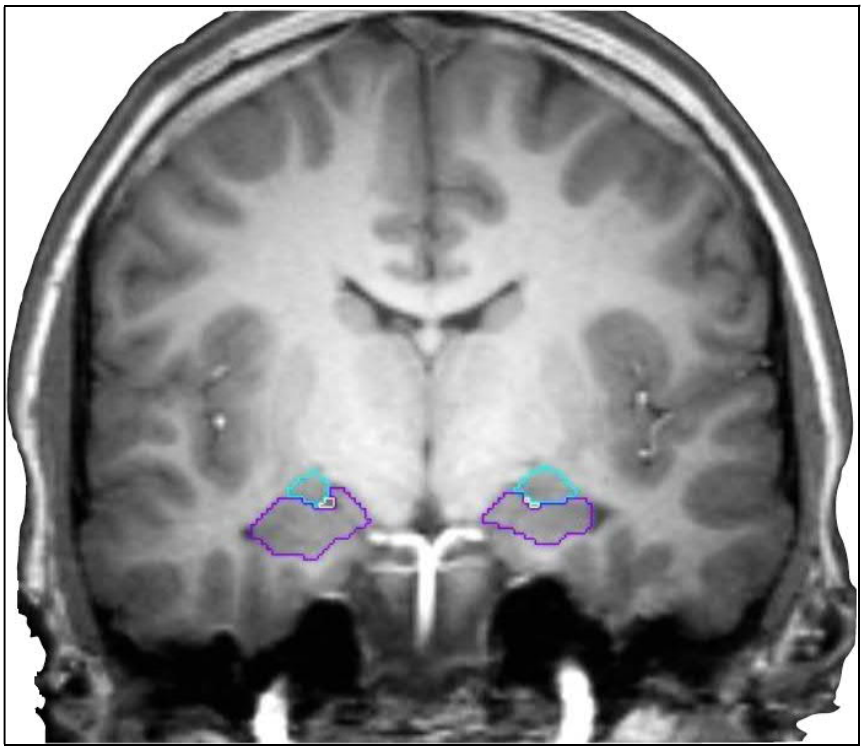
Image acquisition. MRI's were performed on a 3 Tesla GE scanner at the Brain Research Institute, Austin and Repatriation Medical Centre, Melbourne, Australia, using a gradient echo volumetric acquisition sequence (repetition time =36 ms; echo time =9 ms; flip angle =358, field of view=20cm^2, pixel matrix =410×410) to obtain 124 T1-weighted contiguous 1.5 mm-thick slices (voxel dimensions =0.4883×0.4883×1.5mm).

Image pre-processing. Images were transferred to a SGI/Linux workstation for morphometric analysis. Image pre-processing was carried out using tools from the FMRIB software library (http://www.frmib.ox.ac.uk/fsl). Each 3D scan was stripped of all non-brain tissue (1), and aligned to the MNI 152 average template (six-parameter rigid body transform with trilinear interpolation) using FLIRT (2). This registration served to align each image axially along the anterior commissure–posterior commissure (AC–PC) plane and sagittally along the interhemispheric fissure without any deformation. Images were re-sampled to 1mm^3 and then converted to 1cm^3.

Morphometric analysis. All ROIs were traced using the software package ANALYZE (Mayo Clinic, Rochester, USA; http://www.mayo.edu/bir/). Brain tissue was segmented into grey matter, white matter, and cerebrospinal fluid using an automated algorithm, as implemented in FAST (3). An estimate of whole brain volume (WBV) was obtained by summing gray and white matter pixel counts (i.e. WBV included cerebral

gray and white matter, the cerebellum and brainstem, but not the ventricles, cisterns or cerebrospinal fluid). OFC and ACC estimates were based on gray matter pixel counts contained within the defined ROIs, whilst amygdala and hippocampal estimates were based on total voxels within the defined ROI (described below).

The guidelines for tracing the amygdala and hippocampus were adapted from those described by Velakoulis and colleagues (4, 5). Adaptations, designed to maximize reliability, relate to marking the anterior boundary of the amygdala and the boundary between the amygdala and hippocampus. The amygdala and hippocampus were separated according to Watson et al.'s (6) protocol (see Supplementary Figure 1).



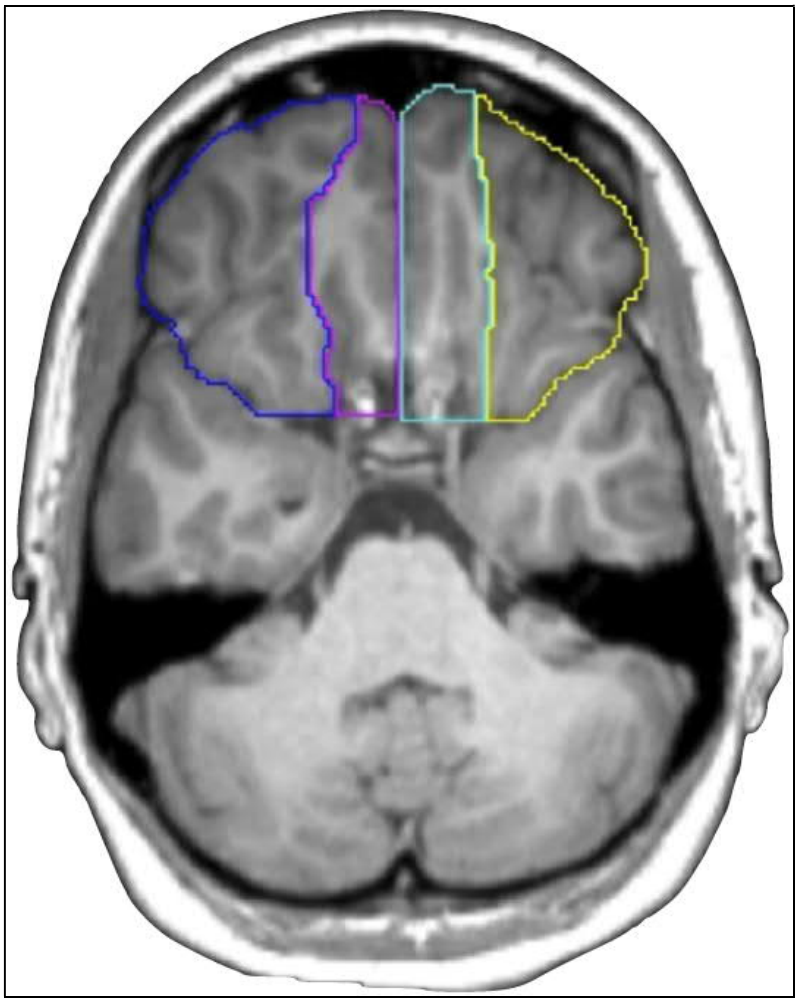
Supplementary Figure 1. Example of manual delineation of bilateral amygdalae (light blue) and hippocampi (purple) on a coronal MR image.

Hippocampal tracings comprised the hippocampus proper, the dendate gyrus, the subiculum, and part of the fimbria and alveus. The posterior border was defined as the section with the greatest length of continuous fornix. The lateral boundary was marked by the temporal horn of the lateral ventricle. The medial boundary was classified by the open end of the hippocampal fissure posteriorly, and by the uncal fissure anteriorly. The superior boundary was defined posteriorly by the fimbria and alveus (which were included in the tracing), and anteriorly by the amygdala.

The posterior boundary of the amygdala was classified according to the first appearance of amygdala gray matter above the temporal horn. The lateral border was marked superiorly by the thin strip of white matter separating the amygdala from the claustrum and tail of the caudate, and inferiorly by the temporal stem and extension of the temporal horn. The medial border was marked superiorly by the semilunar gyrus, and inferiorly by subamygdaloid white matter, which separates the amygdala from the entorhinal cortex. The anterior boundary was identified by the joining of the optic chiasm or the point where the lateral sulcus closes to form the endorhinal sulcus (whichever was more posterior).

The boundaries of the OFC were defined according to a previously published method by Riffkin et al. (7). A line through the AC-PC was drawn to define the superior boundary of the OFC. The posterior border was marked by a coronal plane passing through the most posterior aspect of the olfactory sulcus in each hemisphere. All images were manually edited to eliminate subcortical tissue and artifacts related to the eye sockets and nasal bones. In accordance with Bartholomeusz et al. (8), medial and lateral OFC regions were divided by the first prominent sulcus lateral to the olfactory sulcus

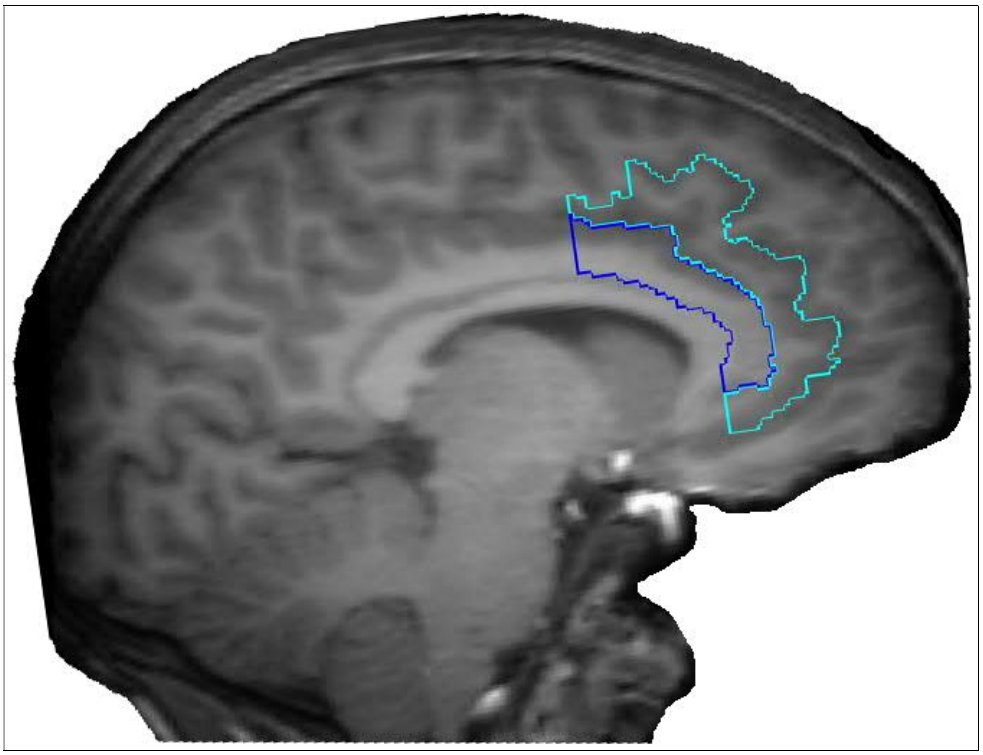
(which in most cases is the medial orbital sulcus) (9). This sulcus was first identified and marked in the coronal plane, with subsequent editing conducted in the transverse plane.



Supplementary Figure 2. Example of manual delineation of the orbitofrontal cortex (OFC) on an axial MR image. The right lateral and right medial regions are highlighted in dark blue and pink, respectively (forming the right OFC), whereas the left lateral and left medial regions are highlighted in yellow and green, respectively (forming the left OFC).

The boundaries of the ACC were based on a previously published method (10), which defines separate limbic and paralimbic regions according to individual differences in the morphology of the cingulate, paracingulate and superior rostral sulci. Briefly, the limbic ACC contained all gray matter in the gyrus bound by the callosal sulcus and the

cingulate sulcus. The paralimbic ACC contained all gray matter in the gyrus bound by the cingulate sulcus and paracingulate sulcus, except in cases where the paracingulate sulcus was absent, for which the paralimbic ACC contained only the gray matter on the upper bank of the cingulate sulcus.



Supplementary Figure 3. Example of manual delineation of limbic (dark blue) and paralimbic (light blue) divisions, as a function of sulcal variability in the anterior cingulate cortex, on a sagittal MR image.

Interrater and intrarater reliabilities were assessed by means of the intraclass correlation coefficient (absolute agreement) using 10 brain images from a separate magnetic resonance imaging database established for this purpose. Intraclass correlation coefficient values were deemed acceptable for all ROIs (29 of 36 the ROIs were <0.90 and none<0.75). All brain structural measures were corrected for whole-brain size

separately by gender by means of a covariance adjustment method (11) and converted from mm^3 to cm^3.

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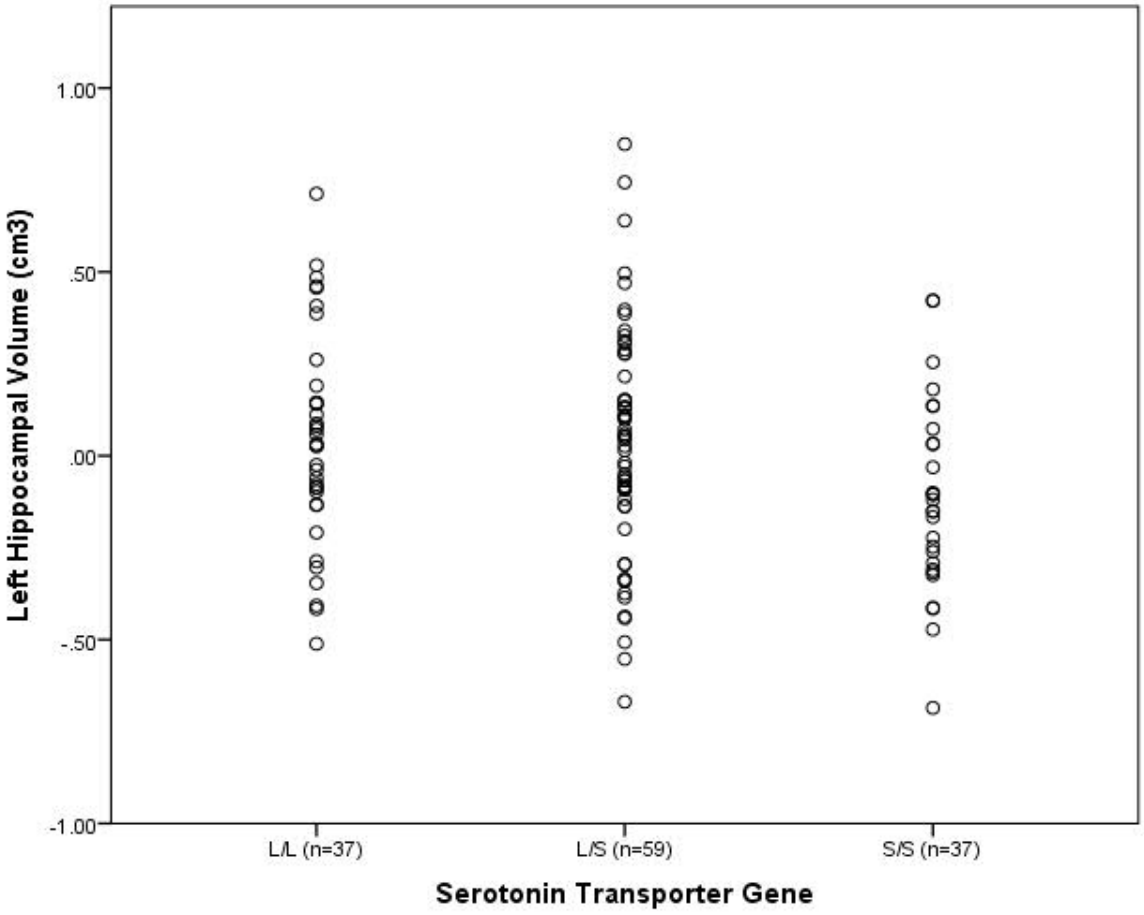
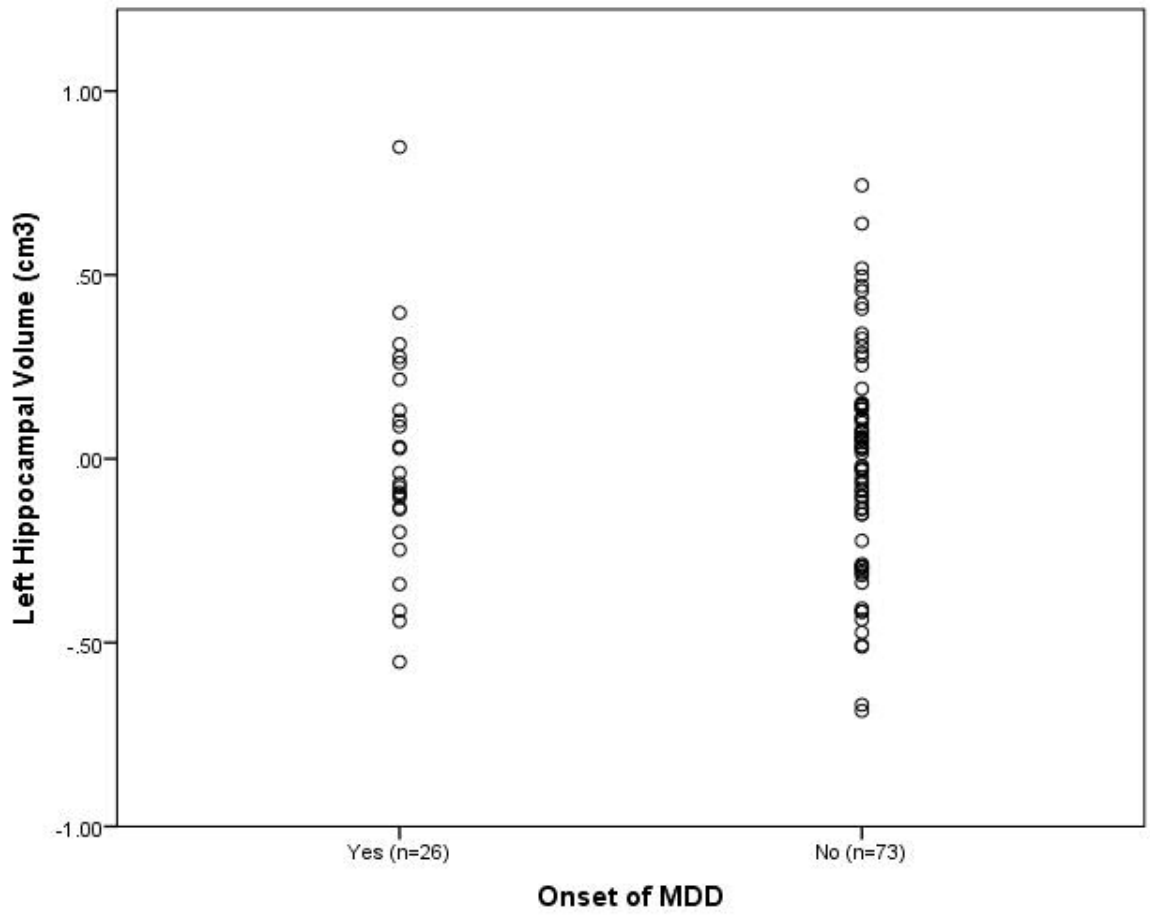
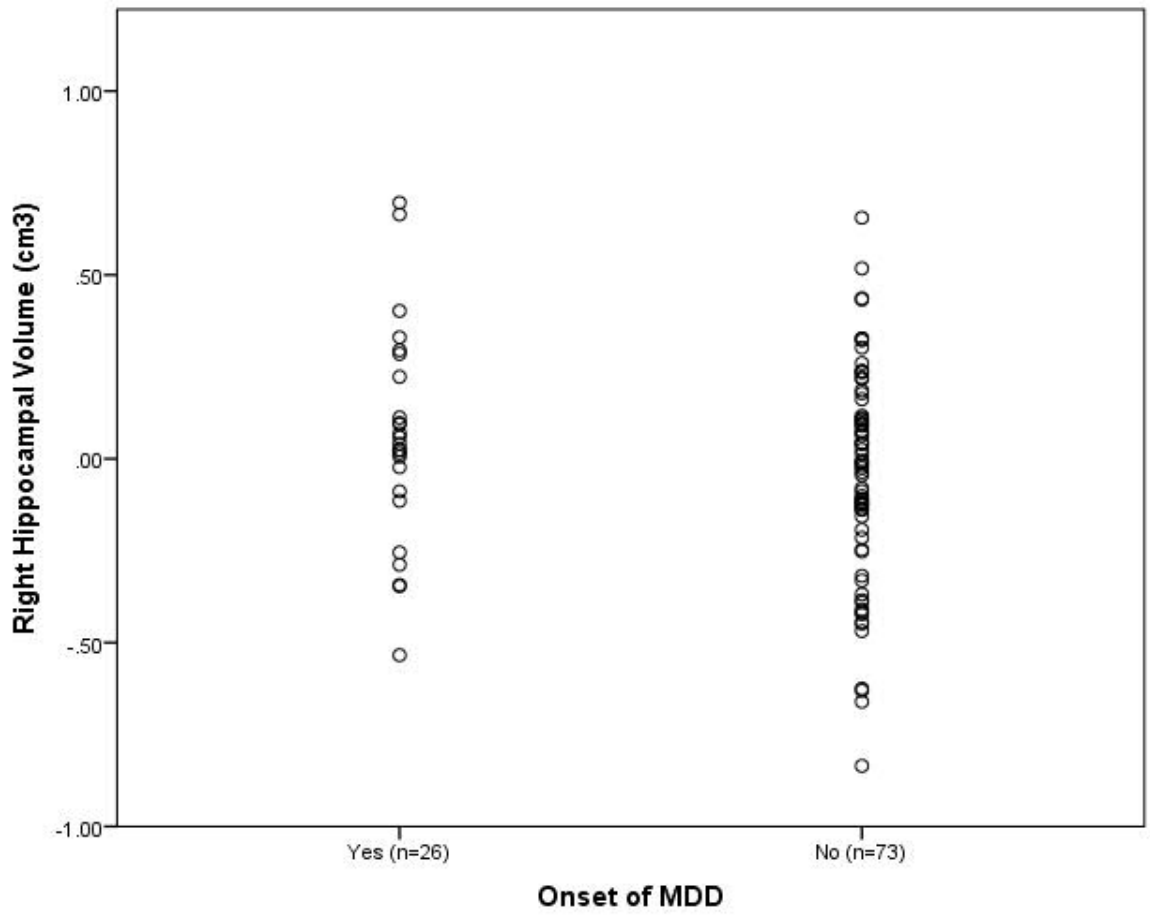


