Bridging the link between trauma, brain development and depression: epigenetic mechanisms

by

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Abstract

Despite its large impact on the individual and society, we currently have only a rudimentary understanding of the biological basis of Major Depressive Disorder, even less so in adolescent populations. This thesis focuses on two research questions. First, how do adolescents with depression differ from adolescents who have never been depressed on (1a) brain morphology and (1b) DNA methylation? We studied differences in the fronto-limbic system (a collection of areas responsible for emotion regulation) and methylation at the serotonin transporter (*SLC6A4*) and FK506 binding protein gene (*FKBP5*) genes (two genes strongly linked to stress regulation and depression). Second, how does childhood trauma, which is known to increase risk for depression, affect (2a) brain development and (2b) *SLC6A4* and *FKBP5* methylation? Further, (2c) how might DNA methylation explain how trauma affects brain development in depression? We studied these questions in 24 adolescent depressed patients and 21 controls. We found that (1a) depressed adolescents had decreased left precuneus volume and greater volume of the left precentral gyrus compared to controls; however, no differences in fronto-limbic morphology were identified. Moreover, (1b) individuals with depression had lower levels of *FKBP5* methylation than controls. In line with our second hypothesis (2a) greater levels of trauma were associated with decreased volume of a number of fronto-limbic regions. Further, we found that (2b) greater trauma was associated with decreased *SLC6A4*, but not *FKBP5*, methylation. Finally, (2c) greater *FKBP5*, but not *SLC6A4*, methylation was associated with decreased volume of a number of fronto-limbic regions. The results of this study suggest an association among trauma, DNA methylation and brain development in youth, but the direction of these relationships appears to be inconsistent. Future studies using a longitudinal design will be
necessary to clarify these results and help us understand how the brain and epigenome change over time in depressed youth.
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Contributions

The current project was conducted in collaboration with several other students and research assistants, supervised by Dr. Booij. Ph.D student Lyndall Schumann and I and with assistance of research assistants Jennifer Thunem, Jennifer Gillies and Jessica Rowe completed the required ethics documentation and created the required project documents (e.g., consent forms, measures etc.). Furthermore, we were all responsible for recruiting participants and for collecting and entering data. Patients were introduced to the study by Dr. Kalid-Khan at Hotel Dieu Hospital or members of her team. Lyndall Schumann and myself conducted the K-SADS and CECA interviews. In addition, I was responsible for developing the research hypothesis of my thesis, for checking and inputting results from the methylation analysis, for analyzing and interpreting the final methylation data set and for the structural analyses of the imaging data. The imaging analyses were done by myself, under the guidance of post-doctoral fellow Florence Pomares and PhD student Elmira Ismaylova, and supervised by Dr. Booij. Patricia Yu, and Dr. Farida Vaisheva at McGill University were responsible for conducting the methylation analysis under the supervision of Dr. Booij and Dr. Szyf. This thesis was written under the supervision of Dr. Booij.
Bridging the link between trauma, brain development and depression: epigenetic mechanisms

Chapter 1 Introduction

This thesis focuses on the relationship between childhood and adolescent trauma, epigenetics and brain structure, with the overarching hypothesis that early trauma can alter brain development through epigenetic mechanisms. In the first chapter, I will first review the epidemiology of major depressive disorder (MDD) as well as its associated symptoms and risk factors. Next, I will discuss brain mechanisms in depression, with an emphasis on what is currently known about depression in youth populations. I will then discuss the impact of childhood trauma on brain development and the relationship between trauma and major depressive disorder, as well the methodological issues with measuring childhood trauma. Subsequently, I will review the role of DNA methylation at specific depression relevant genes as a potential mechanism by which trauma may affect brain development and pose people at greater risk to develop depression. Finally, I will end this chapter by presenting a study that I conducted as part of this Master’s thesis aimed at studying the link among trauma, DNA methylation and depression in adolescents.

Depression: Epidemiology, Symptomatology and Risk Factors

According to the Diagnostics and Statistics Manual 5th edition (DSM 5) criteria, depression is characterized by a period of depressed mood or loss of interest or pleasure in nearly all activities lasting at least two weeks (American Psychiatric Association, 2013). Further, this occurs along with the presence of a number of other secondary symptoms such as a change in appetite or sleep, psychomotor agitation, feelings of worthlessness or guilt, difficulty concentrating, a loss of energy or thoughts of death and/or suicide. According to the World
Health Organization, depression is the leading cause of disability worldwide, affecting an estimated 350 million people. In the United States, the twelve-month prevalence of depression is 7%, with the prevalence being much higher for young adults than those over 60 years (American Psychiatric Association, 2013). Although children can experience depression, the likelihood of developing clinical depression increases greatly with the onset of puberty (American Psychiatric Association, 2013).

Risk factors for depression include environmental factors such as stressful life events and abuse, temperamental factors such as a high degree of negative affectivity, as well as genetic factors, with depression having a heritability of approximately 40% (American Psychiatric Association, 2013). Another important predictor of depressive episodes is the number of previous episodes, with one study showing that the risk of occurrence of a future depressive episode increases by 16% with each previous episode (Soloman et al., 2000).

Depression frequently co-occurs with substance use disorders, anxiety disorders, eating disorders and borderline personality disorders. Individuals with pre-existing mental health disorders are at greater risk for developing depression and for a poorer prognosis (American Psychiatric Association, 2013). Depression in adolescence in particular is also associated with a wide range of mental and physical health problems, often predicting a chronic disorder. For instance, one study found that 43% of adolescents with MDD also had at least one other mental health disorder, and that the MDD episode developed after the other disorder in 80% of the cases (Lewinsohn, Rohde & Seeley, 1998). They also found a number of risk factors associated with becoming depressed during adolescence such poor coping skills, low social support, low self-esteem and self-consciousness.
Interestingly, the incidence of depression rises sharply for girls during adolescence, with females being twice as likely to develop depression after puberty than males (Thapar, Collishaw, Pine & Thapar, 2012). Adolescent girls are also more likely than boys to report disturbances of weight and/or appetite, as well as worthlessness and guilt and girls who reach puberty early or late may be at a greater risk for developing depression (Lewinsohn, Rohde, & Seeley; 1998). Of adolescents who develop depression, 60-90% will remit within a year, although 50-70% of those individuals will experience further depressive episodes within 5 years (Thapar, Collishaw, Pine, & Thapar, 2012). Hence, it is imperative to better understand depression during adolescence, as this is the period when it often emerges and depression during this period is highly predictive of recurrent depression, which can have an enormous impact on both the individual and society. More specifically, the incremental economic burden of depression was estimated at $210.5 billion in 2010, with $77.5 billion in direct costs (i.e., medical services and prescription costs; Greenberg et al., 2015). Further, it is the leading cause of disability in individuals 15-44 years old and results in approximately 400 million disability days per year (Greenberg et al., 2015). On the individual level, depression during adolescents is predictive of later difficulties with unemployment, early parenthood, lower educational achievement, and substance abuse (Fergusson & Woodward, 2002).

**Brain Mechanisms in Depression**

Given the chronicity and pervasiveness of depression, as well as its immense cost to society, it is very important that research be done in order to better understand the underlying biology of depression, even more so in youth populations. To date, much effort has been expended in an attempt to characterize the neural basis of depression. One of the most consistent structural alterations found in affected individuals is a smaller hippocampal volume, with studies
one study reporting up to a 19% smaller left hippocampal volume in individuals with depression compared to controls (Bremner et al., 2014; Campbell, Marriott, Nahmias & MacQueen, 2014). However, decreased volume does not appear to be restricted to the hippocampus, as one meta-analysis combining 64 studies found evidence in the literature for volume reductions in the anterior cingulate, orbitofrontal cortex and prefrontal cortex in depressed individuals (Koolschijn et al., 2009). Another meta-analysis combining 23 studies found that the most consistent result was reduced grey matter in the rostral ACC, with less consistent results in the dorsolateral and dorsomedial prefrontal cortices (Bora, Fornity, Pantelis & Yücel, 2012). Moreover, they also found evidence of decreased amygdala and parahippocampal volume, but only in studies in patients who also had co-morbid anxiety disorders or in first-episode drug free samples.

In addition to alterations in brain volume, researchers have also identified altered neural activity during emotion processing tasks in individuals with depression (Clark & Chamberlain; 2009). For example, depressed individuals show increased activity in the amygdala, an area responsible for processing the emotional significance of stimuli, during emotion processing tasks (Siegle et al., 2002). They also show decreased activity in various regions of the prefrontal cortex, such as the anterior cingulate cortex (ACC), which are important for inhibiting amygdala activity and thus regulating emotion (Carter et al., 1998). Together, these results suggest an alteration in the function of the fronto-limbic circuitry of the brain, a network of brain regions involved in, among other things, emotion regulation. More specifically, these results have led to the current model of emotional functioning, which describes depression as resulting from hyperactivity of the limbic centres responsible for the production of negative affective states (e.g., the amgydala), in combination with hypoactivity of the frontal and pre-frontal regions (e.g., anterior cingulate, orbitofrontal cortex) responsible for dampening this heightened state
De Raedt & Koster, 2010; Disner, Beevers, Haigh & Beck, 2011; Gould, 2012). It is of great interest, therefore, to determine which factors might lead to alterations in brain development that might ultimately explain the altered functioning of the fronto-limbic system seen in depression.