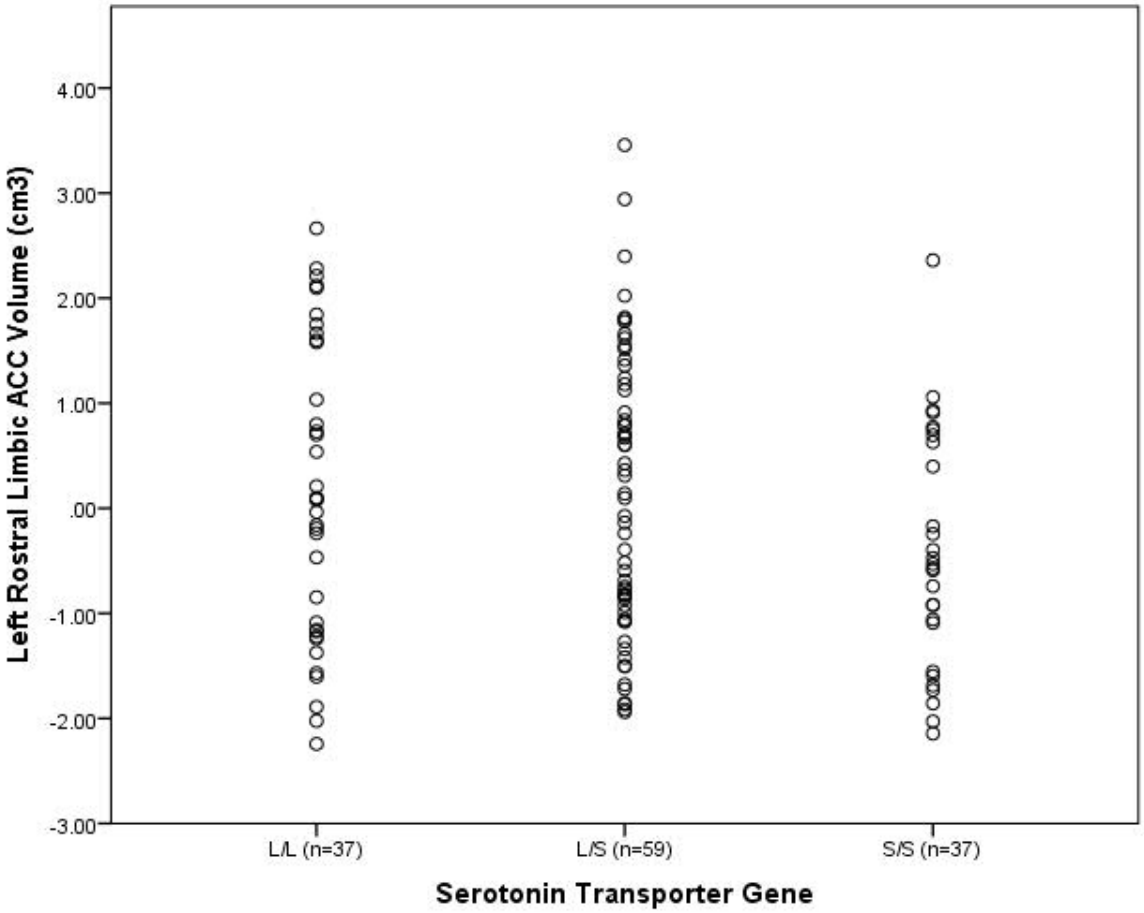
Supplementary Figure 4. Left hippocampal volume for individuals with LL, LS and SS genotypes.



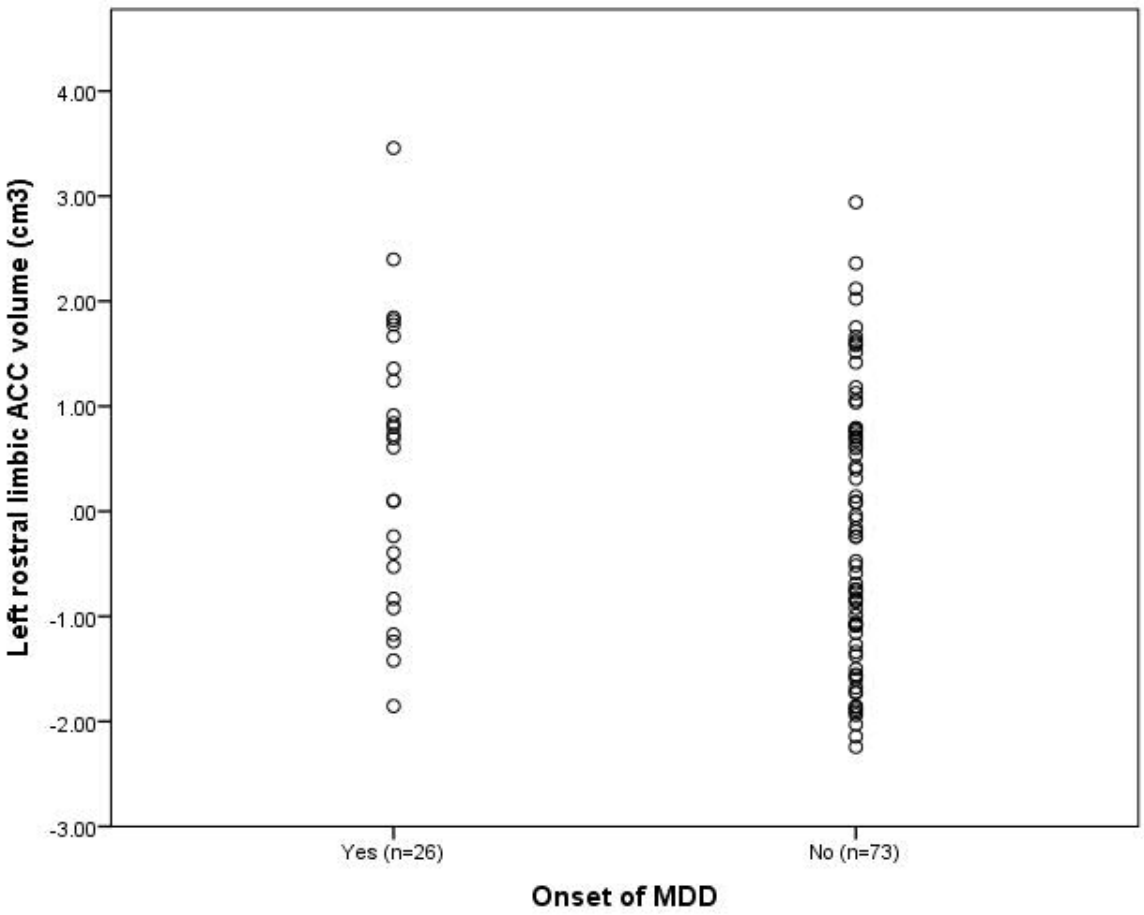
Supplementary Figure 5. Left hippocampal volumes for patients who experienced a first onset of Major Depression during adolescent and healthy comparison subjects



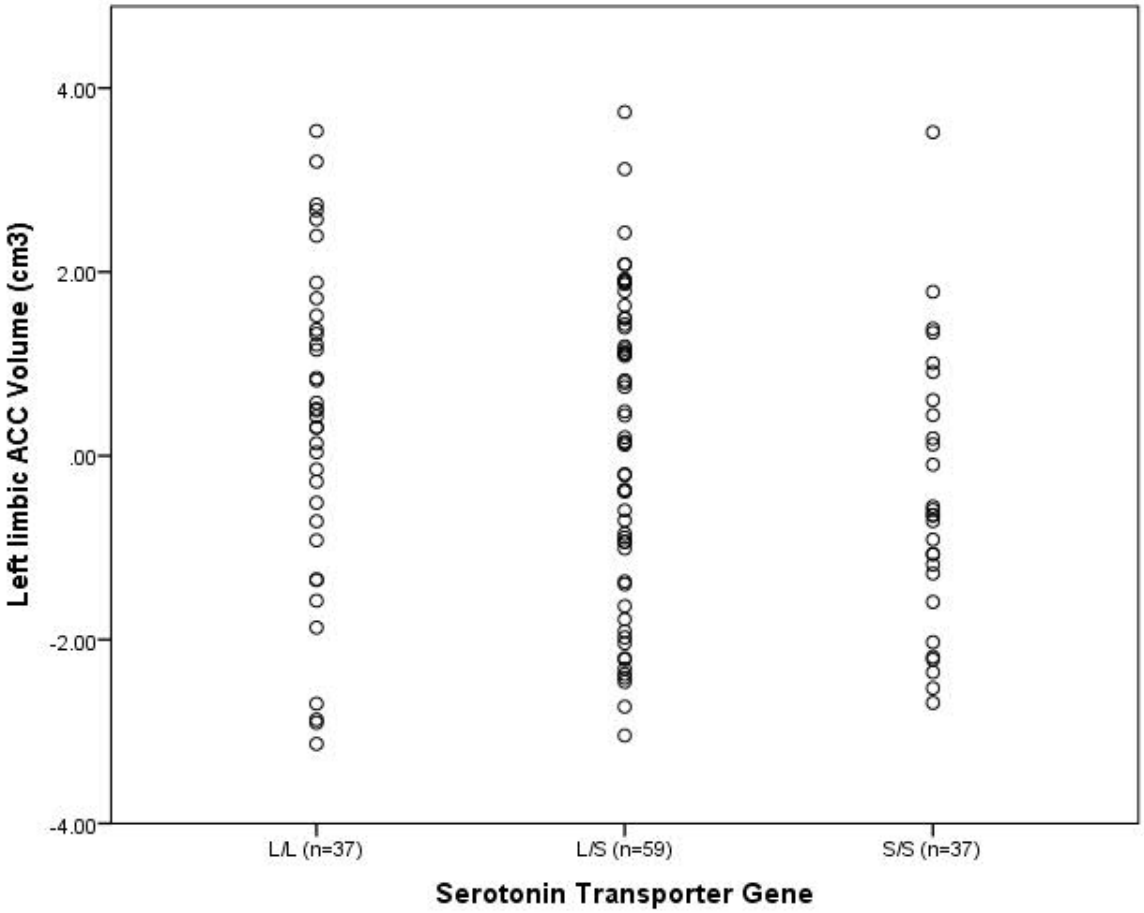
Supplementary Figure 6. Right hippocampal volumes for patients who experienced a first onset of Major Depression during adolescent and healthy comparison subjects



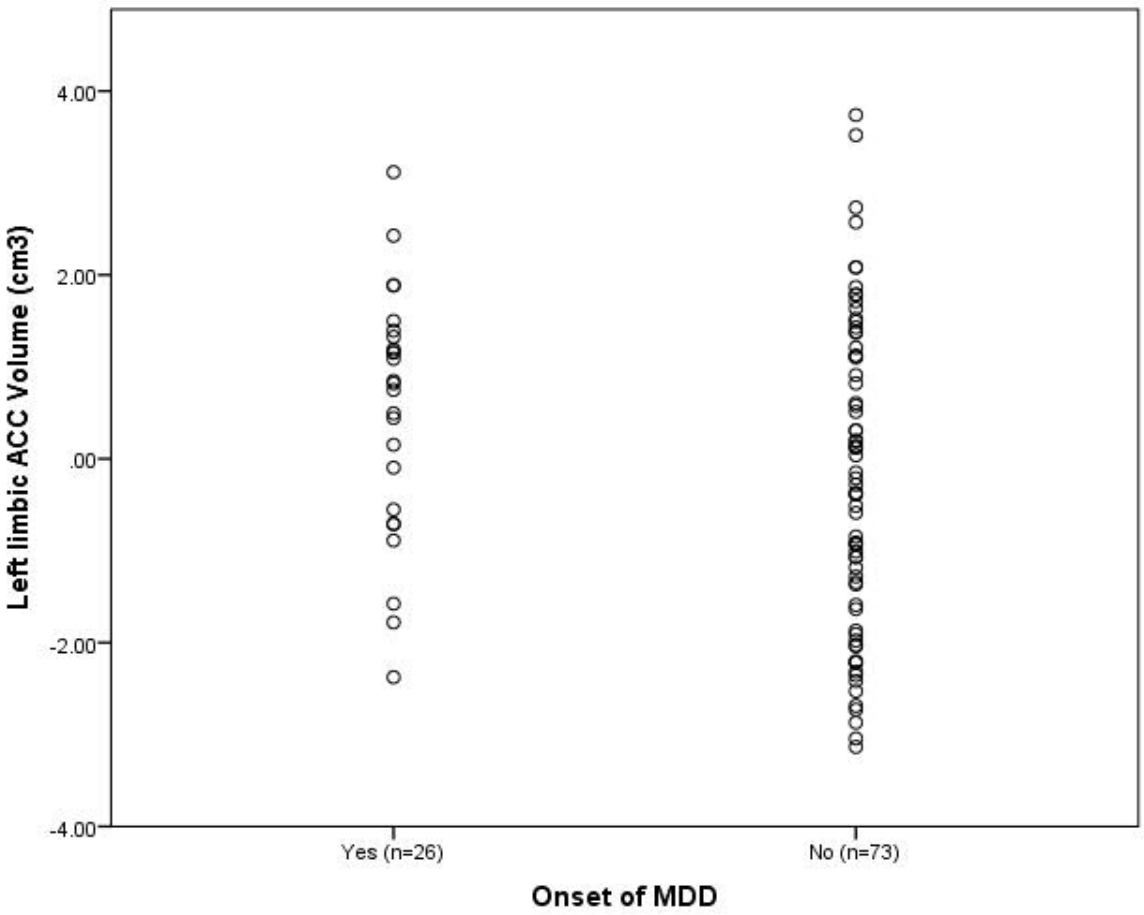
Supplementary Figure 7. Left rostral limbic ACC volumes of individuals with LL, LS and SS genotypes.



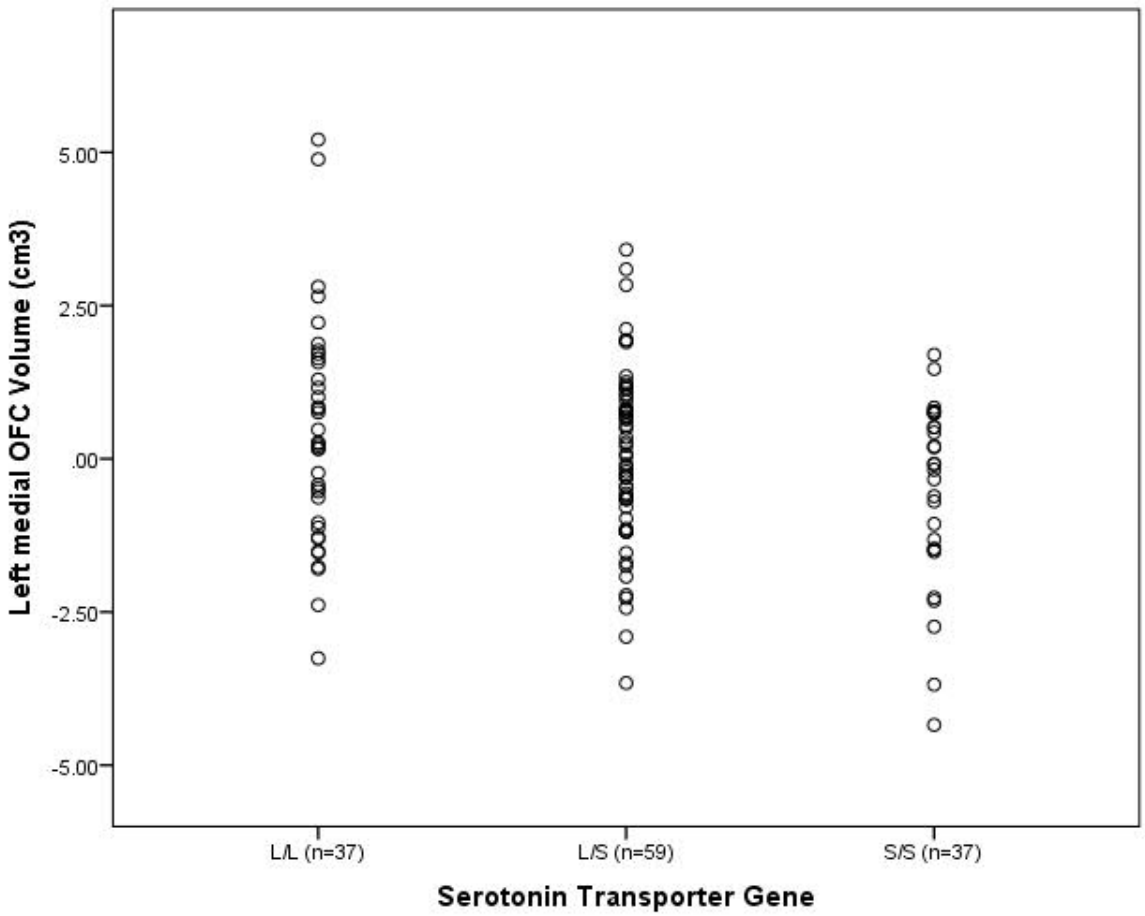
Supplementary Figure 8. Left rostral limbic ACC volumes for patients who experienced a first onset of Major Depression during adolescent and healthy comparison subjects



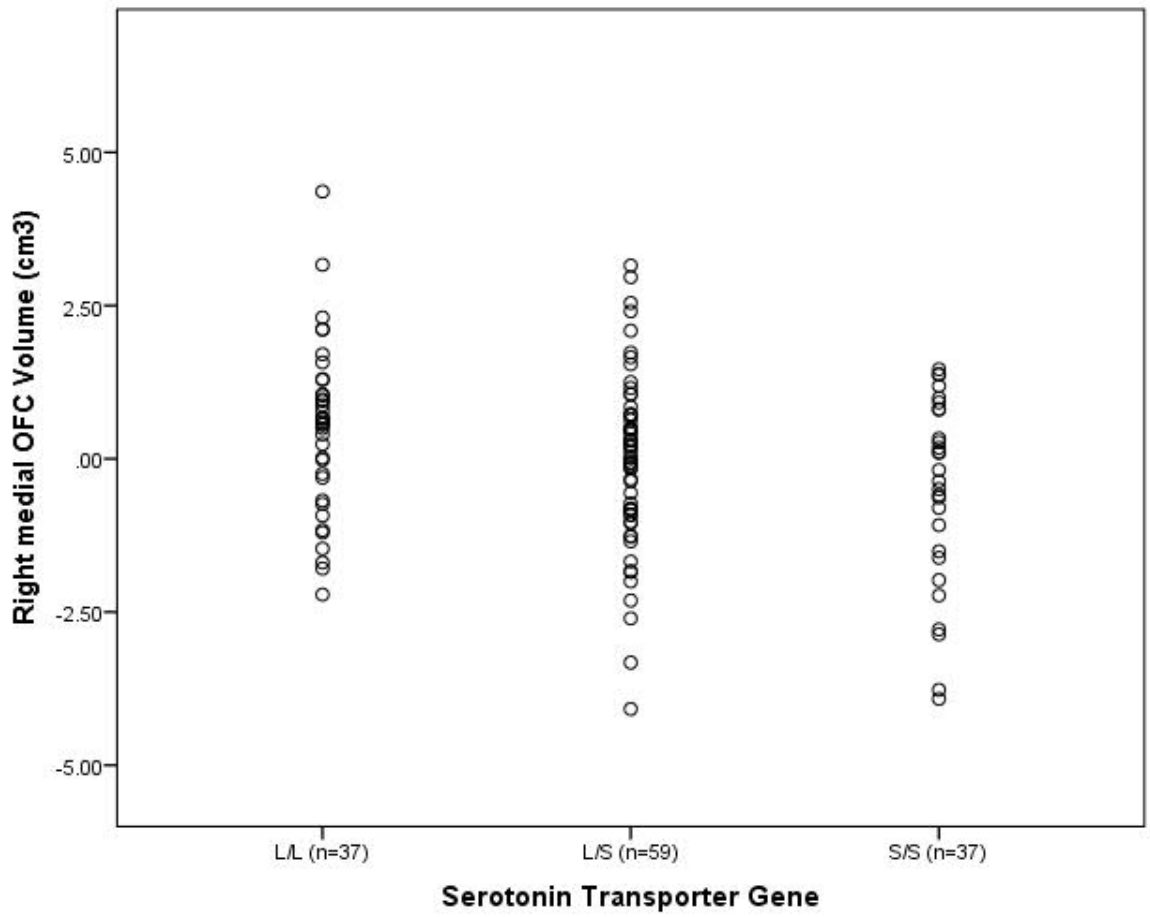
Supplementary Figure 9. Left limbic ACC volumes for individuals with LL, LS and SS genotypes.



Supplementary Figure 10. Left limbic volumes for patients who experienced a first onset of Major Depression during adolescent and healthy comparison subjects



Supplementary Figure 11. Left OFC volumes for individuals of LL, LS and SS genotypes.



Supplementary Figure 12. Right OFC volumes of individuals with LL, LS and SS genotypes.

Appendix C: Supplementary Material for Chapter 9

(Paper: Little, K., Olsson, C. A., Youssef, G. J., Whittle, S., Simmons, J. G., Yucel, M., . . . Allen, N. B. (2015). Linking the serotonin transporter gene, family environments, hippocampal volume and depression onset: A prospective imaging gene x environment analysis. *Journal of Abnormal Psychology, 124*(4), 834-849. doi:10.1037/abn0000101)

Supplemental Materials

Linking the Serotonin Transporter Gene, Family Environments, Hippocampal Volume and Depression Onset: A Prospective Imaging Gene x Environment Analysis

by K. Little et al., 2015, *Journal of Abnormal Psychology*

http://dx.doi.org/10.1037/abn0000101

Table S1

Specific Lifetime Psychopathology Recorded at Completed Assessments

	Overall (*N*=174)	MDD onset (*n*=36)	No MDD onset (*n*=101)	MDD onset undetermined (missing) (*n*=37)
No other diagnosis given*	87	6	67	20
Depression NOS	7	4	3	0
Adjustment disorder	11	6	3	2
PTSD/ASD	6	5	0	1
GAD	7	4	2	1
Social Phobia	20	7	9	4
Specific Phobia	17	10	7	0
Separation Anxiety	3	1	1	1
OCD	5	1	2	2
Panic Disorder	1	1	0	0
ODD	9	2	5	2
Conduct Disorder	12	4	2	6
Attention Deficit Disorders	6	1	0	5
Alcohol Use Disorder	23	8	12	3
Substance Use Disorder	12	8	3	1
Enuresis	5	1	2	2
Eating Disorder	5	2	3	0

GAD, generalized anxiety disorder; NOS, not otherwise specified; OCD, obsessive- compulsive disorder; ODD, oppositional defiant disorder; PTSD, posttraumatic stress disorder; ASD, Acute Stress Disorder.

* Except for Major Depressive Disorder in the MDD onset group.

Table S2

Fit Statistics of Path Models

	χ^2	df	p-value	RMSEA	CFI	WRMR
Model 1: Aggressive parenting	.001	1	.980	.00	1.00	.003
Model 2: Positive parenting	.001	1	.980	.00	1.00	.003

df = degrees of freedom, RMSEA= Root Mean Square Error of Approximation, CFI= Comparative Fit Index, SRMR= Standardized Root Mean Square Residual (available for continuous outcomes), WRMR= Weighted Root Mean Square Residual (available for categorical outcomes).

Table S3

Covariance Coverage Between Variables (Proportion of Participants With Data Available on Both Variables), With Proportion of Participants With Data on a Particular Variable Indicated

	1	2	3	4	5	6	7	8	9	10
1. Gender	1.00									
2. Ethnicity	.98	.98								
3. MDD onset	.78	.79	.79							
4. Left hippocampus	.71	.70	.56	.71						
5. Right hippocampus	.71	.70	.56	.71	.71					
6. Positive parenting EPI	.71	.70	.56	.523	.52	.71				
7. Aggressive parenting EPI	.71	.70	.56	.523	.52	.71	.71			
8. Positive parenting PSI	.71	.70	.56	.523	.52	.71	.71	.71		
9. Aggressive parenting PSI	.71	.70	.56	.523	.52	.71	.71	.71	.71	
10. 5-HTTLPR	1.00	.98	.79	.71	.71	.71	.71	.71	.71	1.00

on the Diagonal

Table S4

Complete Results of the Two Separate Path Models Investigating the Moderating Effect of the Different Parenting Variables of Interest (Aversive Behaviour in the PSI Task and Positive Behaviour in the EPI Task)

	b	SE	95% CI		β	p
			Upper	Lower		
Aggressive Parenting in the PSI						
5-HTTLPR → MDD onset (n=137)	-.12	.18	-.46	.23	-.08	.511
Aggressive parenting → MDD onset (n=98)	.24	.38	-.49	1.00	.15	.522
5-HTTLPR X Aggressive Parenting → MDD onset (n=98)	-.33	.36	-1.11	.33	-.22	.360
5-HTTLPR → Left hippocampus (n=123)	**-.08**	**.04**	**-.16**	**-.01**	**-.20**	**.035**
Aggressive parenting → Left hippocampus (n=91)	.02	.08	-.14	.17	.05	.767
5-HTTLPR X Aggressive Parenting → Left hippocampus (n=98)	-.05	.07	-.18	.08	-.11	.448
Left hippocampus → MDD onset (n=98)	**-1.78**	**.89**	**-3.62**	**-.12**	**-.53**	**.044**
5-HTTLPR → Right hippocampus (n=123)	-.06	.04	-.15	.02	-.15	.126
Aggressive parenting → Right hippocampus (n=91)	.08	.09	-.10	.26	.15	.411
5-HTTLPR X Aggressive Parenting →Right hippocampus (n=91)	-.10	.08	-.26	.04	-.23	.169
Right hippocampus → MDD onset (n=98)	**2.15**	**.81**	**.54**	**3.75**	**.65**	**.008**
Left hippocampus ↔ Right hippocampus (n=123)	**.07**	**.01**	**.05**	**.09**	**.78**	**.000**
5-HTTLPR ↔ Aggressive parenting (n=123)	.07	.05	-.02	.16	.14	.131
5-HTTLPR ↔ 5-HTTLPR X Aggressive Parenting (n=124)	-.02	.05	-.11	.07	-.04	.702
Aggressive parenting ↔ 5-HTTLPR X Aggressive Parenting (n=124)	**.32**	**.05**	**.24**	**.41**	**.80**	**.000**
Positive parenting → MDD onset (n=98)	-.45	.23	-.87	.04	-.31	.054
Positive parenting → Left hippocampus (n=91)	.02	.08	-.14	.17	.05	.767
Positive parenting → Right hippocampus (n=91)	.08	.09	-.10	.26	.15	.411
Positive parenting ↔ 5-HTTLPR (n=124)	.07	.05	-.02	.16	.14	.131
Positive parenting ↔ Aggressive parenting (n=124)	**-.18**	**.03**	**-.25**	**-.12**	**-.44**	**.000**
Positive parenting ↔ 5-HTTLPR X Aggressive parenting (n=124)	**-.12**	**.04**	**-.20**	**-.06**	**-.28**	**.001**
Positive parenting ↔ Gender (n=124)	-.02	.03	-.07	.04	-.04	.628
Positive parenting ↔ Ethnicity (n=122)	-.03	.02	-.08	.02	-.13	.220
Gender → MDD onset (n=137)	.08	.24	-.37	.57	.04	.733
Gender → Left hippocampus (n=123)	.02	.06	-.10	.13	.03	.792
Gender → Right hippocampus (n=123)	.02	.06	-.09	.13	.03	.760
Gender ↔ 5-HTTLPR (n=174)	.03	.03	-.02	.09	.10	.209
Gender ↔ Aggressive parenting (n=124)	-.02	.03	-.07	.04	-.06	.538
Gender ↔ 5-HTTLPR X Aggressive parenting (n=124)	.003	.03	-.05	.06	.01	.928
Gender ↔ Ethnicity (n=171)	.000	.01	-.03	.02	-.002	.980
Ethnicity → MDD onset (n=137)	-.49	.58	-1.84	.30	-.16	.400
Ethnicity → Left hippocampus (n=122)	.07	.10	-.12	.26	.07	.488