Given the importance of the adolescent period in brain development and the malleability of the adolescent brain, one potentially fruitful approach to understanding these processes is to study depression in adolescents, a population that has been relatively understudied. Some of the few studies that have studied adolescents have found results which fall in line with the adult literature, although inconsistencies are also apparent. For example, there is good evidence from a number of studies that reductions in hippocampal volume seen in adults are apparent in depressed adolescents, as well as adolescents at risk for developing depression (Caetano et al., 2007; Macmaster et al., 2003; MacMaster & Kusumakar, 2004; Rao et al., 2010). However, not all studies have found such a reduction (Shad, Muddasani & Rao, 2012). Further, there is some evidence for a reduction in frontal/prefrontal volume in adolescents with depression, but studies demonstrating this are few (Shad, Muddasani & Rao, 2012; Steingard et al., 2002). Moreover, findings of reduced amygdala volume in adolescent depression have been inconsistent as well (Caetano et al., 2007; Shad, Muddasani & Rao, 2012). Interestingly, there is some evidence for potential gender differences in morphology arising in adolescents. One study followed adolescents longitudinally and found evidence of exaggerated growth of the amygdala in female and attenuated growth in male adolescents; which is puzzling given the more consistent direction of decreased amygdala volume in adults (Whittle et al., 2014). At the functional level, depressed adolescents show elevated activity during emotion processing, cognitive control, affective cognition and reward processing tasks, as well as during resting-state in areas such as the ACC, amygdala, hippocampus and orbitofrontal cortex (Kerestes, 2014).
Thus, while a number of frontolimbic regions have been linked with depression, results have been somewhat inconsistent both within adults and adolescents, and across the two groups, and further research is necessary to clarify these findings.

*Serotonin and Depression*

Another important system that has been implicated in depression is the serotonergic system. The serotonergic system has long been linked to depression, with early antidepressants such as monoamine inhibitors and tricyclic antidepressants acting, at least in part, by affecting serotonergic activity, and with the newest class of antidepressants, Selective Serotonin Reuptake Inhibitors (SSRIs), acting primarily to increase serotonergic neurotransmission. More recently, PET studies and tryptophan depletion studies have further confirmed the role of serotonin in depression and mood. (Bell, Abrams & Nutt, 2001; Booij, Van der Does & Riedel, 2003; Rosa-Neto et al., 2004).

The serotonin hypothesis of depression is likely the most widely studied biological theory of depression. While initial theories postulated that low serotonin levels were the cause of depression, later research emphasized the serotonin vulnerability model, postulating that low serotonin is not the cause of depression, but rather a risk factor, evolving from genetic and environmental factors. According to this model, altered serotonergic functioning might only lead to the development of depression in the presence of specific precipitating factors such as life stressors. Recently a neurodevelopmental version of this diathesis-stress model has been proposed, highlighting the dual role of serotonin in both regulating brain development and mood. According to this model, genetic and environmental factors influence serotonin homeostasis which in turn influences the development of the brain and ultimately lead to the development of depression. Alterations in serotonin homeostasis could alter brain development which may lead to increased risk for depression (See Booij et al., 2015 for a review).
In part because it is the target of SSRIs, the serotonin transporter gene (SLC6A4), a protein responsible for the uptake of serotonin from the synaptic cleft, has been of particular interest to depression researchers. Interestingly, variation in a particular Single Nucleotide Polymorphism (SNP) in the promoter region of the serotonin transporter gene has been shown to explain variation in susceptibility to developing depression in the context of stressful life events, with the short allele conferring a greater vulnerability than the long allele (Caspi et al., 2003).

Further, the SLC6A4 gene, along with a number of other serotonin genes, plays an important role in guiding brain development and is highly expressed in a number of brain regions implicated in depression such as the cingulate cortex, amygdala, hippocampus and insular cortex (Kish et al., 2005; Varnäs, Halldin & Hall, 2004; Booij et al., 2015, review). Given the role of the SLC6A4 in brain development and its role of moderating the effects of life stressors in depression, it is an important target for depression studies aimed at understanding the causes and characteristics of depression.

The Hypothalamic-Pituitary-Adrenal (HPA) Axis and Depression

In addition to understanding the structural and functional alterations seen in individuals in depression, it is equally important to understand the neurotransmitter and hormonal systems that guide brain development. One system which is thought to be crucial for the development and regulation of brain systems relevant to depression is the HPA axis. The HPA axis is involved in the regulation of the response to stressful stimuli through the release of a cascade of hormones starting at the pituitary gland and ending at the adrenal gland, and ultimately leading to the release of glucocorticoids and mineralocorticoids, the most relevant of which to depression is cortisol. Given the role of stress as a predisposing and precipitating factor in depression and the role of glucocorticoids in guiding brain development, it is unsurprising that HPA axis dysregulation
has been implicated in depression (Booij et al., 2013). For example, individuals with depression appear to have overactive corticotropin releasing hormone activity, an imbalance of glucocorticoids and mineralocorticoids, decreased glucocorticoid receptor sensitivity, and deficits in the ability to downregulate HPA axis activity (Maletic et al., 2007; Murgatroyd & Spendgler, 2011). This link between cortisol and depression has been found in adolescents as well. For example, one study found that the presence of a daily peak in cortisol above the 80th percentile was predictive of subsequent MDD in high risk adolescents (Goodyer, Tamplin, Herbert & Altham, 2000). Further, another study found that mean morning cortisol levels also predicted the development of depression in women (Harris et al., 2000). Guerry and Hastings (2011) suggest in their review paper that HPA axis dysfunction precedes the development of depression and that this dysfunction is present from childhood and adolescence and worsens into adulthood. Thus, further understanding how and why the HPA axis is dysregulated in depression will be vital to understanding the neural profile of depressed individuals, and especially adolescents given that the development of the adolescent brain is ongoing and may be particularly sensitive to excessive HPA-axis activity.

_Trauma, Depression and the Brain_

As reviewed above, one of the most commonly studied environmental risk factors in depression is exposure to early life trauma. In addition to understanding the neural profile which characterizes depression, it is equally important to understand the environmental factors which, when present, can lead to the development of this profile. One of such factors which is postulated to explain much of the findings seen in imaging experiments conducted on individuals with MDD is childhood trauma. It is now well understood that there is a strong link between childhood abuse and depression, with childhood abuse and neglect predicting the onset of mood
and anxiety of disorders in adulthood (Brown et al., 1999; Clark, Caldwell, Power & Stansfeld, 2010). One study showed that adolescents with a history of abuse or neglect were 2 to 5 times more likely to develop depression compared to those without (Harkness & Lumley, 2008).

Further, childhood maltreatment and abuse is associated with a poorer course characterized by a decreased likelihood of remitting, an increased likelihood of recurrence and a decreased chance of responding to treatment (Nanni, Uher & Danese, 2012; Zlotnick, Mattia, Zimmerman, 2001). Abuse is thought to both trigger episodes of depression, and also sensitize individuals to developing depression in the face of stressors during adulthood (Tenannt, 2002). Like many other type of stressors, childhood trauma is believed to act on the HPA axis. Repeated, excessive activation of the stress response can lead to an excessive and sustained overabundance of glucocorticoids, such as cortisol, in the brain, which is thought to have a deleterious effect on brain development and lead to maladaptive traits associated with anxiety and depression (Booij et al., 2013). Moreover, child abuse has been linked with decreased levels of SLC6A4 in depressed individuals as assessed by PET imaging and early-life stress has been linked with decreased density of the serotonin 1A receptor in rhesus monkeys (Miller et al., 2009; Spinelli et al., 2010). In accordance with this knowledge, there is strong evidence that childhood trauma is also associated with altered brain development, particularly in the fronto-limbic circuitry. For example, childhood trauma is linked with decreased volume in the prefrontal cortex grey matter in adulthood (Frodl et al., 2008; Hart & Rubia, 2012; van Harmelen et al., 2010). More specifically, decreased volume in the dorsolateral PFC (prefrontal cortex), medial PFC, insula and anterior cingulate cortex has been observed in individuals who have experienced childhood trauma (Ansel et al., 2012; Cohen et al., 2006; Cotter et al., 2002; van Harmelen et al., 2010; Dannlowski et al., 2012). In addition, like in depression, a strong link has been identified
between early trauma and reduced hippocampal volume (Frodl et al., 2010).

Childhood trauma also seems to be associated with altered connectivity between various regions in the fronto-limbic circuitry (Hart & Rubia, 2012; Frodl et al., 2010). In particular, these studies have found decreased density in white matter tracts connecting prefrontal circuitry as well as decreased functional connectivity between the orbital frontal cortex and the anterior cingulate cortex.

As with studies in depressed populations, few studies have been done investigating the effect of trauma on the brain in adolescents. There is, however, extensive evidence from both animal and human literature to suggest that the adolescent brain is particularly vulnerable to the effects of stress and trauma (Romeo et al., 2016). For example, both humans and animal models, it has been shown that the adolescent brain is more sensitive to the effect of glucocorticoids (Dziedzic et al., 2014; Lee, Brady and Koenig, 2013). In humans, areas of the brain such as the hippocampus, amygdala and prefrontal-cortex which continue to develop during adolescence are particularly dense with glucocorticoid receptors (Giedd & Rapoport, 2010; Romeo et al., 2006). Like in adults, researchers studying adolescents have found reduced grey matter volume of a number of frontal-limbic regions in those with a history of trauma including reduced PFC and hippocampal volume (Edmiston et al., 2011; Rao et al., 2010). Further, a study looking at individuals in young adulthood (18-21 years of age) found that greater early adversity was associated with decreased anterior cingulate grey matter volume and greater precuneus volume (Jensen et al., 2015)

Given that these are all findings which agree with the depression literature, this would suggest that the neural profile seen in individuals with depression may be explained, at least in part, by the impact of trauma during childhood and adolescence. Moreover, because the fronto-
limbic circuitry remains relatively plastic up until adulthood, adolescents are an extremely valuable group to study as they can provide insight into how the limbic system is affected by trauma during some of its most plastic periods. Further, knowing that depression is most likely to emerge in adolescence, studying the brain at this time period would allow us to obtain a more complete picture of how the brain changes over time in depressed individuals when combined with the adult literature.