Ethnicity → Right hippocampus (n=122)	.10	.09	-.07	.28	.11	.242
Ethnicity ↔ 5-HTTLPR (n=171)	.03	.02	-.004	.07	.13	.107
Ethnicity ↔ Aggressive parenting (n=122)	.01	.02	-.03	.06	.07	.494
Ethnicity ↔5-HTTLPR x Aggressive parenting (n=122)	.02	.02	-.03	.07	.08	.482
<u>Positive Parenting in the EPI</u>						
5-HTTLPR → MDD onset (n=137)	-.18	.17	-.50	.16	-.13	.289
Positive parenting → MDD onset (n=98)	-.50	.36	-1.19	.25	-.24	.168
5-HTTLPR X Positive Parenting → MDD onset (n=98)	.66	.38	-.07	1.47	.32	.086
5-HTTLPR → Left hippocampus (n=123)	**-.08**	**.04**	**-.15**	**.002**	**-.18**	**.048**
Positive parenting → Left hippocampus (n=91)	-.14	.08	-.27	.05	-.22	.090
5-HTTLPR X Positive Parenting → Left hippocampus (n=91)	.11	.08	-.04	.28	.17	.180
Left hippocampus → MDD onset (n=98)	**-1.79**	**.89**	**-3.58**	**-.08**	**-.53**	**.044**
5-HTTLPR → Right hippocampus (n=123)	-.06	.04	-.13	.03	-.13	.168
Positive parenting → Right hippocampus (n=91)	-.15	.09	-.32	.04	-.24	.106
5-HTTLPR X Positive Parenting → Right hippocampus (n=91)	.16	.09	-.01	.34	.25	.075
Right hippocampus → MDD onset (n=98)	**2.03**	**.76**	**.49**	**3.51**	**.61**	**.008**
Left hippocampus ↔ Right hippocampus (n=123)	**.07**	**.01**	**.05**	**.09**	**.78**	**.000**
5-HTTLPR ↔ Positive parenting (n=124)	.03	.03	-.04	.09	.09	.376
5-HTTLPR ↔ 5-HTTLPR X Positive Parenting (n=124)	.02	.04	-.06	.09	.05	.669
Positive parenting ↔ 5-HTTLPR X Positive Parenting (n=124)	**.17**	**.03**	**.12**	**.23**	**.70**	**.000**
Aggressive parenting → MDD onset (n=98)	**.85**	**.31**	**.18**	**1.41**	**.35**	**.006**
Aggressive parenting → Left hippocampus (n=91)	-.04	.09	-.23	.13	-.06	.665
Aggressive parenting → Right hippocampus (n=91)	-.04	.09	-.22	.13	-.05	.670
Aggressive parenting ↔ 5-HTTLPR (n=124)	-.01	.03	-.06	.05	-.02	.847
Aggressive parenting ↔ Positive parenting (n=124)	**-.06**	**.02**	**-.11**	**-.02**	**-.32**	**.005**
Aggressive parenting ↔ 5-HTTLPR X Positive parenting (n=124)	**-.05**	**.02**	**-.10**	**-.01**	**-.24**	**.029**
Aggressive parenting ↔ Gender (n=124)	.01	.02	-.03	.05	.05	.556
Aggressive parenting ↔ Ethnicity (n=123)	.004	.02	-.03	.04	.03	.807
Gender → MDD onset (n=137)	.05	.23	-.40	.51	.02	.839
Gender → Left hippocampus (n=123)	.01	.06	-.10	.12	.01	.908
Gender → Right hippocampus (n=123)	.00	.06	-.10	.12	.01	.940
Gender ↔ 5-HTTLPR (n=174)	.03	.03	-.02	.09	.10	.209
Gender ↔ Positive parenting (n=124)	-.02	.02	-.06	.03	-.06	.500
Gender ↔ 5-HTTLPR X Positive parenting (n=124)	-.01	.02	-.05	.04	-.03	.780
Gender ↔ Ethnicity (n=171)	.00	.01	-.03	.02	.00	.980
Ethnicity → MDD onset (n=137)	-.30	.53	-1.22	.46	-.10	.568
Ethnicity → Left hippocampus (n=122)	.06	.10	-.13	.25	.06	.556
Ethnicity → Right hippocampus (n=122)	.10	.09	-.07	.28	.11	.255
Ethnicity ↔ 5-HTTLPR (n=171)	.03	.02	-.004	.07	.13	.107
Ethnicity ↔ Positive parenting (n=122)	-.03	.01	-.05	.00	-.15	.074
Ethnicity ↔5-HTTLPR x Positive parenting (n=122)	-.03	.02	-.07	.00	-.21	.055

The number of participants used to calculate each statistic, as a result of the use of pairwise analysis, is provided in brackets (n=).

***** Each path analysis contained the serotonin transporter gene, parenting and the specific serotonin transporter gene X parenting interaction of interest as independent variables, left and right hippocampal volume as mediating variables, and MDD onset as the dependent variable, with adolescent gender, ethnicity and the other parenting variable recorded during the same task as covariates**.**

Supplemental Materials

Linking the Serotonin Transporter Gene, Family Environments, Hippocampal Volume and Depression Onset: A Prospective Imaging Gene x Environment Analysis

by K. Little et al., 2015, *Journal of Abnormal Psychology*

http://dx.doi.org/10.1037/abn0000101

Table S5

IGxE Path Models Testing Associations Between 5-HTTLPR Genotype, Hippocampal Volume and Parenting at 11-13 Years and Later MDD Onset During a Six Year Follow Up Period, Controlling for an Interaction Between 5-HTTLPR x Gender

	b	SE	95% CI		β	p
			Lower	Upper		
Aggressive Parenting in the PSI						
5-HTTLPR → MDD onset	-.12	.18	-.46	.25	-.08	.522
Aggressive parenting → MDD onset	.24	.41	-.56	1.04	.14	.565
5-HTTLPR X Aggressive Parenting → MDD onset	-.34	.36	-1.06	.34	-.22	.343
5-HTTLPR → Left hippocampus	**-.08**	**.04**	**-.16**	**-.01**	**-.20**	**.038**
Aggressive parenting → Left hippocampus	.01	.08	-.17	.15	.01	.947
5-HTTLPR X Aggressive Parenting → Left hippocampus	-.06	.07	-.20	.07	-.13	.385
Left hippocampus → MDD onset	-1.79	.94	-3.76	-.15	-.53	.056
5-HTTLPR → Right hippocampus	-.06	.04	-.15	.02	-.15	.129
Aggressive parenting → Right hippocampus	.06	.09	-.12	.25	.12	.531
5-HTTLPR X Aggressive Parenting →Right hippocampus	-.11	.08	-.27	.03	-.24	.143
Right hippocampus → MDD onset	**2.15**	**.87**	**.53**	**3.87**	**.65**	**.014**
Left hippocampus ↔ Right hippocampus	**.07**	**.01**	**.05**	**.09**	**.78**	**.000**
5-HTTLPR ↔ Aggressive parenting	-.03	.04	-.10	.04	-.07	.396
5-HTTLPR ↔ 5-HTTLPR X Aggressive parenting	-.02	.05	-.11	.07	-.04	.702
Aggressive parenting ↔ 5-HTTLPR X Aggressive parenting	**.32**	**.05**	**.24**	**.41**	**.80**	**.000**
Positive parenting → MDD onset	-.45	.25	-.90	.07	-.31	.069
Positive parenting → Left hippocampus	.02	.05	-.09	.12	.04	.762
Positive parenting → Right hippocampus	.03	.05	-.07	.13	.08	.491
Positive parenting ↔ 5-HTTLPR	.07	.05	-.02	.16	.14	.131
Positive parenting ↔ Aggressive parenting	**-.18**	**.03**	**-.25**	**-.12**	**-.44**	**.000**
Positive parenting ↔ 5-HTTLPR X Aggressive parenting	**-.12**	**.04**	**-.20**	**-.06**	**-.28**	**.001**
Positive parenting ↔ Gender	-.02	.03	-.07	.04	-.04	.628

Positive parenting ↔ Ethnicity	-.03	.02	-.08	.02	-.13	.220
Gender → MDD onset	.08	.25	-.38	.60	.04	.753
Gender → Left hippocampus	.01	.06	-.11	.12	.02	.877
Gender → Right hippocampus	.01	.06	-.10	.13	.02	.839
Gender ↔ 5-HTTLPR	.03	.03	-.02	.09	.10	.209
Gender ↔ Aggressive parenting	-.02	.03	-.07	.04	-.06	.538
Gender ↔ 5-HTTLPR X Aggressive parenting	.00	.03	-.05	.06	.01	.928
Gender ↔ Ethnicity	.02	.02	-.03	.07	.08	.481
Ethnicity → MDD onset	-.50	.78	-2.03	.45	-.16	.523
Ethnicity → Left hippocampus	.04	.12	-.17	.27	.05	.711
Ethnicity → Right hippocampus	.08	.10	-.13	.28	.09	.435
Ethnicity ↔ 5-HTTLPR	.03	.02	.00	.07	.13	.107
Ethnicity ↔ Aggressive parenting	.01	.02	-.03	.06	.07	.493
Ethnicity ↔5-HTTLPR x Aggressive parenting	.02	.02	-.03	.07	.08	.481
5-HTTLPR x Gender → MDD	.11	2.15	-3.59	5.28	.02	.961
5-HTTLPR x Gender → Left hippocampus	.23	.25	-.13	.78	.15	.357
5-HTTLPR x Gender → Right hippocampus	.21	.26	-.06	.87	.13	.411
5-HTTLPR x Gender ↔ 5-HTTLPR	.00	.01	-.01	.03	.02	.831
5-HTTLPR x Gender ↔ Aggressive parenting	**.04**	**.02**	**.01**	**.08**	**.32**	**.024**
5-HTTLPR x Gender ↔ Gender	.01	.01	-.01	.02	.05	.571
5-HTTLPR x Gender ↔ Positive parenting	-.01	.01	-.02	.00	-.07	.144
5-HTTLPR x Gender ↔ 5-HTTLPR x Aggressive parenting	.04	.02	.01	.09	.32	.043
5-HTTLPR x Gender ↔ Ethnicity	.01	.02	-.03	.05	.20	.511
<u>Positive Parenting in the EPI</u>						
5-HTTLPR → MDD onset	-.30	.24	-.78	.18	-.21	.225
Positive parenting → MDD onset	-.51	.37	-1.21	.25	-.25	.161
5-HTTLPR X Positive Parenting → MDD onset	.68	.39	-.08	1.48	.33	.082
5-HTTLPR → Left hippocampus	-.10	.06	-.21	.01	-.25	.063
Positive parenting → Left hippocampus	-.14	.08	-.28	.05	-.23	.090
5-HTTLPR X Positive Parenting → Left hippocampus	.11	.08	-.04	.28	.18	.168
Left hippocampus → MDD onset	**-1.77**	**.90**	**-3.58**	**-.04**	**-.53**	**.048**
5-HTTLPR → Right hippocampus	**-.11**	**.06**	-.22	.00	**-.26**	**.048**
Positive parenting → Right hippocampus	-.15	.10	-.33	.04	-.25	.107
5-HTTLPR X Positive Parenting → Right hippocampus	.16	.09	.00	.34	.26	.060
Right hippocampus → MDD onset	**1.98**	**.77**	**.42**	**3.47**	**.60**	**.010**
Left hippocampus ↔ Right hippocampus	**.06**	**.01**	**.05**	**.09**	**.78**	**.000**
5-HTTLPR ↔ Positive parenting	.03	.03	-.04	.09	.09	.376
5-HTTLPR ↔ 5-HTTLPR X Positive Parenting	.02	.04	-.06	.09	.05	.669
Positive parenting ↔ 5-HTTLPR X Positive Parenting	**.17**	**.03**	**.12**	**.23**	**.70**	**.000**
Aggressive parenting → MDD onset	**.86**	**.31**	**.19**	**1.42**	**.35**	**.005**
Aggressive parenting → Left hippocampus	-.04	.09	-.23	.14	-.05	.687
Aggressive parenting → Right hippocampus	-.03	.09	-.21	.14	-.05	.707
Aggressive parenting ↔ 5-HTTLPR	-.01	.03	-.06	.05	-.02	.847

Aggressive parenting ↔ Positive parenting	**-.06**	**.02**	**-.11**	**-.02**	**-.32**	**.005**
Aggressive parenting ↔ 5-HTTLPR X Positive parenting	**-.05**	**.02**	**-.10**	**-.01**	**-.24**	**.029**
Aggressive parenting ↔ Gender	.01	.02	-.03	.05	.05	.556
Aggressive parenting ↔ Ethnicity	.00	.02	-.03	.04	.03	.807
Gender → MDD onset	-.15	.38	-.92	.57	-.08	.689
Gender → Left hippocampus	-.04	.09	-.21	.13	-.07	.623
Gender → Right hippocampus	-.09	.09	-.26	.08	-.15	.311
Gender ↔ 5-HTTLPR	.03	.03	-.02	.09	.10	.209
Gender ↔ Positive parenting	.03	.03	-.04	.09	.09	.376
Gender ↔ 5-HTTLPR X Positive parenting	-.01	.02	-.05	.04	-.03	.780
Gender ↔ Ethnicity	.00	.01	-.03	.02	.00	.980
Ethnicity → MDD onset	-.29	.53	-1.22	.46	-.10	.579
Ethnicity → Left hippocampus	.06	.10	-.13	.26	.06	.551
Ethnicity → Right hippocampus	.10	.09	-.06	.27	.11	.235
Ethnicity ↔ 5-HTTLPR	.03	.03	-.02	.09	.10	.209
Ethnicity ↔ Positive parenting	-.03	.01	-.05	.00	-.15	.074
Ethnicity ↔5-HTTLPR x Positive parenting	-.03	.02	-.07	.00	-.21	.055
5-HTTLPR x Gender → MDD	.22	.34	-.43	.90	.16	.515
5-HTTLPR x Gender → Left hippocampus	.06	.07	-.08	.20	.13	.438
5-HTTLPR x Gender → Right hippocampus	.10	.07	-.04	.25	.24	.164
5-HTTLPR x Gender ↔ 5-HTTLPR	**.30**	**.04**	**.22**	**.39**	**.60**	**.000**
5-HTTLPR x Gender ↔ Positive parenting	.01	.03	-.05	.07	.03	.769
5-HTTLPR x Gender ↔ Gender	**.24**	**.02**	**.21**	**.28**	**.68**	**.000**
5-HTTLPR x Gender ↔ Aggressive parenting	.00	.03	-.05	.06	.00	.980
5-HTTLPR x Gender ↔ 5-HTTLPR x Positive parenting	.00	.05	-.10	.09	.00	.982
5-HTTLPR x Gender ↔ Ethnicity	.01	.02	-.02	.06	.06	.464

Table S6

IGxE Path Models Testing Associations Between 5-HTTLPR Genotype, Hippocampal Volume and Parenting at 11-13 Years and Later MDD Onset During a Six Year Follow Up Period, Controlling for an Interaction Between 5-HTTLPR x Ethnicity

	b	SE	95% CI		β	p
			Lower	Upper		
Aggressive Parenting in the PSI						
5-HTTLPR → MDD onset	-.12	.24	-.54	.36	-.09	.622
Aggressive parenting → MDD onset	.25	.40	-.49	1.08	.15	.538
5-HTTLPR X Aggressive Parenting → MDD onset	-.34	.36	-1.09	.34	-.22	.354
5-HTTLPR → Left hippocampus	**-.09**	**.04**	**-.17**	**-.01**	**-.22**	**.035**
Aggressive parenting → Left hippocampus	.03	.08	-.13	.18	.06	.715
5-HTTLPR X Aggressive Parenting → Left hippocampus	-.06	.07	-.20	.07	-.12	.414
Left hippocampus → MDD onset	-1.78	.97	-3.79	.03	-.53	.066
5-HTTLPR → Right hippocampus	-.07	.05	-.15	.03	-.15	.154
Aggressive parenting → Right hippocampus	.08	.09	-.10	.28	.15	.422
5-HTTLPR X Aggressive Parenting →Right hippocampus	-.11	.08	-.28	.04	-.23	.187
Right hippocampus → MDD onset	**2.16**	**.90**	**.38**	**3.91**	**.65**	**.016**
Left hippocampus ↔ Right hippocampus	**.07**	**.01**	**.05**	**.09**	**.78**	**.000**
5-HTTLPR ↔ Aggressive parenting	-.03	.04	-.10	.04	-.07	.396
5-HTTLPR ↔ 5-HTTLPR X Aggressive parenting	-.02	.05	-.11	.07	-.04	.702
Aggressive parenting ↔ 5-HTTLPR X Aggressive parenting	**.32**	**.05**	**.24**	**.41**	**.80**	**.000**
Positive parenting → MDD onset	-.45	.27	-.91	.11	-.31	.092
Positive parenting → Left hippocampus	.03	.05	-.08	.13	.06	.639
Positive parenting → Right hippocampus	.04	.05	-.06	.14	.09	.428
Positive parenting ↔ 5-HTTLPR	.07	.05	-.02	.16	.14	.131
Positive parenting ↔ Aggressive parenting	**-.18**	**.03**	**-.25**	**-.12**	**-.44**	**.000**
Positive parenting ↔ 5-HTTLPR X Aggressive parenting	**-.12**	**.04**	**-.20**	**-.06**	**-.28**	**.001**
Positive parenting ↔ Gender	-.02	.03	-.07	.04	-.04	.628
Positive parenting ↔ Ethnicity	-.03	.02	-.08	.02	-.13	.220
Gender → MDD onset	.08	.25	-.38	.59	.04	.741
Gender → Left hippocampus	.02	.06	-.10	.13	.03	.779
Gender → Right hippocampus	.02	.06	-.09	.13	.03	.761
Gender ↔ 5-HTTLPR	.03	.03	-.02	.09	.10	.209
Gender ↔ Aggressive parenting	-.02	.03	-.07	.04	-.06	.538
Gender ↔ 5-HTTLPR X Aggressive parenting	.00	.03	-.05	.06	.01	.928
Gender ↔ Ethnicity	.02	.02	-.03	.07	.08	.482
Ethnicity → MDD onset	-.52	1.43	-2.60	3.00	-.17	.715

Ethnicity → Left hippocampus	.01	.21	-.37	.48	.01	.962
Ethnicity → Right hippocampus	.10	.24	-.36	.57	.10	.685
Ethnicity ↔ 5-HTTLPR	.03	.02	.00	.07	.13	.107
Ethnicity ↔ Aggressive parenting	.01	.02	-.03	.06	.07	.494
Ethnicity ↔5-HTTLPR x Aggressive parenting	.02	.02	-.03	.07	.08	.482
5-HTTLPR x Ethnicity → MDD	.03	1.37	-3.68	1.53	.01	.982
5-HTTLPR x Ethnicity → Left hippocampus	.05	.15	-.27	.33	.08	.725
5-HTTLPR x Ethnicity → Right hippocampus	.01	.16	-.32	.33	.01	.964
5-HTTLPR x Ethnicity ↔ 5-HTTLPR	**.10**	**.03**	**.04**	**.17**	**.30**	**.002**
5-HTTLPR x Ethnicity ↔ Aggressive parenting	.02	.03	-.04	.08	.07	.548
5-HTTLPR x Ethnicity ↔ Gender	.00	.02	-.03	.04	.01	.900
5-HTTLPR x Ethnicity ↔ Positive parenting	-.04	.04	-.11	.03	-.13	.253
5-HTTLPR x Ethnicity ↔ 5-HTTLPR x Aggressive parenting	.04	.04	-.03	.14	.13	.354
Positive Parenting in the EPI	**.12**	**.03**	**.07**	**.18**	**.83**	**.000**
5-HTTLPR → MDD onset	-.16	.24	-.58	.30	-.12	.491
Positive parenting → MDD onset	-.48	.39	-1.28	.22	-.24	.214
5-HTTLPR X Positive Parenting → MDD onset	.64	.42	-.13	1.53	.31	.133
5-HTTLPR → Left hippocampus	**-.09**	**.04**	**-.17**	**.00**	**-.21**	**.045**
Positive parenting → Left hippocampus	-.15	.08	-.29	.05	-.24	.085
5-HTTLPR X Positive Parenting → Left hippocampus	.12	.09	-.03	.32	.20	.173
Left hippocampus → MDD onset	-1.78	.98	-3.74	.09	-.53	.069
5-HTTLPR → Right hippocampus	-.06	.05	-.15	.03	-.14	.186
Positive parenting → Right hippocampus	-.15	.10	-.34	.05	-.25	.121
5-HTTLPR X Positive Parenting → Right hippocampus	.16	.10	-.02	.37	.26	.097
Right hippocampus → MDD onset	**2.03**	**.85**	**.29**	**3.63**	**.61**	**.017**
Left hippocampus ↔ Right hippocampus	.07	.01	.05	.09	.78	.000
5-HTTLPR ↔ Positive parenting	.03	.03	-.04	.09	.09	.376
5-HTTLPR ↔ 5-HTTLPR X Positive Parenting	.02	.04	-.06	.09	.05	.669
Positive parenting ↔ 5-HTTLPR X Positive Parenting	**.17**	**.03**	**.12**	**.23**	**.70**	**.000**
Aggressive parenting → MDD onset	**.87**	**.37**	**.12**	**1.52**	**.35**	**.018**
Aggressive parenting → Left hippocampus	-.05	.10	-.27	.13	-.07	.609
Aggressive parenting → Right hippocampus	-.04	.10	-.24	.14	-.06	.659
Aggressive parenting ↔ 5-HTTLPR	-.01	.03	-.06	.05	-.02	.847
Aggressive parenting ↔ Positive parenting	**-.06**	**.02**	**-.11**	**-.02**	**-.32**	**.005**
Aggressive parenting ↔ 5-HTTLPR X Positive parenting	**-.05**	**.02**	**-.10**	**-.01**	**-.24**	**.029**
Aggressive parenting ↔ Gender	.01	.02	-.03	.05	.05	.556
Aggressive parenting ↔ Ethnicity	.00	.02	-.03	.04	.03	.807
Gender → MDD onset	.05	.24	-.43	.52	.02	.849
Gender → Left hippocampus	.01	.06	-.10	.12	.01	.895
Gender → Right hippocampus	.01	.06	-.11	.11	.01	.935
Gender ↔ 5-HTTLPR	.03	.03	-.02	.09	.10	.209
Gender ↔ Positive parenting	.03	.03	-.04	.09	.09	.376
Gender ↔ 5-HTTLPR X Positive parenting	-.01	.02	-.05	.04	-.03	.780

Gender ↔ Ethnicity	.00	.01	-.03	.02	.00	.980
Ethnicity → MDD onset	-.17	1.44	-2.11	3.23	-.06	.906
Ethnicity → Left hippocampus	-.03	.23	-.45	.45	-.04	.882
Ethnicity → Right hippocampus	.06	.24	-.37	.58	.07	.799
Ethnicity ↔ 5-HTTLPR	.03	.03	-.02	.09	.10	.209
Ethnicity ↔ Positive parenting	-.03	.01	-.05	.00	-.15	.074
Ethnicity ↔5-HTTLPR x Positive parenting	-.03	.02	-.07	.00	-.21	.055
5-HTTLPR x Ethnicity → MDD	-.12	1.30	-3.26	1.16	-.06	.925
5-HTTLPR x Ethnicity → Left hippocampus	.09	.17	-.26	.42	.13	.620
5-HTTLPR x Ethnicity → Right hippocampus	.04	.18	-.33	.36	.05	.838
5-HTTLPR x Ethnicity ↔ 5-HTTLPR	**.10**	**.03**	**.04**	**.17**	**.30**	**.002**
5-HTTLPR x Ethnicity ↔ Positive parenting	-.03	.02	-.08	.00	-.16	.073
5-HTTLPR x Ethnicity ↔ Gender	.00	.02	-.03	.04	.01	.900
5-HTTLPR x Ethnicity ↔ Aggressive parenting	.03	.03	-.03	.10	.16	.352
5-HTTLPR x Ethnicity ↔ 5-HTTLPR x Positive parenting	**-.06**	**.03**	**-.13**	**-.01**	**-.29**	**.033**
5-HTTLPR x Ethnicity ↔ Ethnicity	**.12**	**.03**	**.07**	**.18**	**.83**	**.000**

Table S7

IGxE Path Models Testing Associations Between 5-HTTLPR Genotype, Hippocampal Volume and Parenting at 11-13 Years and Later MDD Onset During a Six Year Follow Up Period, Controlling for an Interaction Between 5-HTTLPR x the Other Parenting Variable of Interest (Positive Parenting in the Model Examining the Effect of Aggressive Parenting on the Indirect Pathway from 5-HTTLPR→ Hippocampal Volume→ MDD Onset, and Aggressive Parenting in the Model Examining the Effect of Positive Parenting on the Indirect Pathway From 5-HTTLPR→ Hippocampal Volume→ MDD Onset)

	b	SE	95% CI		β	p
			Lower	Upper		
Aggressive Parenting in the PSI						
5-HTTLPR → MDD onset	-.08	.18	-.42	.27	-.06	.661
Aggressive parenting → MDD onset	-.15	.44	-1.00	.74	-.09	.731
5-HTTLPR X Aggressive Parenting → MDD onset	.001	.41	-.87	.73	.001	.998
5-HTTLPR → Left hippocampus	**-.08**	**.04**	**-.16**	**-.01**	**-.20**	**.036**
Aggressive parenting → Left hippocampus	.03	.09	-.15	.19	.06	.755
5-HTTLPR X Aggressive Parenting → Left hippocampus	-.05	.08	-.21	.09	-.11	.496
Left hippocampus → MDD onset	-1.61	.88	-3.44	.00	-.48	.066
5-HTTLPR → Right hippocampus	-.06	.04	-.14	.02	-.14	.147
Aggressive parenting → Right hippocampus	.04	.10	-.14	.23	.08	.695
5-HTTLPR X Aggressive Parenting →Right hippocampus	-.07	.08	-.25	.08	-.16	.394
Right hippocampus → MDD onset	**1.95**	**.80**	**.40**	**3.56**	**.59**	**.015**
Left hippocampus ↔ Right hippocampus	**.07**	**.01**	**.05**	**.09**	**.79**	**.000**
5-HTTLPR ↔ Aggressive parenting	-.03	.04	-.10	.04	-.07	.396
5-HTTLPR ↔ 5-HTTLPR X Aggressive parenting	.03	.07	-.10	.17	.05	.658
Aggressive parenting ↔ 5-HTTLPR X Aggressive parenting	**-.12**	**.04**	**-.20**	**-.06**	**-.25**	**.001**
Positive parenting → MDD onset	**-1.08**	**.41**	**-1.89**	**-.28**	**-.73**	**.008**
Positive parenting → Left hippocampus	.03	.08	-.13	.19	.07	.718
Positive parenting → Right hippocampus	-.02	.07	-.17	.12	-.04	.810
Positive parenting ↔ 5-HTTLPR	.07	.05	-.02	.16	.14	.131
Positive parenting ↔ Aggressive parenting	**-.18**	**.03**	**-.25**	**-.12**	**-.44**	**.000**
Positive parenting ↔ 5-HTTLPR X Aggressive parenting	**.43**	**.08**	**.30**	**.60**	**.78**	**.000**
Positive parenting ↔ Gender	-.02	.03	-.07	.04	-.04	.628
Positive parenting ↔ Ethnicity	-.03	.02	-.08	.02	-.13	.220
Gender → MDD onset	.03	.24	-.43	.52	.02	.899
Gender → Left hippocampus	.02	.06	-.10	.13	.03	.791
Gender → Right hippocampus	.01	.06	-.10	.13	.02	.830
Gender ↔ 5-HTTLPR	.03	.03	-.02	.09	.10	.209

Gender ↔ Aggressive parenting	-.02	.03	-.07	.04	-.06	.538
Gender ↔ 5-HTTLPR X Aggressive parenting	-.01	.04	-.08	.06	-.02	.812
Gender ↔ Ethnicity	-.04	.03	-.12	.01	-.17	.175
Ethnicity → MDD onset	-.42	.58	-1.57	.36	-.14	.466
Ethnicity → Left hippocampus	.07	.10	-.12	.27	.07	.502
Ethnicity → Right hippocampus	.11	.09	-.06	.29	.12	.224
Ethnicity ↔ 5-HTTLPR	.03	.02	.00	.07	.13	.107
Ethnicity ↔ Aggressive parenting	.01	.02	-.03	.06	.07	.494
Ethnicity ↔5-HTTLPR x Aggressive parenting	-.04	.03	-.12	.01	-.17	.175
5-HTTLPR x Positive parenting → MDD	.60	.33	-.01	1.31	.48	.069
5-HTTLPR x Positive parenting → Left hippocampus	-.01	.06	-.12	.12	-.02	.926
5-HTTLPR x Positive parenting → Right hippocampus	.06	.06	-.05	.18	.15	.347
5-HTTLPR x Positive parenting ↔ 5-HTTLPR	-.02	.05	-.11	.07	-.04	.702
5-HTTLPR x Positive parenting ↔ Aggressive parenting	**.32**	**.05**	**.24**	**.41**	**.80**	**.000**
5-HTTLPR x Positive parenting ↔ Gender	.00	.03	-.05	.06	.01	.928
5-HTTLPR x Positive parenting ↔ Positive parenting	**-.12**	**.04**	**-.20**	**-.06**	**-.28**	**.001**
5-HTTLPR x Positive parenting ↔ 5-HTTLPR x Aggressive Parenting	**-.16**	**.06**	**-.29**	**-.06**	**-.31**	**.006**
5-HTTLPR x Positive parenting ↔ Ethnicity	.02	.02	-.03	.07	.08	.482
<u>Positive Parenting in the EPI</u>						
5-HTTLPR → MDD onset	-.16	.17	-.49	.18	-.12	.336
Positive parenting → MDD onset	-.43	.36	-1.10	.32	-.21	.231
5-HTTLPR X Positive Parenting → MDD onset	.58	.38	-.18	1.35	.28	.130
5-HTTLPR → Left hippocampus	-.07	.04	-.14	.01	-.17	.066
Positive parenting → Left hippocampus	-.12	.09	-.27	.08	-.20	.172
5-HTTLPR X Positive Parenting → Left hippocampus	.09	.09	-.07	.27	.15	.303
Left hippocampus → MDD onset	**-1.81**	**.91**	**-3.65**	**-.06**	**-.54**	**.047**
5-HTTLPR → Right hippocampus	-.05	.04	-.13	.03	-.12	.206
Positive parenting → Right hippocampus	-.13	.09	-.30	.06	-.22	.147
5-HTTLPR X Positive Parenting → Right hippocampus	.14	.09	-.03	.33	.23	.119
Right hippocampus → MDD onset	**2.03**	**.79**	**.46**	**3.57**	**.61**	**.010**
Left hippocampus ↔ Right hippocampus	**.07**	**.01**	**.05**	**.09**	**.78**	**.000**
5-HTTLPR ↔ Positive parenting	.03	.03	-.04	.09	.09	.376
5-HTTLPR ↔ 5-HTTLPR X Positive Parenting	.02	.04	-.06	.09	.05	.669
Positive parenting ↔ 5-HTTLPR X Positive Parenting	**.17**	**.03**	**.12**	**.23**	**.70**	**.000**
Aggressive parenting → MDD onset	**1.17**	**.50**	**.07**	**2.08**	**.48**	**.019**
Aggressive parenting → Left hippocampus	.03	.14	-.27	.30	.04	.831
Aggressive parenting → Right hippocampus	.02	.14	-.27	.29	.03	.870
Aggressive parenting ↔ 5-HTTLPR	-.01	.03	-.06	.05	-.02	.847
Aggressive parenting ↔ Positive parenting	**-.06**	**.02**	**-.11**	**-.02**	**-.32**	**.005**
Aggressive parenting ↔ 5-HTTLPR X Positive parenting	**-.05**	**.02**	**-.10**	**-.01**	**-.24**	**.029**
Aggressive parenting ↔ Gender	.01	.02	-.03	.05	.05	.556
Aggressive parenting ↔ Ethnicity	.00	.02	-.03	.04	.03	.807

Gender → MDD onset	.04	.24	-.41	.51	.02	.876
Gender → Left hippocampus	.00	.06	-.10	.12	.01	.941
Gender → Right hippocampus	.00	.06	-.10	.12	.00	.969
Gender ↔ 5-HTTLPR	.03	.03	-.02	.09	.10	.209
Gender ↔ Positive parenting	.03	.03	-.04	.09	.09	.376
Gender ↔ 5-HTTLPR X Positive parenting	-.01	.02	-.05	.04	-.03	.780
Gender ↔ Ethnicity	.00	.01	-.03	.02	.00	.980
Ethnicity → MDD onset	-.24	.54	-1.08	.59	-.08	.663
Ethnicity → Left hippocampus	.07	.10	-.12	.28	.08	.492
Ethnicity → Right hippocampus	.11	.09	-.06	.30	.12	.227
Ethnicity ↔ 5-HTTLPR	.03	.03	-.02	.09	.10	.209
Ethnicity ↔ Positive parenting	-.03	.01	-.05	.00	-.15	.074
Ethnicity ↔5-HTTLPR x Positive parenting	-.03	.02	-.07	.00	-.21	.055
5-HTTLPR x Aggressive parenting → MDD	-.36	.54	-1.54	.54	-.17	.502
5-HTTLPR x Aggressive parenting → Left hippocampus	-.08	.11	-.29	.12	-.13	.459
5-HTTLPR x Aggressive parenting → Right hippocampus	-.07	.11	-.30	.13	-.11	.543
5-HTTLPR x Aggressive parenting ↔ 5-HTTLPR	.03	.04	-.05	.11	.07	.540
5-HTTLPR x Aggressive parenting ↔ Positive parenting	**-.05**	**.02**	**-.10**	**-.01**	**-.20**	**.026**
5-HTTLPR x Aggressive parenting ↔ Gender	.00	.02	-.04	.05	.01	.922
5-HTTLPR x Aggressive parenting ↔ Aggressive parenting	**.15**	**.03**	**.10**	**.23**	**.76**	**.000**
5-HTTLPR x Aggressive parenting ↔ 5-HTTLPR x Positive parenting	-.07	.04	-.15	-.01	-.29	.060
5-HTTLPR x Aggressive parenting ↔ Ethnicity	.03	.03	-.02	.10	.17	.349