**DNA methylation**

While brain development is to a large extent determined by DNA sequence (genetics), the strong environmental component to depression would suggest that environmental factors affect brain development as well. With knowledge of the evidence linking trauma to both brain development and depression, the next logical question is as follows: what are the molecular mechanisms by which trauma might affect brain development and ultimately lead to the development of depressive symptoms? One of the mechanisms which may provide an answer to these questions is DNA methylation, an epigenetic modification which is believed to mediate gene X environment interactions (Meaney, 2010).

Epigenetics is defined as the study of inheritable changes in gene expression that do not involve alterations in the DNA sequence itself (Meaney, 2010). DNA methylation is one of the most well researched epigenetic mechanisms. It refers to a process in which the enzyme DNA methyltransferase transfers methyl groups onto the nucleotide cytosine at sites where it is found next to a guanine (CpG sites). This can lead to an alteration of gene expression in one of two ways. Firstly, methylation of CpG islands can lead to chromatin (the complex formed by DNA tightly bound to histone proteins) remodelling by recruiting histone deacetylases and other chromatin remodelling proteins. CpG islands are regions of the DNA which are particularly
abundant with CpG sites. Methylation of these sites allows for binding by methyl-CpG-binding domain proteins (MBDs), which are responsible for recruiting proteins involved in chromatic remodelling and the formation of heterochromatin, a more compact form of chromatin which is much less accessible to transcription factors. Secondly, when CpG islands in or near promoter regions of genes are methylated, this can directly inhibit binding of transcription factors to these regions. In this way, both of these mechanisms can lead to the silencing of gene expression that is both relatively stable, but also reversible, allowing for the long term programming and re-programming of gene expression (Bestor, 1998; Bird, 2002).

Findings from animal research suggest that early life experience can directly influence methylation at specific genes, and that altered methylation at these genes is associated with greater stress sensitivity. For example, in their seminal study, Weaver and colleagues showed that rats exposed to high levels of maternal licking and grooming behaviour (L/G) had lower methylation levels at the glucocorticoid receptor (GR) gene promoter, relative to rats exposed to low levels of maternal licking/grooming (Weaver et al., 2004). This is interesting given the role of the GR in binding cortisol and regulating the HPA axis response to stressful stimuli. In fact, the decreased methylation levels in animals exposed to high maternal licking and grooming behaviour displayed a decreased magnitude of the HPA-axis response to stress. Further, there is evidence that epigenetic regulation of the glucocorticoid receptor is relevant to the regulation of stress related behaviours and outcomes in humans as well, with on study finding increased methylation at a glucocorticoid receptor gene in suicide victims who also experienced childhood abuse compared to those who did not and to healthy controls. These results provided evidence for a possible mechanism by which early environmental factors could alter gene expression and thus phenotype.
While the studies above focused on genes regulating the HPA-axis system, of particular interest in the context of depression are serotonergic genes, in particular the serotonin transporter. Especially relevant to depression are studies in human samples, that have found associations between peripheral levels of SLC6A4 DNA methylation and childhood trauma (e.g., Kang et al., 2013). However, findings have been inconsistent; while some studies have found an association between SLC6A4 methylation and childhood trauma, others have not (see Booij et al., 2013 for a review). Further, the direction of the effect appears to differ across studies. Furthermore, DNA methylation at this gene is also associated with aggression, depression and human brain serotonin synthesis (Booij et al., 2015; Booij 2013; Frodl et al., 2015; Wang et al., 2012). These findings suggest that early trauma may lead to phenotypical changes by altering DNA methylation at specific genes in humans as well. This is supported by the finding of an association between SLC6A4 methylation and childhood trauma and abuse (Lutz & Turecki, 2014; Mehta et al., 2013). Further, although specific causal links have not been tested, there is also evidence consistent with the hypothesis that these changes in DNA methylation may mediate the relationship between child trauma and brain development, and that these changes in the trajectory of brain development may lead to increased risk for depression. Specifically, Booij and colleagues found that adults with depression exposed to trauma in childhood had a greater degree of SLC6A4 methylation (as assessed in peripheral blood cells) and smaller hippocampi (2015) than those not exposed to trauma, and that greater levels of peripheral SLC6A4 methylation were associated with a smaller hippocampal volume. Further, Dannlowski and colleagues (2014) also found a link between SLC6A4 methylation and hippocampal volume in a sample of healthy volunteers, although the effect was in the other direction. Moreover, they
found a positive association between *SLC6A4* methylation and insula volume bilaterally, right amygdala and putamen, and the left caudate and superior occipital gyrus.

In addition to the *SLC6A4*, DNA methylation at the FK506 binding protein gene (FKBP5) has also been linked to childhood trauma (Binder, 2009). FKBP5 codes for the FKBP5 binding protein, a co-chaperone of hsp90, a protein which regulates sensitivity of glucocorticoid receptors. When FKBP5 binds to the GR complex, cortisol binds to GR with decreased affinity, leading to less translocation of the receptor to the nucleus and thus less of a downstream effect of cortisol. While FKBP5 normally has the adaptive function of establishing a negative feedback loop to regulate GR sensitivity, evidence suggests that this function may go awry in the face of stressful live events, with carriers of FKBP5 risk alleles being significantly more likely to experience PTSD in the face of trauma than carriers of protective alleles (Klengel et al., 2013). Further, another study found that a specific FKBP5 risk allele was associated with HPA-axis hyperactivity, an increased number of depressive episodes and better response to antidepressant treatment (Binder al., 2004).

Although most studies investigating FKBP5 have focused on identifying alleles associated with increased risk for mental health problems in the presence of trauma, one study found that, in carriers of the risk allele who have experienced trauma, DNA methylation was associated with exacerbated risk for developing PTSD in response to trauma (Klengel et al., 2013). The same study also found that increased FKBP5 methylation was associated with decreased volume in the right hippocampus. In another study conducted by Weder and colleagues (2014), FKBP5 methylation was a significant predictor of children’s depression scores, although not when a correction for multiple comparisons was applied (Weder et al., 2014). Further, Yehuda and colleagues (2015) found that survivors of the holocaust had greater
whole blood FKBP5 methylation levels compared to controls and that, interestingly, greater FKBP5 methylation was significantly associated with lower methylation levels in their offspring. Further, FKBP5 methylation significantly predicted wake up cortisol level, suggesting a potential functional impact of FKBP5 methylation on HPA axis activity. Taking together, these findings seem to suggest that extreme stressors such as trauma may lead to increased methylation levels at the FKBP5, which, in turn, may lead to a maladaptive dysregulation of the HPA axis. However, as in the imaging literature, adolescents have been greatly understudied, and more research will need to be done in order to better understand the immediate impact of trauma on the FKBP5 gene and how FKBP5 methylation impacts the developing brain.

Thus, there is strong evidence to suggest the involvement of DNA methylation at various genes in responding to environmental events in order to guide brain development. Depressed adolescents are relatively understudied, especially in the context of DNA methylation.

*Measuring Trauma*

Though many researchers elect to measure trauma using retrospective self-report questionnaires, self-reports can be affected by the desirability bias and other social biases, as well as participants’ mood at the time of responding, leading to issues with reliability and validity. One way to overcome some of these issues is to assess trauma using a (semi-)structured interview. One commonly used interview is the Childhood Experience of Care and Abuse (CECA) interview (Bifulco, Brown & Harris, 1994). The CECA takes a number of measures to reduce biases in responding (Bower, 1981; Van de Mortel, 2008). The CECA uses two steps to exclude participant bias from their ratings of trauma. Firstly, participants are interviewed by a trained interviewer using a semi-structured interview aimed at obtaining only objective information about experiences,
and not the participants' subjective view of them. Secondly, interviews are transcribed and rated independent of the interviewer so as to minimize any subjective influence of the interview on the final rating. Using this method has shown to reduce bias, with one study finding no evidence of any significant bias in the reporting of childhood maltreatment, with the exception of perhaps some slight underreporting (Brown et al., 2007). Further, it has also shown to be reliable, with one study finding very strong inter-rater agreement of trained raters (kappa coefficient of .86- 1.00; Harkness, Bruce & Lumley, 2006).

The Current Study: Objectives

The current study will attempt to answer the three unanswered question previously discussed. First, in order to determine how adolescents with depression differ in brain morphometry from healthy controls, depressed adolescents and aged matched controls will undergo an MRI scan, and morphological analyses will be used to compare the two groups. To determine the effect of childhood trauma and abuse on brain development, an in depth measure of childhood care and abuse will be used to assess associations between abuse and brain structure in depressed adolescents. Finally, to examine the mechanism by which stressors known to lead to depression impact brain development, we will assess the relationship between DNA methylation and brain structure in the depressed group. We will focus on two candidate genes given their relevance to depression and childhood trauma: the SLC6A4 and FKBP5 genes.

The Current Study: Hypotheses

1) Adolescents with depression will differ significantly in brain structure and SLC6A4/FKBP5 methylation from controls.
a. Given the importance of fronto-limbic circuitry in emotion regulation and findings of previous studies in (primarily) adult populations, I hypothesize that the depressed group will show decreased grey matter volume in emotion producing and consolidating regions (insula, amygdala, hippocampus) of the fronto-limbic circuitry as well as in regions involved in emotion regulation (orbitofrontal cortex, anterior cingulate cortex) compared to controls.

b. Because trauma is tightly linked to depression, and because trauma has been linked with increased SLC6A4 and FKBP5 methylation, I predict that peripheral SLC6A4 and FKBP5 methylation will be higher in the depressed group compared to controls.