Table S8

IGxE Path Models Testing Associations Between 5-HTTLPR Genotype, Hippocampal Volume and Parenting at 11-13 Years and Later MDD Onset During a Six Year Follow Up Period, Controlling for an Interaction Between Positive Parenting x Aggressive Parenting

	b	SE	95% CI		β	p
			Lower	Upper		
Aggressive Parenting in the PSI						
5-HTTLPR → MDD onset	-.11	.18	-.46	.26	-.08	.550
Aggressive parenting → MDD onset	.24	.38	-.49	1.02	.14	.540
5-HTTLPR X Aggressive Parenting → MDD onset	-.33	.37	-1.13	.35	-.21	.381
5-HTTLPR → Left hippocampus	**-.09**	**.04**	**-.17**	**-.01**	**-.21**	**.030**
Aggressive parenting → Left hippocampus	.03	.08	-.14	.17	.06	.733
5-HTTLPR X Aggressive Parenting → Left hippocampus	-.05	.06	-.18	.07	-.12	.411
Left hippocampus → MDD onset	-1.77	.91	**-3.65**	**-.08**	-.53	.052
5-HTTLPR → Right hippocampus	-.06	.04	-.14	.02	-.15	.139
Aggressive parenting → Right hippocampus	.07	.09	-.10	.25	.15	.414
5-HTTLPR X Aggressive Parenting →Right hippocampus	-.10	.07	-.26	.03	-.22	.166
Right hippocampus → MDD onset	**2.14**	**.83**	**.49**	**3.78**	**.64**	**.010**
Left hippocampus ↔ Right hippocampus	**.07**	**.01**	**.05**	**.09**	**.78**	**.000**
5-HTTLPR ↔ Aggressive parenting	-.03	.04	-.10	.04	-.07	.396
5-HTTLPR ↔ 5-HTTLPR X Aggressive parenting	-.02	.05	-.11	.07	-.04	.702
Aggressive parenting ↔ 5-HTTLPR X Aggressive parenting	**.32**	**.05**	**.24**	**.41**	**.80**	**.000**
Positive parenting → MDD onset	-.46	.24	-.88	.04	-.31	.051
Positive parenting → Left hippocampus	.03	.05	-.07	.13	.06	.605
Positive parenting → Right hippocampus	.04	.05	-.06	.14	.09	.438
Positive parenting ↔ 5-HTTLPR	.07	.05	-.02	.16	.14	.131
Positive parenting ↔ Aggressive parenting	**-.18**	**.03**	**-.25**	**-.12**	**-.44**	**.000**
Positive parenting ↔ 5-HTTLPR X Aggressive parenting	**-.12**	**.04**	**-.20**	**-.06**	**-.28**	**.001**
Positive parenting ↔ Gender	-.02	.03	-.07	.04	-.04	.628
Positive parenting ↔ Ethnicity	-.03	.02	-.08	.02	-.13	.220
Gender → MDD onset	.09	.25	-.37	.59	.04	.722
Gender → Left hippocampus	.01	.06	-.10	.13	.02	.837
Gender → Right hippocampus	.02	.06	-.09	.14	.03	.748
Gender ↔ 5-HTTLPR	.03	.03	-.02	.09	.10	.209
Gender ↔ Aggressive parenting	-.02	.03	-.07	.04	-.06	.538
Gender ↔ 5-HTTLPR X Aggressive parenting	.00	.03	-.05	.06	.01	.928
Gender ↔ Ethnicity	.02	.02	-.03	.07	.08	.482
Ethnicity → MDD onset	-.49	.59	-1.75	.31	-.16	.408

Ethnicity → Left hippocampus	.07	.10	-.12	.27	.07	.501
Ethnicity → Right hippocampus	.10	.09	-.07	.29	.11	.242
Ethnicity ↔ 5-HTTLPR	.03	.02	.00	.07	.13	.107
Ethnicity ↔ Aggressive parenting	.01	.02	-.03	.06	.07	.494
Ethnicity ↔5-HTTLPR x Aggressive parenting	.02	.02	-.03	.07	.08	.482
Positive parenting x Aggressive parenting → MDD	-.08	.40	-.88	.67	-.03	.837
Positive parenting x Aggressive parenting → Left hippocampus	.04	.09	-.13	.24	.05	.687
Positive parenting x Aggressive parenting → Right hippocampus	-.02	.10	-.21	.18	-.02	.837
Positive parenting x Aggressive parenting ↔ 5-HTTLPR	.04	.02	.00	.08	.15	.073
Positive parenting x Aggressive parenting ↔ Aggressive parenting	.01	.03	-.04	.07	.04	.746
Positive parenting x Aggressive parenting ↔ Gender	.03	.02	-.01	.06	.14	.108
Positive parenting x Aggressive parenting ↔ Positive parenting	-.04	.04	-.12	.03	-.15	.357
Positive parenting x Aggressive parenting ↔ 5-HTTLPR x Aggressive parenting	.02	.03	-.03	.08	.10	.424
Positive parenting x Aggressive parenting ↔ Ethnicity	.01	.01	-.01	.02	.07	.254
<u>Positive Parenting in the EPI</u>						
5-HTTLPR → MDD onset	-.18	.17	-.50	.17	-.13	.307
Positive parenting → MDD onset	-.47	.39	-1.19	.35	-.23	.235
5-HTTLPR X Positive Parenting → MDD onset	.65	.40	-.13	1.46	.31	.106
5-HTTLPR → Left hippocampus	-.08	.04	-.15	.00	-.18	.052
Positive parenting → Left hippocampus	-.13	.09	-.28	.07	-.21	.147
5-HTTLPR X Positive Parenting → Left hippocampus	.10	.08	-.05	.28	.17	.211
Left hippocampus → MDD onset	-1.71	1.00	-3.66	.15	-.51	.086
5-HTTLPR → Right hippocampus	-.05	.04	-.13	.03	-.13	.190
Positive parenting → Right hippocampus	-.12	.09	-.27	.06	-.19	.166
5-HTTLPR X Positive Parenting → Right hippocampus	.14	.09	-.02	.32	.22	.105
Right hippocampus → MDD onset	**1.92**	**.90**	**.13**	**3.55**	**.58**	**.032**
Left hippocampus ↔ Right hippocampus	**.06**	**.01**	**.05**	**.09**	**.79**	**.000**
5-HTTLPR ↔ Positive parenting	.03	.03	-.04	.09	.09	.376
5-HTTLPR ↔ 5-HTTLPR X Positive Parenting	.02	.04	-.06	.09	.05	.669
Positive parenting ↔ 5-HTTLPR X Positive Parenting	**.17**	**.03**	**.12**	**.23**	**.70**	**.000**
Aggressive parenting → MDD onset	**.76**	**.36**	**.04**	**1.45**	**.31**	**.035**
Aggressive parenting → Left hippocampus	-.06	.11	-.29	.15	-.08	.622
Aggressive parenting → Right hippocampus	-.11	.10	-.33	.06	-.15	.267
Aggressive parenting ↔ 5-HTTLPR	-.01	.03	-.06	.05	-.02	.847
Aggressive parenting ↔ Positive parenting	**-.06**	**.02**	**-.11**	**-.02**	**-.32**	**.005**
Aggressive parenting ↔ 5-HTTLPR X Positive parenting	**-.05**	**.02**	**-.10**	**-.01**	**-.24**	**.028**
Aggressive parenting ↔ Gender	.01	.02	-.03	.05	.05	.556
Aggressive parenting ↔ Ethnicity	.00	.02	-.03	.04	.03	.807
Gender → MDD onset	.05	.24	-.41	.51	.02	.848
Gender → Left hippocampus	.01	.06	-.11	.12	.01	.914
Gender → Right hippocampus	.00	.06	-.11	.11	.00	.965
Gender ↔ 5-HTTLPR	.03	.03	-.02	.09	.10	.209

Gender ↔ Positive parenting	.03	.03	-.04	.09	.09	.376
Gender ↔ 5-HTTLPR X Positive parenting	-.01	.02	-.05	.04	-.03	.780
Gender ↔ Ethnicity	.00	.01	-.03	.02	.00	.980
Ethnicity → MDD onset	-.28	.54	-1.15	.51	-.09	.608
Ethnicity → Left hippocampus	.06	.10	-.13	.26	.07	.543
Ethnicity → Right hippocampus	.11	.09	-.06	.30	.12	.213
Ethnicity ↔ 5-HTTLPR	.03	.03	-.02	.09	.10	.209
Ethnicity ↔ Positive parenting	-.03	.01	-.05	.00	-.15	.074
Ethnicity ↔5-HTTLPR x Positive parenting	-.03	.02	-.07	.00	-.21	.055
Positive parenting x Aggressive parenting → MDD	-.33	.68	-1.67	.95	-.08	.626
Positive parenting x Aggressive parenting → Left hippocampus	-.06	.17	-.37	.31	-.05	.734
Positive parenting x Aggressive parenting → Right hippocampus	-.28	.16	-.59	.06	-.23	.093
Positive parenting x Aggressive parenting ↔ 5-HTTLPR	.01	.02	-.02	.05	.05	.593
Positive parenting x Aggressive parenting ↔ Positive parenting	.03	.02	-.01	.09	.26	.193
Positive parenting x Aggressive parenting ↔ Gender	-.01	.01	-.03	.02	-.04	.632
Positive parenting x Aggressive parenting ↔ Aggressive parenting	**-.05**	**.02**	**-.10**	**-.01**	**-.46**	**.020**
Positive parenting x Aggressive parenting ↔ 5-HTTLPR x Positive parenting	.02	.02	-.02	.06	.12	.428
Positive parenting x Aggressive parenting ↔ Ethnicity	.00	.01	-.02	.02	.05	.666

Table S9

IGxE Path Models Testing Associations Between 5-HTTLPR Genotype, Hippocampal Volume and Parenting at 11-13 Years and Later MDD Onset During a Six Year Follow Up Period, Controlling for an Interaction Between the Parenting Variable of Interest x Gender

	b	SE	95% CI		β	p
			Lower	Upper		
Aggressive Parenting in the PSI						
5-HTTLPR → MDD onset	-.12	.18	-.46	.25	-.08	.522
Aggressive parenting → MDD onset	.24	.41	-.56	1.04	.14	.565
5-HTTLPR X Aggressive Parenting → MDD onset	-.34	.36	-1.06	.34	-.22	.343
5-HTTLPR → Left hippocampus	**-.08**	**.04**	**-.16**	**-.01**	**-.20**	**.038**
Aggressive parenting → Left hippocampus	.01	.08	-.17	.15	.01	.947
5-HTTLPR X Aggressive Parenting → Left hippocampus	-.06	.07	-.20	.07	-.13	.385
Left hippocampus → MDD onset	-1.79	.94	**-3.76**	**-.15**	-.53	.056
5-HTTLPR → Right hippocampus	-.06	.04	-.15	.02	-.15	.129
Aggressive parenting → Right hippocampus	.06	.09	-.12	.25	.12	.531
5-HTTLPR X Aggressive Parenting →Right hippocampus	-.11	.08	-.27	.03	-.24	.143
Right hippocampus → MDD onset	**2.15**	**.87**	**.53**	**3.87**	**.65**	**.014**
Left hippocampus ↔ Right hippocampus	**.07**	**.01**	**.05**	**.09**	**.78**	**.000**
5-HTTLPR ↔ Aggressive parenting	-.03	.04	-.10	.04	-.07	.396
5-HTTLPR ↔ 5-HTTLPR X Aggressive parenting	-.02	.05	-.11	.07	-.04	.702
Aggressive parenting ↔ 5-HTTLPR X Aggressive parenting	**.32**	**.05**	**.24**	**.41**	**.80**	**.000**
Positive parenting → MDD onset	-.45	.25	-.90	.07	-.31	.069
Positive parenting → Left hippocampus	.02	.05	-.09	.12	.04	.762
Positive parenting → Right hippocampus	.03	.05	-.07	.13	.08	.491
Positive parenting ↔ 5-HTTLPR	.07	.05	-.02	.16	.14	.131
Positive parenting ↔ Aggressive parenting	**-.18**	**.03**	**-.25**	**-.12**	**-.44**	**.000**
Positive parenting ↔ 5-HTTLPR X Aggressive parenting	**-.12**	**.04**	**-.20**	**-.06**	**-.28**	**.001**
Positive parenting ↔ Gender	-.02	.03	-.07	.04	-.04	.628
Positive parenting ↔ Ethnicity	-.03	.02	-.08	.02	-.13	.220
Gender → MDD onset	.08	.25	-.38	.60	.04	.753
Gender → Left hippocampus	.01	.06	-.11	.12	.02	.877
Gender → Right hippocampus	.01	.06	-.10	.13	.02	.839
Gender ↔ 5-HTTLPR	.03	.03	-.02	.09	.10	.209
Gender ↔ Aggressive parenting	-.02	.03	-.07	.04	-.06	.538
Gender ↔ 5-HTTLPR X Aggressive parenting	.00	.03	-.05	.06	.01	.928
Gender ↔ Ethnicity	.02	.02	-.03	.07	.08	.481
Ethnicity → MDD onset	-.50	.78	-2.03	.45	-.16	.523

Ethnicity → Left hippocampus	.04	.12	-.17	.27	.05	.711
Ethnicity → Right hippocampus	.08	.10	-.13	.28	.09	.435
Ethnicity ↔ 5-HTTLPR	.03	.02	.00	.07	.13	.107
Ethnicity ↔ Aggressive parenting	.01	.02	-.03	.06	.07	.493
Ethnicity ↔5-HTTLPR x Aggressive parenting	.02	.02	-.03	.07	.08	.481
Aggressive parenting x Gender → MDD	.11	2.15	-3.59	5.28	.02	.961
Aggressive parenting x Gender → Left hippocampus	.23	.25	-.13	.78	.15	.357
Aggressive parenting x Gender → Right hippocampus	.21	.26	-.06	.87	.13	.411
Aggressive parenting x Gender ↔ 5-HTTLPR	.00	.01	-.01	.03	.02	.831
Aggressive parenting x Gender ↔ Aggressive parenting	**.04**	**.02**	**.01**	**.08**	**.32**	**.024**
Aggressive parenting x Gender ↔ Gender	.01	.01	-.01	.02	.05	.571
Aggressive parenting x Gender ↔ Positive parenting	-.01	.01	-.02	.00	-.07	.144
Aggressive parenting x Gender ↔ 5-HTTLPR x Aggressive parenting	**.04**	**.02**	**.01**	**.09**	**.32**	**.043**
Aggressive parenting x Gender ↔ Ethnicity	.01	.02	-.03	.05	.20	.511
<u>Positive Parenting in the EPI</u>						
5-HTTLPR → MDD onset	-.18	.17	-.50	.17	-.13	.300
Positive parenting → MDD onset	-.31	.46	-1.23	.59	-.15	.504
5-HTTLPR X Positive Parenting → MDD onset	.66	.39	-.09	1.47	.32	.093
5-HTTLPR → Left hippocampus	-.08	.04	-.15	.00	-.18	.051
Positive parenting → Left hippocampus	-.11	.11	-.30	.13	-.18	.313
5-HTTLPR X Positive Parenting → Left hippocampus	.11	.08	-.04	.28	.17	.191
Left hippocampus → MDD onset	**-1.79**	**.90**	**-3.62**	**-.06**	**-.53**	**.048**
5-HTTLPR → Right hippocampus	-.05	.04	-.13	.03	-.13	.179
Positive parenting → Right hippocampus	-.11	.10	-.31	.10	-.18	.276
5-HTTLPR X Positive Parenting → Right hippocampus	.16	.09	-.01	.34	.25	.081
Right hippocampus → MDD onset	**2.02**	**.78**	**.45**	**3.53**	**.61**	**.009**
Left hippocampus ↔ Right hippocampus	**.07**	**.01**	**.05**	**.09**	**.78**	**.000**
5-HTTLPR ↔ Positive parenting	.03	.03	-.04	.09	.09	.376
5-HTTLPR ↔ 5-HTTLPR X Positive Parenting	.02	.04	-.06	.09	.05	.669
Positive parenting ↔ 5-HTTLPR X Positive Parenting	**.17**	**.03**	**.12**	**.23**	**.70**	**.000**
Aggressive parenting → MDD onset	**.84**	**.31**	**.17**	**1.41**	**.34**	**.007**
Aggressive parenting → Left hippocampus	-.04	.09	-.24	.13	-.06	.661
Aggressive parenting → Right hippocampus	-.04	.09	-.22	.13	-.05	.662
Aggressive parenting ↔ 5-HTTLPR	-.01	.03	-.06	.05	-.02	.847
Aggressive parenting ↔ Positive parenting	**-.06**	**.02**	**-.11**	**-.02**	**-.32**	**.005**
Aggressive parenting ↔ 5-HTTLPR X Positive parenting	**-.05**	**.02**	**-.10**	**-.01**	**-.24**	**.029**
Aggressive parenting ↔ Gender	.01	.02	-.03	.05	.05	.556
Aggressive parenting ↔ Ethnicity	.00	.02	-.03	.04	.03	.807
Gender → MDD onset	.05	.24	-.41	.51	.02	.835
Gender → Left hippocampus	.01	.06	-.10	.12	.01	.906
Gender → Right hippocampus	.00	.06	-.10	.12	.01	.937
Gender ↔ 5-HTTLPR	.03	.03	-.02	.09	.10	.209
Gender ↔ Positive parenting	.03	.03	-.04	.09	.09	.376

Gender ↔ 5-HTTLPR X Positive parenting	-.01	.02	-.05	.04	-.03	.780
Gender ↔ Ethnicity	.00	.01	-.03	.02	.00	.980
Ethnicity → MDD onset	-.27	.54	-1.17	.51	-.09	.610
Ethnicity → Left hippocampus	.06	.10	-.13	.26	.07	.536
Ethnicity → Right hippocampus	.10	.09	-.07	.29	.11	.243
Ethnicity ↔ 5-HTTLPR	.03	.03	-.02	.09	.10	.209
Ethnicity ↔ Positive parenting	-.03	.01	-.05	.00	-.15	.074
Ethnicity ↔5-HTTLPR x Positive parenting	-.03	.02	-.07	.00	-.21	.055
Positive parenting x Gender → MDD	-.35	.45	-1.25	.54	-.12	.447
Positive parenting x Gender → Left hippocampus	-.05	.10	-.25	.16	-.06	.636
Positive parenting x Gender → Right hippocampus	-.07	.11	-.27	.15	-.08	.535
Positive parenting x Gender ↔ 5-HTTLPR	.02	.03	-.03	.08	.09	.400
Positive parenting x Gender ↔ Positive parenting	**.13**	**.03**	**.09**	**.19**	**.74**	**.000**
Positive parenting x Gender ↔ Gender	-.01	.02	-.04	.02	-.04	.648
Positive parenting x Gender ↔ Aggressive parenting	**-.04**	**.02**	**-.08**	**-.01**	**-.26**	**.029**
Positive parenting x Gender ↔ 5-HTTLPR x Positive parenting	**.09**	**.02**	**.05**	**.14**	**.50**	**.000**
Positive parenting x Gender ↔ Ethnicity	-.01	.01	-.02	.01	-.05	.462

Table S10

IGxE Path Models Testing Associations Between 5-HTTLPR Genotype, Hippocampal Volume and Parenting at 11-13 Years and Later MDD Onset During a Six Year Follow Up Period, Controlling for an Interaction Between the Parenting Variable of Interest x Ethnicity

	b	SE	95% CI		β	p
			Lower	Upper		
Aggressive Parenting in the PSI						
5-HTTLPR → MDD onset	-.11	.18	-.46	.26	-.08	.550
Aggressive parenting → MDD onset	.24	.38	-.49	1.02	.14	.540
5-HTTLPR X Aggressive Parenting → MDD onset	-.33	.37	-1.13	.35	-.21	.381
5-HTTLPR → Left hippocampus	**-.09**	**.04**	**-.17**	**-.01**	**-.21**	**.030**
Aggressive parenting → Left hippocampus	.03	.08	-.14	.17	.06	.733
5-HTTLPR X Aggressive Parenting → Left hippocampus	-.05	.06	-.18	.07	-.12	.411
Left hippocampus → MDD onset	-1.77	.91	**-3.65**	**-.08**	-.53	.052
5-HTTLPR → Right hippocampus	-.06	.04	-.14	.02	-.15	.139
Aggressive parenting → Right hippocampus	.07	.09	-.10	.25	.15	.414
5-HTTLPR X Aggressive Parenting →Right hippocampus	-.10	.07	-.26	.03	-.22	.166
Right hippocampus → MDD onset	**2.14**	**.83**	**.49**	**3.78**	**.64**	**.010**
Left hippocampus ↔ Right hippocampus	**.07**	**.01**	**.05**	**.09**	**.78**	**.000**
5-HTTLPR ↔ Aggressive parenting	-.03	.04	-.10	.04	-.07	.396
5-HTTLPR ↔ 5-HTTLPR X Aggressive parenting	-.02	.05	-.11	.07	-.04	.702
Aggressive parenting ↔ 5-HTTLPR X Aggressive parenting	**.32**	**.05**	**.24**	**.41**	**.80**	**.000**
Positive parenting → MDD onset	-.46	.24	-.88	.04	-.31	.051
Positive parenting → Left hippocampus	.03	.05	-.07	.13	.06	.605
Positive parenting → Right hippocampus	.04	.05	-.06	.14	.09	.438
Positive parenting ↔ 5-HTTLPR	.07	.05	-.02	.16	.14	.131
Positive parenting ↔ Aggressive parenting	**-.18**	**.03**	**-.25**	**-.12**	**-.44**	**.000**
Positive parenting ↔ 5-HTTLPR X Aggressive parenting	**-.12**	**.04**	**-.20**	**-.06**	**-.28**	**.001**
Positive parenting ↔ Gender	-.02	.03	-.07	.04	-.04	.628
Positive parenting ↔ Ethnicity	-.03	.02	-.08	.02	-.13	.220
Gender → MDD onset	.09	.25	-.37	.59	.04	.722
Gender → Left hippocampus	.01	.06	-.10	.13	.02	.837
Gender → Right hippocampus	.02	.06	-.09	.14	.03	.748
Gender ↔ 5-HTTLPR	.03	.03	-.02	.09	.10	.209
Gender ↔ Aggressive parenting	-.02	.03	-.07	.04	-.06	.538
Gender ↔ 5-HTTLPR X Aggressive parenting	.00	.03	-.05	.06	.01	.928
Gender ↔ Ethnicity	.02	.02	-.03	.07	.08	.482
Ethnicity → MDD onset	-.49	.59	-1.75	.31	-.16	.408
Ethnicity → Left hippocampus	.07	.10	-.12	.27	.07	.501

Ethnicity → Right hippocampus	.10	.09	-.07	.29	.11	.242
Ethnicity ↔ 5-HTTLPR	.03	.02	.00	.07	.13	.107
Ethnicity ↔ Aggressive parenting	.01	.02	-.03	.06	.07	.494
Ethnicity ↔5-HTTLPR x Aggressive parenting	.02	.02	-.03	.07	.08	.482
Aggressive parenting x Ethnicity → MDD	-.08	.40	-.88	.67	-.03	.837
Aggressive parenting x Ethnicity → Left hippocampus	.04	.09	-.13	.24	.05	.687
Aggressive parenting x Ethnicity → Right hippocampus	-.02	.10	-.21	.18	-.02	.837
Aggressive parenting x Ethnicity ↔ 5-HTTLPR	.04	.02	.00	.08	.15	.073
Aggressive parenting x Ethnicity ↔ Aggressive parenting	.01	.03	-.04	.07	.04	.746
Aggressive parenting x Ethnicity ↔ Gender	.03	.02	-.01	.06	.14	.108
Aggressive parenting x Ethnicity ↔ Positive parenting	-.04	.04	-.12	.03	-.15	.357
Aggressive parenting x Ethnicity ↔ 5-HTTLPR x Aggressive parenting	.02	.03	-.03	.08	.10	.424
Aggressive parenting x Ethnicity ↔ Ethnicity	.01	.01	-.01	.02	.07	.254
<u>Positive Parenting in the EPI</u>						
5-HTTLPR → MDD onset	-.20	.17	-.52	.14	-.14	.248
Positive parenting → MDD onset	-.45	.35	-1.11	.31	-.22	.208
5-HTTLPR X Positive Parenting → MDD onset	**.76**	**.37**	**.03**	**1.50**	**.37**	**.041**
5-HTTLPR → Left hippocampus	**-.08**	**.04**	**-.15**	**.00**	**-.18**	**.050**
Positive parenting → Left hippocampus	-.13	.08	-.27	.05	-.22	.097
5-HTTLPR X Positive Parenting → Left hippocampus	.11	.08	-.04	.29	.18	.177
Left hippocampus → MDD onset	-1.70	.92	-3.67	-.02	-.51	.066
5-HTTLPR → Right hippocampus	-.06	.04	-.13	.02	-.14	.156
Positive parenting → Right hippocampus	-.14	.09	-.31	.06	-.23	.132
5-HTTLPR X Positive Parenting → Right hippocampus	.17	.09	.00	.36	.27	.059
Right hippocampus → MDD onset	1.89	.80	.37	3.49	.57	.018
Left hippocampus ↔ Right hippocampus	**.07**	**.01**	**.05**	**.09**	**.78**	**.000**
5-HTTLPR ↔ Positive parenting	.03	.03	-.04	.09	.09	.376
5-HTTLPR ↔ 5-HTTLPR X Positive Parenting	.02	.04	-.06	.09	.05	.669
Positive parenting ↔ 5-HTTLPR X Positive Parenting	**.17**	**.03**	**.12**	**.23**	**.70**	**.000**
Aggressive parenting → MDD onset	**.87**	**.31**	**.23**	**1.44**	**.36**	**.005**
Aggressive parenting → Left hippocampus	-.04	.09	-.24	.13	-.05	.676
Aggressive parenting → Right hippocampus	-.03	.09	-.21	.13	-.05	.699
Aggressive parenting ↔ 5-HTTLPR	-.01	.03	-.06	.05	-.02	.847
Aggressive parenting ↔ Positive parenting	**-.06**	**.02**	**-.11**	**-.02**	**-.32**	**.005**
Aggressive parenting ↔ 5-HTTLPR X Positive parenting	**-.05**	**.02**	**-.10**	**-.01**	**-.24**	**.029**
Aggressive parenting ↔ Gender	.01	.02	-.03	.05	.05	.556
Aggressive parenting ↔ Ethnicity	.00	.02	-.03	.04	.03	.807
Gender → MDD onset	.09	.24	-.37	.56	.04	.709
Gender → Left hippocampus	.01	.06	-.10	.12	.01	.886
Gender → Right hippocampus	.01	.06	-.10	.12	.02	.861
Gender ↔ 5-HTTLPR	.03	.03	-.02	.09	.10	.209
Gender ↔ Positive parenting	.03	.03	-.04	.09	.09	.376

Gender ↔ 5-HTTLPR X Positive parenting	-.01	.02	-.05	.04	-.03	.780
Gender ↔ Ethnicity	.00	.01	-.03	.02	.00	.980
Ethnicity → MDD onset	-.63	.68	-1.76	.32	-.21	.353
Ethnicity → Left hippocampus	.04	.14	-.25	.28	.05	.754
Ethnicity → Right hippocampus	.05	.12	-.22	.26	.06	.669
Ethnicity ↔ 5-HTTLPR	.03	.03	-.02	.09	.10	.209
Ethnicity ↔ Positive parenting	-.03	.01	-.05	.00	-.15	.074
Ethnicity ↔5-HTTLPR x Positive parenting	-.03	.02	-.07	.00	-.21	.054
Positive parenting x Ethnicity → MDD	-1.90	2.53	-6.74	.33	-.25	.451
Positive parenting x Ethnicity → Left hippocampus	-.08	.41	-.68	.79	-.04	.847
Positive parenting x Ethnicity → Right hippocampus	-.26	.27	-.73	.21	-.12	.323
Positive parenting x Ethnicity ↔ 5-HTTLPR	-.01	.01	-.03	.01	-.07	.399
Positive parenting x Ethnicity ↔ Positive parenting	**.02**	**.01**	**.00**	**.04**	**.28**	**.045**
Positive parenting x Ethnicity ↔ Gender	.01	.01	-.01	.02	.07	.457
Positive parenting x Ethnicity ↔ Aggressive parenting	.00	.01	-.02	.01	-.05	.737
Positive parenting x Ethnicity ↔ 5-HTTLPR x Positive parenting	.02	.01	.00	.05	.32	.053
Positive parenting x Ethnicity ↔ Ethnicity	-.02	.01	-.05	.00	-.49	.073

Table S11

Bootstrapping Estimates of the Indirect Effect (5-HTTLPR → Hippocampal Volume → MDD Onset) at Varying Levels of Parenting Behaviour and the Index of Moderated Mediation (the Extent to Which the Indirect Effect of 5-HTTLPR → Hippocampal Volume → MDD Onset Varies as a Linear Function of Parenting Behaviour) for the Model Controlling for an Interaction Between 5-HTTLPR x Gender

	Left Hippocampus						Right Hippocampus					
	Raw *M*	Raw *SE*	Bootstrapped 95% CI		Bootstrapped 90% CI		Raw *M*	Raw *SE*	Bootstrapped 95% CI		Bootstrapped 90% CI	
			Lower	Upper	Lower	Upper			Lower	Upper	Lower	Upper
<u>Aggressive Parenting (PSI)</u>												
High (+1SD)	**.24**	**.18**	**.01**	**.76**	**.04**	**.66**	**-.37**	**.19**	**-.99**	**-.05**	**-.87**	**-.08**
Average (M)	.19	.16	-.002	.64	**.02**	**.56**	**-.24**	**.17**	**-.70**	**-.01**	**-.61**	**-.04**
Low (-1SD)	.14	.18	-.06	.67	-.02	.56	-.11	.24	-.60	.17	-.49	.11
Index of Moderated Mediation	.08	.13	-.12	.45	-.07	.37	-.21	.20	-.74	.08	-.64	.03
<u>Positive Parenting (EPI)</u>												
High (+1SD)	.07	.15	-.16	.46	-.11	.38	-.02	.16	-.37	.27	-.30	.21
Average (M)	.18	.16	-.01	.61	**.01**	**.53**	**-.22**	**.16**	**-.62**	**-.01**	**-.54**	**-.03**
Low (-1SD)	**.30**	**.24**	**.01**	**.96**	**.04**	**.83**	**-.41**	**.25**	**-1.06**	**-.06**	**-.93**	**-.11**
Index of Moderated Mediation	-.19	.20	-.82	.03	-.68	.000	**.32**	**.23**	**.003**	**.92**	**.04**	**.81**

Table S12

Bootstrapping Estimates of the Indirect Effect (5-HTTLPR → Hippocampal Volume → MDD Onset) at Varying Levels of Parenting Behaviour and the Index of Moderated Mediation (the Extent to Which the Indirect Effect of 5-HTTLPR → Hippocampal Volume → MDD Onset Varies as a Linear Function of Parenting Behaviour) for the Model Controlling for an Interaction Between 5-HTTLPR x Ethnicity

	Left Hippocampus						Right Hippocampus					
	Raw *M*	Raw *SE*	Bootstrapped 95% CI		Bootstrapped 90% CI		Raw *M*	Raw *SE*	Bootstrapped 95% CI		Bootstrapped 90% CI	
			Lower	Upper	Lower	Upper			Lower	Upper	Lower	Upper
<u>Aggressive Parenting (PSI)</u>												
High (+1SD)	**.22**	**.17**	**.01**	**.71**	**.04**	**.62**	-.28	.14	-.88	.000	**-.76**	**-.03**
Average (M)	.16	.13	.000	.53	**.02**	**.46**	-.14	.13	-.49	.02	-.43	.001
Low (-1SD)	.10	.14	-.06	.53	-.03	.44	-.002	.21	-.28	.28	-.22	.22
Index of Moderated Mediation	.10	.14	-.08	.54	-.03	.45	-.23	.20	-.81	.04	-.70	.003
<u>Positive Parenting (EPI)</u>												
High (+1SD)	.02	.13	-.20	.33	-.14	.26	.08	.25	-.14	.43	-.08	.37
Average (M)	.15	.13	-.01	.50	**.01**	**.43**	-.12	.12	-.44	.04	-.37	.01
Low (-1SD)	**.28**	**.24**	**.01**	**.98**	**.05**	**.84**	**-.32**	**.14**	**-1.03**	**-.01**	**-.88**	**-.05**
Index of Moderated Mediation	-.22	.23	-.99	.03	**-.83**	**-.01**	.33	.26	-.01	1.09	**.03**	**.94**

Table S13

Bootstrapping Estimates of the Indirect Effect (5-HTTLPR → Hippocampal Volume → MDD Onset) at Varying Levels of Parenting Behaviour and the Index of Moderated Mediation (the Extent to Which the Indirect Effect of 5-HTTLPR → Hippocampal Volume → MDD Onset Varies as a Linear Function of Parenting Behaviour) for the Model Controlling for an Interaction Between 5-HTTLPR x the Other Parenting Variable of Interest

	Left Hippocampus						Right Hippocampus					
	Raw *M*	Raw *SE*	Bootstrapped 95% CI		Bootstrapped 90% CI		Raw *M*	Raw *SE*	Bootstrapped 95% CI		Bootstrapped 90% CI	
			Lower	Upper	Lower	Upper			Lower	Upper	Lower	Upper
<u>Aggressive Parenting (PSI)</u>												
High (+1SD)	**.19**	**.14**	**.004**	**.61**	**.03**	**.53**	**-.20**	**.14**	**-.71**	**.01**	**-.61**	**-.01**
Average (M)	.14	.11	.000	.45	**.01**	**.39**	-.12	.11	-.41	.02	-.35	.001
Low (-1SD)	.08	.14	-.08	.50	-.05	.41	-.03	.17	-.35	.22	-.28	.17
Index of Moderated Mediation	.08	.14	-.11	.50	-.06	.42	-.14	.19	-.68	.12	-.58	.07
<u>Positive Parenting (EPI)</u>												
High (+1SD)	.03	.14	-.20	.36	-.15	.29	.07	.21	-.16	.41	-.11	.35
Average (M)	.13	.11	-.01	.42	**.01**	**.36**	-.11	.11	-.37	.04	-.32	.02
Low (-1SD)	.23	.19	**.002**	**.79**	**.03**	**.67**	**-.28**	**.14**	**-.85**	**-.01**	**-.74**	**-.04**
Index of Moderated Mediation	-.16	.20	-.82	.09	-.67	.04	.29	.24	-.03	.95	**.01**	**.81**

Table S14

Bootstrapping Estimates of the Indirect Effect (5-HTTLPR → Hippocampal Volume → MDD Onset) at Varying Levels of Parenting Behaviour and the Index of Moderated Mediation (the Extent to Which the Indirect Effect of 5-HTTLPR → Hippocampal Volume → MDD Onset Varies as a Linear Function of Parenting Behaviour) for the Model Controlling for an Interaction Between Positive Parenting x Aggressive Parenting

	Left Hippocampus						Right Hippocampus					
	Raw *M*	Raw *SE*	Bootstrapped 95% CI		Bootstrapped 90% CI		Raw *M*	Raw *SE*	Bootstrapped 95% CI		Bootstrapped 90% CI	
			Lower	Upper	Lower	Upper			Lower	Upper	Lower	Upper
<u>Aggressive Parenting (PSI)</u>												
High (+1SD)	**.21**	**.15**	**.01**	**.63**	**.03**	**.55**	**-.27**	**.14**	**-.78**	**-.01**	**-.69**	**-.04**
Average (M)	**.15**	**.12**	**.003**	**.49**	**.02**	**.42**	-.13	.12	-.45	.02	-.39	.000
Low (-1SD)	.10	.14	-.07	.50	-.04	.41	.000	.19	-.28	.28	-.22	.22
Index of Moderated Mediation	.09	.13	-.09	.46	-.05	.39	-.22	.19	-.75	.04	-.65	.002
<u>Positive Parenting (EPI)</u>												
High (+1SD)	.02	.14	-.21	.33	-.15	.26	.06	.21	-.16	.40	-.11	.33
Average (M)	.13	.12	-.01	.43	**.01**	**.37**	-.10	.11	-.38	.04	-.32	.01
Low (-1SD)	**.24**	**.21**	**.002**	**.83**	**.03**	**.70**	**-.26**	**.14**	**-.84**	**-.01**	**-.73**	**-.04**
Index of Moderated Mediation	-.18	.21	-.87	.05	-.72	.01	.27	.23	-.02	.92	**.02**	**.79**