2) Consistent with previous findings in brain imaging literature, both trauma and DNA methylation will be associated with brain development in depressed individuals.

a. Increased trauma will be linked to decreased grey matter volume of emotion producing and consolidating regions (insula, amygdala, hippocampus) of the fronto-limbic circuitry as well as in regions involved in emotion regulation (orbitofrontal cortex, anterior cingulate cortex)

b. In line with previous research in adult populations, trauma will be associated with increased methylation of the serotonin transporter (SLC6A4) and FK506 binding protein 5 (FKBP5) genes.

c. DNA Methylation at both the SLC6A4 and FKBP5 gene will be associated with fronto-limbic brain morphology. Specifically, increased SLC6A4 and FKBP5 methylation will be associated with decreased grey matter volume in emotion producing and consolidating regions (insula, amygdala,
hippocampus) of the fronto-limbic circuitry as well as in regions involved in emotion regulation (orbitofrontal cortex, anterior cingulate cortex)
Chapter 2 Methods

Participants

Adolescents between the ages of 14-18 who met the diagnostic criteria for current depression were referred to the current study by Dr. S. Khan, psychiatrist at Hotel Dieu Hospital. Participants were then contacted and underwent a phone screening interview to ensure eligibility for the study. Control participants were recruited by word of mouth and through the Queen’s University Developmental database. Controls also underwent a phone screening interview to determine eligibility. Participants were excluded if they were found in the screening interview to be actively suicidal, psychotic, or abusing drugs or alcohol (indicated by the K-SADS, see below). Furthermore, controls were excluded if they had previously taken or were currently taking any psychiatric medication and patients were excluded if they were taking any stimulant medications. Further, all patients were stabilized on antidepressant medication for 4-6 weeks. Patients were excluded if they did not receive a diagnosis of Major Depressive Disorder during the K-SADS interview. Finally, participants were not eligible for the study if they did not meet the MRI safety criteria, which include not having any non-removable metal in their body (e.g., electronic implant, prosthesis, pacemaker, aneurysm clip), as well as not being pregnant.

In total, 24 controls and 23 depressed patients were recruited.

Measures and Assessments

In order to confirm the presence or absence of a psychiatric diagnosis, the Schedule for Affective Disorders and Schizophrenia for School Age Children (K-SADS; Kaufman et al., 1997) was administered. The K-SADs is a semi-structured interview
based on the DSM-IV criteria used to collect symptom information for a variety of psychiatric disorders in children and adolescents ages 6-18. The interview was also used to collect demographic and clinical information such as medication use and detailed history of depressive episodes. The interview was administered directly to the participant; parent reports were not conducted. Interviews were conducted by graduate students in clinical psychology who were trained by Dr. Booij. Prior to conducting the interviews autonomously the interviewer observed another interviewer at least 2 times and both scored the interview. Every case was also discussed with Dr. Booij prior to determining the final diagnosis.

Participants also completed the Beck Depression Inventory for Youth (BDI-Y), a subscale of the Beck Youth Inventories of Emotional and Social Impairment, second edition (Beck, Beck, Jolly, & Steer, 2005). The BDI-Y is a 20 item self-report measure designed to measure the presence and severity of depressive symptoms in children and adolescents ranging from 7-18 years old. Individuals are instructed to rate the extent to which their thoughts or feelings have been consistent with a number statements in the last two weeks. The items are rated on a 4-point scale with responses ranging from 0 (never) to 3 (always). Examples of items include “I feel empty inside” and “I think my life will be bad”. Scores on each item are summed together to obtain a final score ranging from 0 to 60, with higher scores indicating greater depressive symptomatology. The measure has been shown to be both reliable and valid in the study of depression, with a mean coefficient alpha of 0.86 in psychiatric patients and mean correlations with clinical ratings of 0.72 (Beck, Steer & Carbin, 1988).
In addition, participants underwent the Childhood Experience of Care and Abuse (CECA) interview (Bifulco, Brown & Harris, 2002), an interview designed to measure trauma. The CECA is an approximately 45 minute long semi-structured, retrospective interview made up of 6 core subscales and 10 optional subscales which assess the quality of parental care experienced by the interviewee throughout their life from childhood to the present. Recordings of the interview are scored by trained objective raters who are instructed to score each subscale on a scale ranging from 1 to 4, with 1 indicating marked evidence of a given experience, 2 indicating moderate evidence, 3 indicating some evidence and 4 indicating little/none (see appendix A for an example rating form). Hence, a higher score indicates less/no trauma. In addition to total trauma scores, the subscales of interest in the current study were the antipathy and neglect (hostility or coldness toward the child and indifference and physical and/or emotional neglect), abuse (physical, sexual and psychological) and bullying subscales. An example of an item on the antipathy scale is “How did you get along with your mother growing up” and “Could you have a laugh together”. Parents were rated separately and ratings were combined to produce a score of the collective experience. An overall trauma score was calculated by summing the individual trauma scores (emotional, physical, sexual, psychological and bullying), each of which range from 1 to 4, forming and aggregate trauma score which ranges from 0 to 20. Scores were then reverse coded so that higher scores indicated higher trauma. Under the training of Dr. K. Harkness, who trained the students for the current study, the CECA has previously been shown to have excellent inter-rater reliability, with kappa values ranging from .86–1.00 (Harkness et al., 2006).
MRI Data Acquisition

Participants underwent an approximately 40:00 minute long functional and structural MRI scan at the Queen’s Centre for Neuroscience Studies’ MRI facility using a Siemens 3.0 Tesla TIM Trio scanner with a 12-channel head coil. The scanning protocol consisted of an approximately 30 second long three-dimensional (sagittal, transversal, and coronal planes) localizer scan to acquire head position for subsequent scans (repetition time (TR) = 20 ms; echo time (TE) = 5 ms; field of view (FOV) = 280 mm; slice thickness = 10 mm; voxel size = 1.5 mm x 1.1 mm x 10.0 mm), followed by an approximately 7 minute and 30 second long high-resolution three-dimensional $T_1$-weighted structural scan (TR = 1760 ms; TE = 2.2 ms; FOV anterior to posterior = 256 mm; slice thickness = 1.0 mm; voxel size = 1.0 mm x 1.0 mm x 1.0 mm).

The scanning protocol also included a 18 and a half minute functional MRI scan using an emotional attention-shifting task, an approximately 10:00 minute diffusor tensor imaging scan and a 5 minutes resting state measure. These data are beyond the scope of the topic of this thesis and therefore will not be described here.

DNA methylation assessment

*DNA Methylation Protocol*

Participants donated 3-6 ml of saliva into a tube through a passive drooling method, using the Oragene Kit (DNA Genotek), and as used in previous work in Dr. Booij’s laboratory (Levesque et al., 2014). Samples were then stored in Dr. Booij’s laboratory until further analyses were performed. DNA and RNA extraction were performed using Trizol (Invitrogen) according to the manufacturer’s instructions and
stored at 220uC and 280uC, respectively. RNA qualities were analyzed using Agilent 2100 Bioanalyzer.

In order to analyse SLC6A4 methylation, we used the assay previously developed and validated by Dr. Linda Booij and collaborators (Wang et al., 2012). Specifically, three sets of outside primers and four sets of nested primers were used as follows: Out F1&2 59-TGTAGTTGGTTAATAA AATGAGAATTAGTT-39, Out R1&2 59-AAATCCTA ACTTTCTACTCTTTAAC TTTA-39, Out F3 59-TTTAGGAAGAAAGAGAG-AGTAGTTTTT-39, Out R3 59-CAAAAAACT CTTAAAAAATTTTTAC-39, Out F4 59-TTTGT- TTTTTTGTGTA GTTTTTTTTT-39, Out R4 59-CTCACATAATCTAATCTCT AAATAACC-39, Nest F1 59- TTTTTTATTGTGGAAGTTTTTATTGTG-39, Nest R1 59-CTCTCTCTTTCTTCTCCT- AAAACCTAACA-39, Nest F2 59-TGTAGGTTTTTA GGAAGAAAGAGAGA-39, Nest R2 59-AAAAAAAACTACACAAAAAAACAAA TATAC-39, Nest F3 59- TTTTAGGAAGAAAGAG-AGAGTAGTTTT-39, Nest R3 59-AAATCCTAAACTTTTC CTACTCTTTAACTTTA-39, Nest F4 59-TAAAG TTAA AGAGTAGGAAAAG TTAGGATT-39, and Nest R4 59-ACCCCAAAACCA-AAAAAAAAA-39. The nested reverse primers were biotinylated for pyrosequencing. DNA was treated with sodium bisulfite and two rounds of PCR amplification will be performed. 15ml of the PCR products was be used to perform pyrosequencing using PyroMarkQ24 (Qiagen) according to the manufacturer’s protocol. The methylation percentage at each CpG site was analyzed using the PyroMark Q24 software (Qiagen) in the laboratory of Dr Moshe Szyf at McGill University. Three replicates were performed for each site, and the average of the three replicates at each CpG site was used as the final methylation value.
for that CpG site. All analyses were done by a qualified lab technician (Dr. F. Vaisheva) in Dr. Szyf’s laboratory. I chose to investigate DNA methylation patterns in predetermined CpG sites upstream of the SLC6A4 gene promoter (CpG5 to CpG14; see Figure 1) as they have been most strongly associated with in vivo human brain measures of serotonin synthesis and environmental variability in previous studies done by the team of Dr. Linda Booij, especially the average methylation of CpG5 and CpG6, and CpG11 and CpG12 (see Wang et al., 2012). However, due to technical errors, sites 8 and 9 had to be excluded from the analyses.

Figure 1. A schematic of the SLC6A4 promoter showing the location of the CpG sites analyzed within the SLC6A4 promoter. CpG sites are represented by solid circles. The transcription start site (TSS) is represented by the arrow. Figure adapted from Wang et al. (2012).

For the FKB5 gene, we chose to analyse two CpG sites in the intron 7 region of the gene based on findings from a previous study (Klengel et al., 2013). We used two PCR primers (in_7F2TGGGATAATAATTTGGAGTTATAGTGTAGG, in7_R2 biot /5Biosg/AAATTATCTCTTACCTCCAACACTAC) and two sequencing primers
(in7_S3 TTTAAGGAGTTATTTGTGAGA, in7_S4 GTTGATATATAGGAATAAAATAAGA). The same procedures for bisulfite treatment, PCR amplification and pyrosequencing used for *SLC6A4* were used for *FKBP5*.

**Procedures**

For each participant, the study took approximately 4 hours to complete and was completed in a single session. The interviews and questionnaires were all completed at the Stress Neurodevelopment and Emotions Lab (SNEL) at Queen’s University. When participants arrived, informed consent and/or assent were obtained where applicable (see Appendices B, C, D, and E for the consent and assent forms). Participants then completed the BDI-Y (approximately 20 minutes), followed by the K-SADS (approximately 20 minutes) and CECA (approximately 45 minutes) interviews. Participants then went to the MRI facility where they first underwent a safety screen (20 minutes) followed by a 40 minute scan. A saliva sample for DNA methylation assessment was also collected. For this, participants were asked to donate 3-6 ml of saliva into a tube, using the Oragene Kit (DNA genotek). For this, participants were told not to drink food or drink for half an hour before donating the sample to avoid contamination of the sample. Samples were stored in Dr. Booij’s laboratory until further analyses were performed. DNA and RNA extraction was performed using Trizol (Invitrogen) according to the manufacturer’s instructions and stored at 220uC and 280uC respectively. RNA qualities were analyzed using Agilent 2100 Bioanalyzer. After completing the study participants were thanked for their participation, and were given a $50 gift certificate, as well as $10 cash to offset travel expenses.
The study was approved by the Health Sciences Research Ethics Board of Queen’s University in Kingston, Ontario. Written informed consent was obtained from the adolescent or a parent or guardian if the participant was under 16 years of age. If under the age of 16, assent was obtained from the participant.

Statistics

A total trauma score was calculated by summing the trauma scores of each individual subscale (emotional, psychology, physical, sexual, bullying), each of which range from a score of 1 to 4, with greater scores indicating less trauma. A total \( SLC6A4 \) methylation score was obtained by summing together methylation at CpG sites 5 to 14 (excluding sites 8 and 9). Further, \( FKBP5 \) methylation was determined separately for site 5 and 6. Independent t-tests were then performed to compare total trauma and the individual methylation outcome measures (i.e., total \( SLC6A4 \) methylation, \( FKBP5 \) methylation at site 5, \( FKBP5 \) methylation at site 6) between controls and depressed patients.

MRI data pre-processing and analyses:

Pre-processing was done using the VBM12 toolbox (Christian Gaser, University of Jena Department of Psychiatry, Germany) in Statistical Parametric Mapping (SPM12, Wellcome Department of Cognitive Neurology, London UK) implemented in MATLAB R2010a (Mathworks, Sherborne, MA).

First, MRI images were segmented into gray matter (GM), white matter (WM) and cerebrospinal fluid (CSF) using the segmentation model in VBM12. GM population templates were then generated from the entire image dataset using the Diffeomorphic Anatomical Registration Through Exponentiated Lie Algebra (DARTEL) technique.
(Ashburner, 2007). Then, after an initial affine registration of the GM DARTEL templates to the tissue probability maps in Montreal Neurological Institute (MNI) space, warped versions of the images were generated. These steps improve computational anatomy providing more easily interpreted voxel-based morphometry (VBM), and better parameterization of brain shapes. Following these steps, the normalized gray matter (GM) was modulated with the resulting Jacobian determinant maps and was spatially smoothed with an 8-mm full width at half maximum (FWHM) Gaussian kernel. The segmented and normalized grey matter images were used in the next steps of the analyses.

We first conducted Whole Brain VBM analyses, which allow for exploration of the relationships between depression, trauma and methylation and brain volume across different regions of the brain. VBM is not limited to one particular structure, which permits an even-handed and comprehensive assessment of anatomical differences throughout the brain (Ashburner & Friston, 2001). Consistent with the first objective of the study, t-tests were performed in SPM 12 in order to determine whether there were any differences in GM volume between adolescents with depression and controls.

In line with the second study hypotheses, regression analyses were performed in SPM12 in the depressed group only. First, GM was regressed onto each of the DNA methylation measures. Next, GM morphology was regressed onto each of the Trauma measures. Statistical threshold was set at an uncorrected $p < 0.001$, with a minimum cluster size threshold of 10 voxels, to balance between Type 1 and Type 2 errors, (Lieberman & Cunningham, 2009) for all group-level contrast analyses. All peak coordinates are reported in Montreal Neurological Institute (MNI) format. Using the
Automated Anatomical Labeling Atlas II (Rolls, Joliot & Tzourio-Mazoyer; 2015) the anatomic location of significant clusters was detected.

Each whole brain analyses was followed by Region of Interest (ROI) analyses. Based on previous findings implicating them in depression and the effects of chronic stress, the following fronto-limbic regions were investigated: amygdala, hippocampus, insula, cingulate cortex, orbitofrontal cortex, prefrontal cortex. These ROIs were derived by constructing masks for each of the a priori determined regions using the neuromorphometrics atlas as provided by Neuromorphometrics, Inc. (http://Neuromorphometrics.com/) under academic subscription. The ROI analyses are the primary outcome measures of interest, and therefore will be reported first when presenting results for each of the tested hypothesis.

In order to investigate the first hypothesis, independent t-tests using a threshold of \( p < .05 \) were used to compare ROI volume between depressed patients and controls. As part of the second hypothesis, ROI volumes were regressed first onto trauma and then DNA methylation using the criterion of \( p < .05 \). For both of these analyses; however, \( p \) values between .05 and .1 were considered trends.

**Chapter 3 Results**

**Study sample**

Forty-seven participants, including 23 MDD patients and 24 healthy controls were recruited for the current study. Of these 47 individuals, two controls were excluded, one due to a past diagnosis of an anxiety disorder for which they were being treated, and another because she met criteria for obsessive compulsive disorder.
Further, another control was found to meet criteria for depression and was moved to the depression group.

The final sample consisted of 24 depressed individuals and 21 controls that were tested. However, one patient was excluded from the methylation analyses due to technical errors during processing of DNA while a separate patient was excluded from the imaging analyses as the scan had to be stopped prematurely. The groups did not differ significantly in age ($t(43) = 7.59, p = .452$) or gender ($\chi^2 = .022, p = .881$). See Tables 1 and 2 for demographic and clinical information.

Table 1

<table>
<thead>
<tr>
<th>Group Differences</th>
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<th>Test Statistic</th>
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<td>$SD$</td>
<td>$n$</td>
<td>$M$</td>
</tr>
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<td>% SLC6A4 Methylation (Total)</td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CpGTotal</td>
<td>3.02</td>
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<td>21</td>
<td>3.21</td>
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<tr>
<td>% FKBP5 Methylation</td>
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<td></td>
<td></td>
</tr>
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<td>CpG5</td>
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<td>CpG6</td>
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<td>Grey Matter Volume</td>
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<td>963.12</td>
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<td>White Matter Volume</td>
<td>594.45</td>
<td>18.98</td>
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<td>593.64</td>
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</table>

Note. n.s. = not significant

Table 2

Demographic and Clinical Information

<table>
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<tr>
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<th>$n$</th>
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### MDD history

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<tr>
<td>Age of onset of first episode</td>
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<td>1.35</td>
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</tr>
<tr>
<td>Number of episodes</td>
<td>1.75</td>
<td>0.99</td>
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<tr>
<td>Cumulative duration of MDEs (months)</td>
<td>21.75</td>
<td>20.16</td>
<td>24</td>
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</table>

### Comorbidities

- Generalized Anxiety Disorder: 6
- Other: 6

### Psycho-active medication

- Antidepressant: N/A, N/A, 14
- Antipsychotic: N/A, N/A, 5
- Anxiolytic: N/A, N/A, 2
- Stimulant: N/A, N/A, 5
- Other: N/A, N/A, 0

### History of early life trauma

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<tr>
<td>Emotional abuse</td>
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### Beck Depression Inventory II (BDI-II)

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<td>57.86</td>
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### Controls

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<tr>
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<tr>
<td>Bullying</td>
<td>1.33</td>
<td>0.66</td>
<td>24.00</td>
</tr>
</tbody>
</table>

Note. MDD = Major Depressive Disorder; MDE = Major Depressive Episode; N/A = Not Applicable

**Aim 1A: Comparison of Brain Morphology Between Depressed Adolescents and Controls**
There were no significant differences in volume between the two groups in any of the a-priori defined regions as shown by the ROI analyses. However, a further exploration at the whole brain level showed that the depression and control groups did differ in volume in the left precentral gyri (T = 3.8, p < 0.001, k ≥ 10), with depressed individuals having greater volume in these areas. Further, individuals with depression displayed, relative to controls, smaller left precuneus volume (T = 4.21, p < .001, k ≥ 10).

Aim 1B: Comparison of DNA methylation Between Depressed Adolescents and Controls

There was no difference between the depressed participants and controls in peripheral SLC6A4 methylation levels (t(43) = -0.851, p = .399). However, the depressed group had significantly lower levels of FKBP5 methylation at CpG site 5 compared to controls (t(42) = 2.522, p = .016). Further, depressed individuals tended to have lower levels of FKBP5 methylation across sites 6 compared to controls, although this difference was not significant (t(42) = 1.933, p = .06). See Figures 2 and 3 for a graphical representation of these results.
Figure 2. Bar graph comparing the mean $FKBP5$ methylation at site 5 for the control and depressed groups. Controls had significantly higher methylation levels than depressed patients at site 5 ($p < .016$). Standard errors are represented in the figure by the error bars attached to each column.