Table S15

Bootstrapping Estimates of the Indirect Effect (5-HTTLPR → Hippocampal Volume → MDD Onset) at Varying Levels of Parenting Behaviour and the Index of Moderated Mediation (the Extent to Which the Indirect Effect of 5-HTTLPR → Hippocampal Volume → MDD Onset Varies as a Linear Function of Parenting Behaviour) for the Model Controlling for an Interaction Between the Parenting Variable of Interest x Gender

	Left Hippocampus						Right Hippocampus					
	Raw *M*	Raw *SE*	Bootstrapped 95% CI		Bootstrapped 90% CI		Raw *M*	Raw *SE*	Bootstrapped 95% CI		Bootstrapped 90% CI	
			Lower	Upper	Lower	Upper			Lower	Upper	Lower	Upper
<u>Aggressive Parenting (PSI)</u>												
High (+1SD)	**.20**	**.14**	**.01**	**.61**	**.03**	**.53**	**-.27**	**.14**	**-.81**	**-.01**	**-.71**	**-.04**
Average (M)	**.15**	**.12**	**.001**	**.47**	**.02**	**.41**	-.14	.12	-.45	.02	**-.39**	**-.003**
Low (-1SD)	.10	.14	-.07	.50	-.04	.42	.00	.19	-.28	.29	-.22	.23
Index of Moderated Mediation	.08	.13	-.11	.45	-.06	.37	-.22	.20	-.77	.05	-.67	.01
<u>Positive Parenting (EPI)</u>												
High (+1SD)	.02	.13	-.22	.32	-.17	.25	.08	.20	-.15	.43	-.10	.37
Average (M)	.13	.11	-.004	.43	**.01**	**.37**	-.11	.10	-.37	.04	-.32	.01
Low (-1SD)	**.25**	**.19**	**.02**	**.81**	**.05**	**.69**	**-.30**	**.14**	**-.85**	**-.03**	**-.75**	**-.06**
Index of Moderated Mediation	-.19	.20	-.84	.04	-.70	.000	.31	.23	-.01	.95	**.04**	**.83**

Table S16

Bootstrapping Estimates of the Indirect Effect (5-HTTLPR → Hippocampal Volume → MDD Onset) at Varying Levels of Parenting Behaviour and the Index of Moderated Mediation (the Extent to Which the Indirect Effect of 5-HTTLPR → Hippocampal Volume → MDD Onset Varies as a Linear Function of Parenting Behaviour) for the Model Controlling for an Interaction Between the Parenting Variable of Interest x Ethnicity

	Left Hippocampus						Right Hippocampus					
	Raw *M*	Raw *SE*	Bootstrapped 95% CI		Bootstrapped 90% CI		Raw *M*	Raw *SE*	Bootstrapped 95% CI		Bootstrapped 90% CI	
			Lower	Upper	Lower	Upper			Lower	Upper	Lower	Upper
<u>Aggressive Parenting (PSI)</u>												
High (+1SD)	**.21**	**.15**	**.02**	**.65**	**.04**	**.56**	**-.28**	**.14**	**-.83**	**-.02**	**-.73**	**-.05**
Average (M)	**.15**	**.12**	**.01**	**.49**	**.02**	**.43**	-.14	.12	-.46	.01	**-.40**	**-.01**
Low (-1SD)	.09	.13	-.07	.50	-.04	.41	.01	.20	-.25	.31	-.19	.25
Index of Moderated Mediation	.10	.13	-.07	.51	-.03	.43	-.24	.20	-.80	.03	**-.70**	**-.01**
<u>Positive Parenting (EPI)</u>												
High (+1SD)	.02	.12	-.20	.31	-.15	.24	.09	.21	-.11	.42	-.06	.36
Average (M)	.13	.11	-.003	.44	**.01**	**.38**	-.11	.10	-.38	.02	-.33	.001
Low (-1SD)	**.24**	**.20**	**.01**	**.85**	**.04**	**.72**	**-.30**	**.13**	**-.89**	**-.03**	**-.78**	**-.07**
Index of Moderated Mediation	-.19	.20	-.87	.02	**-.73**	**-.01**	**.32**	**.23**	**.02**	**.98**	**.06**	**.86**

Supplemental Materials

Linking the Serotonin Transporter Gene, Family Environments, Hippocampal Volume and Depression Onset: A Prospective Imaging Gene x Environment Analysis

by K. Little et al., 2015, *Journal of Abnormal Psychology*

http://dx.doi.org/10.1037/abn0000101

Table S17

Complete Results of the Two Separate Path Models Investigating the Moderating Effect of the Aversive Behaviour in the PSI Task and Positive Behaviour in the EPI Task for the Group of Participants From an Anglo-European Background

	b	SE	95% CI		β	p
			Upper	Lower		
Aggressive Parenting in the PSI						
5-HTTLPR → MDD onset (n=120)	-0.10	0.19	-0.45	0.28	-0.07	0.596
Aggressive parenting → MDD onset (n=89)	0.38	0.41	-0.39	1.22	0.23	0.354
5-HTTLPR X Aggressive Parenting → MDD onset (n=89)	-0.57	0.36	-1.33	0.12	-0.36	0.118
5-HTTLPR → Left hippocampus (n=106)	**-0.09**	**0.04**	**-0.17**	**-0.01**	**-0.21**	**0.035**
Aggressive parenting → Left hippocampus (n=82)	0.02	0.08	-0.15	0.17	0.04	0.807
5-HTTLPR X Aggressive Parenting → Left hippocampus (n=82)	-0.04	0.07	-0.19	0.09	-0.10	0.523
Left hippocampus → MDD onset (n=86)	-1.26	0.97	-3.28	0.55	-0.37	0.193
5-HTTLPR → Right hippocampus (n=106)	-0.06	0.04	-0.15	0.02	-0.15	0.166
Aggressive parenting → Right hippocampus (n=82)	0.09	0.10	-0.09	0.29	0.19	0.338
5-HTTLPR X Aggressive Parenting →Right hippocampus (n=82)	-0.11	0.08	-0.28	0.04	-0.23	0.193
Right hippocampus → MDD onset (n=86)	1.73	0.89	0.00	3.51	0.51	0.051
Left hippocampus ↔ Right hippocampus (n=106)	**0.07**	**0.01**	**0.05**	**0.09**	**0.79**	**0.000**
5-HTTLPR ↔ Aggressive parenting (n=111)	-0.03	0.04	-0.10	0.04	-0.06	0.479
5-HTTLPR ↔ 5-HTTLPR X Aggressive Parenting (n=111)	-0.03	0.05	-0.13	0.07	-0.06	0.563
Aggressive parenting ↔ 5-HTTLPR X Aggressive Parenting (n=111)	**0.31**	**0.05**	**0.22**	**0.41**	**0.80**	**0.000**
Positive parenting → MDD onset (n=89)	**-0.54**	**0.26**	**-1.00**	**0.01**	**-0.36**	**0.035**
Positive parenting → Left hippocampus (n=82)	0.04	0.06	-0.07	0.15	0.09	0.509
Positive parenting → Right hippocampus (n=82)	0.06	0.06	-0.05	0.17	0.13	0.293
Positive parenting ↔ 5-HTTLPR (n=111)	0.08	0.05	-0.01	0.18	0.17	0.090
Positive parenting ↔ Aggressive parenting (n=111)	**-0.18**	**0.04**	**-0.26**	**-0.12**	**-0.46**	**0.000**
Positive parenting ↔ 5-HTTLPR X Aggressive parenting (n=111)	**-0.13**	**0.04**	**-0.21**	**-0.06**	**-0.29**	**0.002**
Positive parenting ↔ Gender (n=111)	-0.04	0.03	-0.11	0.02	-0.13	0.173
Gender → MDD onset (n=120)	-0.08	0.26	-0.57	0.45	-0.04	0.763

Gender → Left hippocampus (n=106)	0.06	0.06	-0.05	0.19	0.11	0.295
Gender → Right hippocampus (n=106)	0.08	0.06	-0.03	0.20	0.14	0.186
Gender ↔ 5-HTTLPR (n=150)	0.03	0.03	-0.03	0.09	0.09	0.290
Gender ↔ Aggressive parenting (n=111)	-0.03	0.04	-0.10	0.04	-0.06	0.479
Gender ↔ 5-HTTLPR X Aggressive parenting (n=111)	-0.01	0.03	-0.07	0.05	-0.04	0.686
Positive Parenting in the EPI						
5-HTTLPR → MDD onset (n=120)	-0.16	0.18	-0.49	0.21	-0.11	0.379
Positive parenting → MDD onset (n=89)	-0.44	0.36	-1.12	0.30	-0.22	0.222
5-HTTLPR X Positive Parenting → MDD onset (n=89)	**0.86**	**0.40**	**0.11**	**1.70**	**0.41**	**0.031**
5-HTTLPR → Left hippocampus (n=106)	**-0.08**	**0.04**	**-0.16**	**0.00**	**-0.19**	**0.050**
Positive parenting → Left hippocampus (n=82)	-0.13	0.08	-0.28	0.06	-0.22	0.115
5-HTTLPR X Positive Parenting → Left hippocampus (n=82)	0.11	0.08	-0.04	0.30	0.18	0.191
Left hippocampus → MDD onset (n=89)	-1.27	0.98	-3.29	0.56	-0.37	0.196
5-HTTLPR → Right hippocampus (n=106)	-0.05	0.04	-0.13	0.03	-0.12	0.224
Positive parenting → Right hippocampus (n=82)	-0.13	0.08	-0.28	0.06	-0.22	0.115
5-HTTLPR X Positive Parenting → Right hippocampus (n=82)	0.11	0.08	-0.04	0.30	0.18	0.191
Right hippocampus → MDD onset (n=86)	1.57	0.84	-0.14	3.16	0.46	0.061
Left hippocampus ↔ Right hippocampus (n=106)	**0.06**	**0.01**	**0.05**	**0.09**	**0.79**	**0.000**
5-HTTLPR ↔ Positive parenting (n=111)	0.04	0.03	-0.03	0.11	0.11	0.271
5-HTTLPR ↔ 5-HTTLPR X Positive Parenting (n=111)	0.03	0.04	-0.05	0.10	0.09	0.409
Positive parenting ↔ 5-HTTLPR X Positive Parenting (n=111)	**0.16**	**0.03**	**0.11**	**0.23**	**0.69**	**0.000**
Aggressive parenting → MDD onset (n=89)	**1.03**	**0.32**	**0.35**	**1.62**	**0.41**	**0.001**
Aggressive parenting → Left hippocampus (n=82)	-0.05	0.11	-0.27	0.15	-0.06	0.653
Aggressive parenting → Right hippocampus (n=82)	-0.04	0.11	-0.26	0.16	-0.06	0.691
Aggressive parenting ↔ 5-HTTLPR (n=111)	-0.02	0.03	-0.08	0.03	-0.09	0.374
Aggressive parenting ↔ Positive parenting (n=111)	**-0.07**	**0.02**	**-0.12**	**-0.02**	**-0.34**	**0.006**
Aggressive parenting ↔ 5-HTTLPR X Positive parenting (n=111)	**-0.04**	**0.02**	**-0.08**	**-0.01**	**-0.22**	**0.027**
Aggressive parenting ↔ Gender (n=111)	0.00	0.02	-0.03	0.04	0.02	0.819
Gender → MDD onset (n=120)	0.00	0.25	-0.47	0.50	0.00	0.996
Gender → Left hippocampus (n=106)	0.05	0.06	-0.06	0.17	0.08	0.398
Gender → Right hippocampus (n=106)	0.06	0.06	-0.05	0.18	0.10	0.299
Gender ↔ 5-HTTLPR (n=150)	0.03	0.03	-0.03	0.09	0.09	0.290
Gender ↔ Positive parenting (n=111)	-0.02	0.02	-0.07	0.02	-0.09	0.332
Gender ↔ 5-HTTLPR X Positive parenting (n=111)	-0.01	0.02	-0.05	0.03	-0.04	0.667

The number of participants used to calculate each statistic, as a result of the use of pairwise analysis, is provided in brackets (n=).

***** Each path analysis contained the serotonin transporter gene, parenting and the specific serotonin transporter gene X parenting interaction of interest as independent variables, left and right hippocampal volume as mediating variables, and MDD onset as the dependent variable, with adolescent gender and the other parenting variable recorded during the same task as covariates**.**

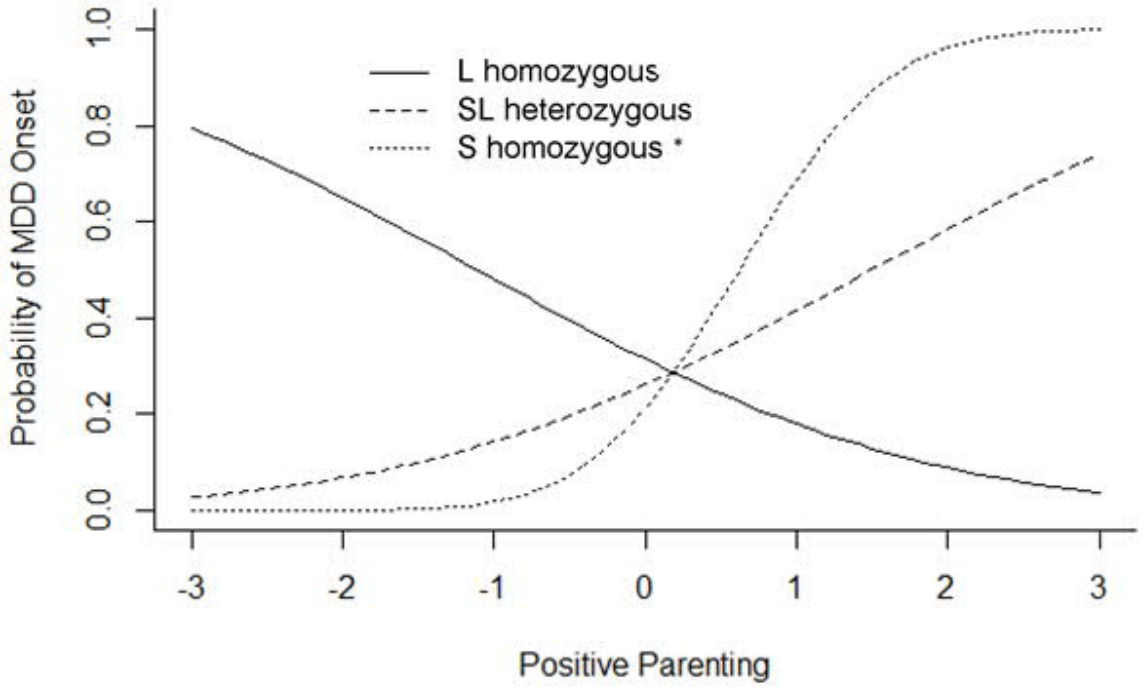


Figure S1. Influence of positive parenting behaviors measured during the EPI task when participants were 11-13 years on probability of MDD onset during adolescence for LL homozygous, SL heterozygous and SS homozygous individuals, controlling for gender and aggressive parenting in the EPI task, and indirect pathways from 5-HTTLPR to MDD through left and right hippocampal volume, for participants of Anglo-European background.

Table S18

Bootstrapping Estimates of the Indirect Path (5-HTTLPR → Hippocampal Volume → MDD Onset) at Varying Levels of Parenting Behaviour and the Index of Moderated Mediation (the Extent to Which the Indirect Effect of 5-HTTLPR → Hippocampal Volume → MDD Onset Varies as a Linear Function of Parenting Behaviour) for the Group of Participants From an Anglo-European Background

	Left Hippocampus						Right Hippocampus					
	Raw *M*	Raw *SE*	Bootstrapped 95% CI		Bootstrapped 90% CI		Raw *M*	Raw *SE*	Bootstrapped 95% CI		Bootstrapped 90% CI	
			Lower	Upper	Lower	Upper			Lower	Upper	Lower	Upper
<u>Aggressive Parenting (PSI)</u>												
High (+1SD)	.15	.14	-.03	.57	-.003	.49	-.22	.12	-.78	.01	**-.67**	**-.02**
Average (M)	.11	.12	-.03	.45	-.01	.39	-.11	.11	-.44	.02	-.37	.003
Low (-1SD)	.08	.13	-.04	.50	-.02	.41	.01	.19	-.22	.27	-.16	.21
Index of Moderated Mediation	.06	.11	-.08	.42	-.04	.35	-.18	.18	-.74	.04	-.63	.01
<u>Positive Parenting (EPI)</u>												
High (+1SD)	.03	.10	-.09	.34	-.06	.27	.05	.18	-.11	.32	-.07	.27
Average (M)	.10	.11	-.03	.42	-.01	.35	-.08	.10	-.35	.03	-.29	.01
Low (-1SD)	.17	.18	-.04	.69	-.01	.57	-.21	.11	-.73	.003	**-.62**	**-.02**
Index of Moderated Mediation	-.14	.19	-.80	.04	-.66	.01	.26	.22	-.02	.92	**.02**	**.79**

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