Figure 3. Bar graph comparing the mean $FKBP5$ methylation at site 6 for the control and depressed groups. Controls had significantly higher methylation levels than depressed patients at site 6 ($p < .06$). Standard errors are represented in the figure by the error bars attached to each column.

**Aim 2A: Trauma and Brain Morphology in Depressed Adolescents**

**Trauma and Brain Morphology**

A ROI Analysis showed that increased total trauma was associated
a trend towards decreased volume of the right anterior insula ($t(22) = -1.83$, $b = -0.049$, $p = .081$).

Looking at specific types of trauma showed that, although the number of people with high levels of physical abuse was small, increased physical abuse was significantly associated with decreased volume of left hippocampus ($t(22) = -2.23$, $b = -0.19$, $p = .037$; See Figure 4. below) and a trend towards decreased volume of the left ($t(22) = -2.06$, $b = -0.19$, $p = .052$) and right ($t(22) = -1.96$, $b = -0.22$, $p = .063$) anterior insula. Further, greater emotional abuse was associated with increased volume of the right middle cingulate ($t(22) = 2.71$, $b = 0.225$, $p = .013$; See Figure 5. below).

*Figure 4.* Scatter plot illustrating the relationship between physical abuse and volume of the left hippocampus. Greater physical abuse was associated with decreased volume of the left hippocampus. ($p = .037$).
Figure 5. Scatter plot illustrating the relationship between emotional abuse and volume of the right middle cingulate. Greater emotional abuse was associated with increased volume of the right middle cingulate ($p = .013$).

Whole Brain Analysis revealed that, greater trauma was associated with the volume of a number of brain regions. Of particular relevance to the current study, greater trauma was associated with decreased volume of BA 47 (pars orbitalis) ($T = 4.02, p < 0.001, k \geq 10$) and increased volume in the right anterior cingulate cortex ($T = 3.88, p < 0.001, k \geq 10$).

Aim 2B: Trauma and DNA Methylation in Depressed Adolescents

There was no significant relationship between trauma and total $SLC6A4$ methylation ($t(22) = -1.12, b = -.76, p = .246$). Further, there was no association between trauma and DNA methylation at sites 5 ($t(21) = 2.90, b = .031, p = .77$) or 6 ($t(21) = - .33, b = -.034, p = .74$) of the $FKBP5$ gene.

Looking at more specific forms of trauma, increased physical abuse was associated with decreased total $SLC6A4$ methylation ($t(22) = -2.39, b = -.328, p = .026$).
There was also a trend towards an association between increased sexual abuse and decreased total $SLC6A4$ methylation ($t(22) = -1.90$, $b= -0.354$, $p = .07$).

**Aim 2C: DNA Methylation and Brain Morphology in Depressed Adolescents**

**$SLC6A4$ Methylation**

A *ROI Analysis* showed that greater total $SLC6A4$ methylation was associated with a trend towards increased volume of the left middle cingulate area ($t(22) = 1.78$, $b = .17$, $p = .089$; See Figure 6. below).

![Figure 6](image)

*Figure 6.* Scatter plot illustrating the relationship between total $SLC6A4$ methylation and left middle cingulate volume. Increased methylation was associated with a trend towards increased volume ($p = .089$).

Further exploration at the *Whole Brain level* showed that, within the MDD group, there were a number of both positive and negative associations between the DNA methylation measures and brain structure in depressed adolescents (See Appendix B). Of particular relevance to the current study were the findings of associations between $SLC6A4$ methylation and fronto-limbic circuitry. Total $SLC6A4$ methylation was associated with
increased volume of the left hippocampus (T = 4.93, p < 0.001, k ≥ 10; Figure 7.), and the left middle cingulate (T = 4.09, p < 0.001, k ≥ 10).

![Figure 7](image.png)

*Figure 7.* Image displaying a significant positive association between volume of the left hippocampus and total SLC6A4 methylation (p < .001).

In addition to findings in the hypothesized fronto-limbic areas, there were also a number of associations between SLC6A4 methylation and regions forming part of the basal ganglia. In particular, greater total SLC6A4 methylation was associated with decreased volume of the putamen (T = 3.92, p < 0.001, k ≥ 10) and pallidum (T = 3.62, p < 0.001, k ≥ 10).

*FKBP5 Methylation*

Further, an ROI analysis showed that increased FKBP5 methylation at site 5 was associated with decreased volume of the right posterior orbitofrontal gyrus (t(21) = -2.20, b = -.027, p = .040; Figure 8.). Further, it was also associated with a trend towards decreased volume of the left amygdala (t(21) = -1.81, b = -.007, p = .086), left (t(21) = -2.03, b = -.020, p = .056; Figure 9.) and right (t(21) = -1.815, b = -.021, p =
.085; Figure 10.) hippocampus and left posterior orbitofrontal gyrus ($t(21) = -1.81$, $b = -.022$, $p = .086$; Figure 11.).

Figure 8. Scatter plot illustrating the relationship between FKBP5 methylation at site 5 and right posterior orbitofrontal gyrus volume. Increased Methylation was associated with decreased volume ($p = .040$).

Figure 9. Scatter plot illustrating the relationship between FKBP5 methylation at site 5 and left amygdala volume. Increased methylation was associated with a trend towards decreased volume ($p = .08$).
Figure 10. Scatter plot illustrating the relationship between FKBP5 methylation at site 5 and right hippocampus volume. Increased methylation was associated with a trend towards decreased volume ($p = .085$).

Figure 11. Scatter plot illustrating the relationship between FKBP5 methylation at site 5 and right posterior orbitofrontal gyrus volume. Increased methylation was associated with a trend towards decreased volume ($p = .086$).

Increased FKBP5 methylation at site 6 was significantly associated with decreased volume of left ($t(21) = -4.08, b = -.031, p = .001$; Figure 12) and right ($t(21) = -3.79, b = -.036, p = .001$; Figure 13) hippocampus as well as the right posterior orbitofrontal gyrus ($t(21) = -2.23, b = -.020, p = .031$; Figure 14). Further, it also
tended to be associated with decreased volume of the left posterior orbitofrontal gyrus\( (t(21) = -2.00, b = -0.023, p = .060; \text{Figure 15}) \).

Figure 12. Scatter plot illustrating the relationship between FKBP5 methylation at site 6 and left hippocampus volume. Increased methylation was associated with decreased volume \( (p = .001) \)

Figure 13. Scatter plot illustrating the relationship between FKBP5 methylation at site 6 and right hippocampus volume. Increased methylation was associated with decreased volume \( (p = .001) \)
Figure 14. Scatter plot illustrating the relationship between FKBP5 methylation at site 6 and right posterior orbitofrontal gyrus volume. Increased methylation was associated with decreased volume ($p = .031$).

Figure 15. Scatter plot illustrating the relationship between FKBP5 methylation at site 6 and left posterior orbitofrontal gyrus volume. Increased methylation was associated with a trend towards decreased volume ($p = .060$).
Whole brain analyses showed that greater *FKBP5* methylation at site 6 was associated with greater volume of the left anterior cingulate (T = 4.26, \( p < .001, k \geq 10 \)) and right insula (T = 3.82, \( p < .001, k \geq 10 \); Figure 16.).

![Figure 16](image16.png)

*Figure 16.* Image displaying a significant association between volume of the right insula and *FKBP5* methylation at site 6 (\( p < .001 \)).

See Appendices A, B and C for a summary of the relationship between DNA methylation and brain morphology.

**Chapter 4 Discussion**

**Aim 1: Differences in Brain Volume and DNA Methylation Between Depressed Patients and Controls**

The first aim of the current study was to determine whether there were any differences in brain morphology between depressed adolescents and controls. We hypothesized specifically that depression would be associated with decreased volume of fronto-limbic areas associated with the production/consolidation (amygdala, hippocampus, insula,) and regulation (cingulate cortex, orbitofrontal cortex, prefrontal
cortex) of emotion. This hypothesis was not confirmed. However, although it was not a pre-hypothesized region, we found that depressed adolescents had a larger left precuneus volume than controls, an area of the brain which, although it is not part of the fronto-limbic circuitry, has previously been implicated in depression and has been shown to be important for depression relevant functions (Grieve, Korgaonkar, Koslow, Gordon, & Williams, 2013). Further, individuals with depression had smaller prefrontal gyri than those without.

**Precuneus**

Although it is not part of the fronto-limbic system, the precuneus has connections with a number of fronto-limbic structures such as the dorsolateral prefrontal cortex, anterior cingulate cortex and thalamus. The precuneus is an area of association cortex, and thus is involved in higher order integrated functions as opposed to more basic visuo-spatial, motor, cognitive or emotional processes. Especially relevant to depression, the precuneus has been implicated in the retrieval of a number of aspects of episodic memory, as well as internal imagery (see Cavanna, & Trimble 2006 for a review of the role of the precuneus). Further, it appears to be active during self-processing tasks and it has been suggested that the precuneus forms part of a neural network necessary for self-awareness and consciousness.

Previous studies have implicated the precuneus in depression. One study looking at depressed adolescents found decreased resting state connectivity between the subgenual anterior cingulate and precuneus in depressed adolescents, and that this decrease in connectivity was associated with increased rumination (Connolly et al., 2013). Further, decreased precuneus volume has been found in depressed individuals
(Grieve, Korgaonkar, Koslow, Gordon, & Williams, 2013) and has also been associated with childhood abuse (Jensen et al., 2015). Thus, the results the current study are consistent with the literature and suggest a potential role of the precuneus in adolescent depression.

*Precentral Gyrus*

Interestingly, we also found that individuals with depression had smaller left precentral gyri. A smaller precentral gyrus has been reported in a previous meta-analysis of voxel based morphometry studies in depressed individuals (Bora, Fornito, Pantelis & Yücel 2012). Further, decreased precentral gyrus volume has also been reported in elderly individuals with sub-threshold depression as well as individuals with a cognitive vulnerability to depression as expressed by a negative cognitive style. Thus, decreased precentral gyrus volume may be associated with a vulnerability to depression and may persist in individuals who go on to develop depression; moreover, our study suggests that this may be apparent as early as adolescence (Taki et al., 2005; Zhang et al., 2012). Whether decreased prefrontal gyrus volume can account for depressive symptomatology, however, is unclear. The main functional area of the precentral gyrus is the primary motor cortex; how this might related to depressive symptoms is not obvious, but it may underlie psychomotor retardation or may simply be a byproduct of some other underlying mechanisms.

Interestingly, although findings in the adult literature of decreased amygdala and hippocampal volume in depressed individuals have been relatively consistent our study failed to identify any such differences (Bora, Fornity, Pantelis & Yücel, 2012; Bremner et al., 2014; Campbell, Marriott, Nahmias & MacQueen, 2014). One important reason why this
may be is that meta-analyses have shown that decreases in hippocampus and amygdala volume seem to be associated with an increase in the total duration of depressive episodes (Hamilton, Siemer, & Gotlib 2008; Videbech & Ravnkilde 2004). Given that our sample consisted of adolescents, it is possible that decreases in hippocampal and amygdala volume were not found as they may not have been depressed long enough for the effects of chronic stress associated with depression to cause significant atrophy of these regions. Longitudinal follow up studies are needed to further test this notion. Further, findings of a decreased amygdala are more consistent in samples with co-morbid anxiety, while generalized anxiety disorder was present in only 6 of the 24 depressed patients in our sample (Bora, Fornity, Pantelis & Yücel, 2012)

**DNA Methylation: Depressed vs. Controls**

As part of the first aim, we also sought to identify differences in peripheral DNA methylation between the depressed adolescents and controls. We did not find any differences in *SLC6A4* methylation between the two groups. We did, however, find that the depressed group had lower levels of *FKBP5* methylation at site 5, and that there was a trend towards lower levels at site 6.

Lower levels of *FKBP5* methylation would be presumed to be indicative of increased transcription of the FK506 binding protein, which regulates sensitivity of the glucocorticoid receptor by decreasing the affinity of cortisol and other glucocorticoids to the receptor. Thus, lower DNA methylation levels would suggest that the depressed group would display decreased sensitivity to glucocorticoids. This is consistent with the depression literature, but only in more severely depressed individuals (Harkness, Stewart, & Wynne-Edwards, 2011). It is possible that this decrease in methylation may
represent an attempt downregulate the sensitivity of the glucocorticoid receptor in response to repeated stress. Interestingly, decreased \textit{FKBP5} methylation has been linked to dysregulation of the stress hormone system in the context of trauma. Thus, it appears that decreased \textit{FKBP5} methylation may play a role in the dysregulation of the stress response in individuals with depression.

Given the importance of the serotonin transporter in guiding the development of brain structures relevant to depression, as well as its role in directly regulating neurotransmission, and based on findings in adult samples, we hypothesized that, relative to controls, depressed individuals would show greater levels of methylation at the \textit{SLC6A4} promoter; however, this hypothesis was not confirmed. There are a number of possible reasons as to why this might be the case. Firstly, it can be argued that the study was underpowered. However, a recent study in depressed adult samples, using the same methylation assay, could also not confirm an association between depressive symptoms and methylation (Booij et al., 2015). It may be that previously reported associations between \textit{SLC6A4} methylation and depression may be due to other clinical characteristics or alternatively, go beyond the the CpG sites that were assessed in our study and those in Booij et al., 2015. Further, there is some evidence that DNA methylation may be influenced by DNA sequence effects (Chiarella et al., 2015). With regard to the \textit{SLC6A4} gene, there is some research showing (albeit not consistently) that the association between methylation and poorer psychological outcome is moderated by the 5-HTTLPR polymorphism of the \textit{SLC6A4} gene (van IJzendoorn et al., 2010). Because of the relatively small sample size of the present study, we did not have enough power to control for genotype in our analysis. Studies of monozygotic (MZ)
twin samples are needed to fully control for DNA sequence effects (Chiarella et al., 2015). However, given the relatively low prevalence of MZ twin birth, these studies are very challenging and are not feasible to conduct, even more so when studying depressed samples.

One limitation of the current study which should be taken into consideration is that peripheral tissue was used. Because brain samples cannot be obtained in a non-invasive way, epigenetic research in living humans is confined to using peripheral measures. However, peripheral methylation of candidate genes has been shown to be related to brain samples, with saliva being a better predictor than blood (Di Sante et al., 2016; Smith et al., 2015) and SLC6A4 methylation has been shown to correlate with brain serotonin synthesis (Wang et al., 2012). Nonetheless, results must be interpreted with caution because of this reason until other non-invasive neural epigenetic measures are better developed and applied, such as epigenetic imaging using Positron Emission Tomography measures with specific radioligands.

In summary, differences in volume of fronto-limbic structures between depressed adolescents and controls were not found. However, differences in volume in the precuneus and precentral gyrus, both of which have previously been identified in the depression literature, were found. Altered volume of these regions in depression may underlie symptoms of depression related to self-awareness and motor functioning, or may simply be a byproduct of some other underlying mechanism. In addition to differences in volume, differences in FKBP5 methylation were also identified, with depressed individuals having decreased FKBP5 methylation. This may play a role in the dysregulation of the HPA axis consistently reported in individuals with depression.
Further, no differences in SLC6A4 methylation were found, which may suggest that SLC6A4 methylation may be related to some other factors related to depression, or that results were unclear because of lack of control for genotype.

Aim 2: Trauma, DNA Methylation and Brain Development in Depressed Individuals.

The second aim of the present study was to test the link between trauma, methylation and brain development in depressed adolescents. Specifically, we hypothesized that: a) increased trauma would be associated with decreased volume of fronto-limbic areas associated with the production/consolidation (amygdala, hippocampus, insula,) and regulation (cingulate cortex, orbitofrontal cortex, prefrontal cortex) of emotion, b) increased trauma would be associated with increased DNA methylation of the SLC6A4 and FKBP5 genes, and c) increased SLC6A4 and FKBP5 methylation would be associated with decreased volume of the fronto-limbic regions mentioned above. Researchers have suggested that DNA methylation may provide a mechanism by which environmental factors such as trauma, may affect brain development (Booij et al., 2013, 2015). Thus, it is of interest to study the relationship between trauma and brain structure in the context of epigenetics.

Trauma and Brain Volume

Using an ROI analysis, we found that, in line with our hypothesis and previous findings, increased total trauma was associated with a trend towards decreased volume of the right anterior insula. Further, in spite of very few people reporting high scores of physical abuse, increased physical abuse in particular was associated with decreased volume of the left hippocampus and a
trend towards decreased volume of the left/right anterior insula and left hippocampus, and
greater emotional abuse was associated with a trend towards increased volume of the right
middle cingulate.

These findings are consistent with past research linking trauma to fronto-limbic
morphology (Ansel et al., 2012; Frodl et al., 2010). Further, these findings are interesting given
the functions of these two regions. The anterior insula is implicated in the experiencing of pain
and other interoceptive feelings such as temperature or arousal. Further, along with the anterior
cingulate cortex, it is activated when subjects experience emotions such as happiness, fear,
sadness, anger and empathy (Craig, 2009). Thus, altered anterior insula volume may underlie an
inability to experience pleasure and a more frequent experience of negative feelings in
depression. The hippocampus, on the other hand, is important for the consolidation of memories
with an emotional component and does so through its interconnections with the amygdala
(Phelps, 2004). Moreover, it is crucial for the modulation of the HPA axis stress response
(Sahay, & Hen 2007). Altered hippocampal volume, therefore, may be both a product of and
cause of HPA axis hyperactivity.

**Trauma and DNA Methylation**

As part of our second aim, we also sought to explore the link between trauma and DNA
methylation in depressed adolescents. Greater total trauma was associated with a trend towards
decreased methylation, as was sexual abuse. Further, physical abuse was associated with
decreased *SLC6A4* methylation. None of the trauma measures were associated with *FKBP5*
methylation levels. These results are not what would be expected given past literature which has
tended to show a positive association between the two variables, with increased trauma being
associated with increased methylation (Booij et al., 2015). However, past research has been
conducted with adults (Booij et al., 2013; Frodl et al., 2010) while the current study used adolescent participants. How exactly adolescents would be expected to differ from adults from a theoretical standpoint is unclear; it may be that increased methylation later in life may indicative of overcompensation for decreased levels in adolescents. Further, the direction of the relationship between trauma and methylation may not be as important as the fact that trauma may lead to a dysregulation of DNA methylation, ultimately affecting brain development. If this view is accepted, it appears that physical abuse may be one of the best predictors of DNA methylation dysregulation. Regardless of the underlying mechanism, this study suggests the relationship between methylation and trauma may be moderated by age.

Interestingly, we did not find a strong association between emotional trauma and DNA methylation, even though there is a strong link between emotional abuse/neglect and depression (Brown et al., 1999; Clark, Caldwell, Power & Stansfeld, 2010). It is possible that the effects of physical trauma are more pronounced, and that the current study, with its modest sample size, may not have been sensitive enough to detect an effect of emotional trauma.

**DNA Methylation and Brain Volume**

Also part of the second aim was to explore the relationship between DNA methylation of the *FKBP5* and *SLC6A4* genes and brain morphology in depressed adolescents as both genes have been implicated in brain development, especially of regions relevant to depression and trauma (Kish et al., 2005; Varnäs, Halldin & Hall, 2004; White et al., 2012). Further, DNA methylation is thought to mediate the effect of the environment on brain development and, ultimately, cognition and behaviour (Booij et al., 2013).

It was hypothesized that *SLC6A4* and *FKBP5* methylation would be associated with decreased volume of both emotion producing regions and emotion regulation regions based on
previous findings in the depression and trauma literature. Consistent with previous research increased *FKBP5* methylation was associated with decreased hippocampal volume, decreased volume of the right posterior orbitofrontal gyrus and trend towards decreased amygdala volume (Edmiston et al., 2011; Rajkowska, 2000).

However, the majority of results were in the opposite direction as was expected, with increased *SLC6A4* methylation being associated with increased left hippocampal volume, increased anterior and middle cingulate volume. Thus, we did find that methylation was associated with altered brain development; however, the direction of this relationship was somewhat inconsistent given the current results and past research (Ansel et al., 2012; Cohen et al., 2006; Cotter et al., 2002; van Harmelen et al., 2010; Dannlowski et al., 2012). Also inconsistent with what would be expected given the link between trauma and brain development and the link between trauma and methylation, increased *FKBP5* methylation was associated with increased left anterior cingulate and right insula volume (Dannlowski et al., 2010).

Thus, there does appear to be a link between DNA methylation and fronto-limbic brain development, however, the direction is not as consistent as it has been in the adult literature. This may reflect the unique nature of the adolescent brain, which is still undergoing significant development.

**Summarizing the results**

Together, these results partly support the possible role of DNA methylation as a regulator of brain development. That is, the results are consistent with the proposed model that trauma may lead to changes in DNA methylation, which ultimately affect brain development and lead to the neuropathophysiology characteristic of depression. In particular it appears that physical trauma may be one of the best predictors of altered brain development.
Although the relationship between trauma and methylation, as well as methylation and brain development, was mostly in the opposite direction of what would be expected based on the adult literature, it was often consistent with itself. For example, increased physical trauma was associated with decreased \textit{SLC6A4} methylation, which in turn was associated with changes in volume that would be expected given the trauma (e.g., decreased anterior cingulate, insula, and hippocampal volume). It is interesting that, while \textit{SLC6A4} methylation was associated with both trauma and brain development, \textit{FKBP5} methylation was only associated with brain development. Thus, it may be that \textit{FKBP5} methylation is an important regulator of brain development in depression, but that it may be regulated by factors other than trauma. However, this lack of findings could also result from other factors, such as a more heterogeneous genotype in the depressed group in regards to the \textit{FKBP5} gene than the \textit{SLC6A4} gene which would obscure results.

In addition to rendering results more difficult to interpret, a lack of control over genotype is likely to have decreased the power of the study due to an increase in noise. It will be important for future studies to control for genotype in future studies. Further, the current study was retrospective in nature. It will be important for future studies to use a longitudinal design to determine how methylation changes over time and how these changes over time correspond to brain development and trauma. Further, it will be important to determine whether major traumatic events can elicit changes in DNA methylation, or if repeated events are necessary. The gold standard design to further investigate the relationship between trauma, DNA methylation and brain development is the longitudinal twin design, which solves both of these problems by comparing following MZ twins over time and analyzing variation within monozygotic twin pairs. This would allow us to look at differences due to the environment while controlling for
genotype (Chiarella et al., 2015). In the case that a twin design is not feasible, it will be important at the very least to conduct future studies in adolescents using a larger sample size which would allow for statistical control of genotype. Double blind placebo controlled intervention studies will also be important to conduct in order to determine how DNA methylation and associated brain outcomes can be reversed by treatment in order to elucidate any causal role of methylation in psychopathology. This would also be another way of overcoming the confound of genotype. Further, it will also be important to obtain more of a range of trauma, as the current study had few participants who had experienced severe trauma compared to those who had not, especially with regard to physical abuse. Moreover, future studies should look at more heterogeneous populations, as the current study consisted mostly of Caucasian females and thus may be hard to generalize to males and other cultures. Finally, the results of the current study may be confounded by medication use, as antidepressants have been shown to act, at least in part, by affecting DNA methylation levels (Tsankova et al., 2006). However, it is also important to recognize that excluding individuals based on medication use may lead to an unrepresentative sample. Further, we did not exclude participants based on comorbid psychopathology (with the exception of substance abuse, suicidality and psychosis), making the results more generalizable to the population. However, in the future, both ecologically valid and more controlled studies will be important in order to gain a holistic picture of the role of DNA methylation and trauma in depression. It is also important to note that when interpreting these results that, in addition to volume and thus number of cells, function and connectivity are also important for which together will account for the strength of the signal. In other words, while the grey volume of a particular region may be diminished, this may lead to compensatory mechanisms such as increased neuronal firing rate or more efficient connectivity. Thus,
functional magnetic resonance imaging and diffusion tensor imaging will be important in clarifying these results.

In conclusion, the results of the current study provide evidence for some differences in brain morphology in adolescents with depression compared to controls, but not in fronto-limbic circuitry. This may be because differences in fronto-limbic morphology do not manifest until adulthood after exposure to more chronic stress, or may be due to methodological or statistical factors such as low sample size or small effect size. Further, some differences in methylation were identified, but only in the FKBP5 gene and not the SLC6A4 gene. Although the mechanism is unclear it could be speculated that the FKBP5 gene may be more strongly associated with youth depression than the SLC6A4 gene.

Further, the study found mixed results for a model which describes depression as arising due to the effects of trauma and life stress on methylation, and, in turn, brain development. Though associations between trauma, DNA methylation and fronto-limbic circuitry were found, they were often in the opposite direction than was predicted. This may result from the unique nature of the adolescent biology, lack of control over genetic factors and/or problems inherent in using peripheral measures of DNA methylation. Future longitudinal follow-up studies, following youth populations over time and throughout the course of treatment and recovery are needed to better understand how DNA methylation and brain morphology of adolescents changes over time and into adulthood.
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Appendix A

Total Trauma and Brain Morphology

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<th>Anatomical Region</th>
<th>L/R</th>
<th>Cluster Size (k)</th>
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<th>MNI coordinates x</th>
<th>y</th>
<th>z</th>
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<tr>
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<td>-35</td>
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<td>-15</td>
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<tr>
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<td>Suplemental Motor Area</td>
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<td>Superior Frontal Gyrus</td>
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Note. * Indicates significance at the cluster level using p < .001. MNI = Montreal Neurological Institute. L = Left, R = Right.
## Appendix B

### SLC6A4 Methylation and Brain Morphology

<table>
<thead>
<tr>
<th>Anatomical Region</th>
<th>L/R</th>
<th>Cluster Size (k)</th>
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<th>z</th>
<th>MNI coordinates x/y/z</th>
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<tr>
<td><strong>Total SLC6A4 methylation</strong></td>
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<td></td>
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<td>4.60</td>
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<td>-35/-38/-12</td>
</tr>
<tr>
<td>Middle Temporal Gyrus</td>
<td>L</td>
<td>6.84</td>
<td>4.91</td>
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<tr>
<td>Middle Occipital Gyrus</td>
<td>L</td>
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<td>4.91</td>
<td>-62</td>
<td>-77/0</td>
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<tr>
<td>Middle Temporal Gyrus</td>
<td>L</td>
<td>6.84</td>
<td>4.91</td>
<td>-62</td>
<td>-77/9</td>
</tr>
<tr>
<td>Superior Frontal Gyrus</td>
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<td>14</td>
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<td>4.91</td>
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</tr>
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<td>35</td>
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<td>4.91</td>
<td>39/63/26</td>
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<td>4.91</td>
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<td>Angular</td>
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<td>342</td>
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<td>4.91</td>
<td>-65/-68/24</td>
</tr>
<tr>
<td>Angular</td>
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<td>4.91</td>
<td>-63</td>
<td>-68/35</td>
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<td>Angular</td>
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<td>8.84</td>
<td>4.91</td>
<td>-63</td>
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<td>Supplemental Motor Area</td>
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<td>55</td>
<td>6.84</td>
<td>4.91</td>
<td>11/27/74</td>
</tr>
<tr>
<td>Supplemental Motor Area</td>
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<td>30/71</td>
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<tr>
<td>Lobule IX of Cerebellar Hemisphere</td>
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<td>17</td>
<td>4.98</td>
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<td>-5/-50/-72</td>
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<tr>
<td>Hippocampus</td>
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<td>4.93</td>
<td>3.97</td>
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<td>24</td>
<td>4.93</td>
<td>3.97</td>
<td>-57/-41/60</td>
</tr>
<tr>
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<td>13</td>
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<td>3.79</td>
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<tr>
<td>Inferior Frontal Gyrus, Pars Trangularis</td>
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<td>86</td>
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<td>3.66</td>
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<td>24</td>
<td>4.31</td>
<td>3.61</td>
<td>24/-81/15</td>
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<tr>
<td>Superior Temporal Gyrus</td>
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<td>3.25</td>
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<td>R</td>
<td>4.18</td>
<td>3.53</td>
<td>38</td>
<td>-65/23</td>
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<td>Middle Occipital Gyrus</td>
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<td>Calcarine Sulcus</td>
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<tr>
<td>Medial Frontal Gyrus</td>
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<td>59</td>
<td>4.05</td>
<td>3.44</td>
<td>9/50/32</td>
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<tr>
<td>Medial Frontal Gyrus</td>
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<td>3.66</td>
<td>3.18</td>
<td>12</td>
<td>48/24</td>
</tr>
<tr>
<td>Angular</td>
<td>R</td>
<td>12</td>
<td>3.92</td>
<td>3.36</td>
<td>65/-59/30</td>
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<td>Middle Cingulate</td>
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<td>121</td>
<td>3.86</td>
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### Negative Correlation
**Total SLC6A4 methylation**

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<tr>
<th>Region</th>
<th>Side</th>
<th>Total</th>
<th>Mean</th>
<th>SEM</th>
<th>Min</th>
<th>Max</th>
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<tr>
<td>Lobule VI of Cerebellar Hemisphere</td>
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<td>296</td>
<td>4.85</td>
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<td>Crus II of the Cerebellar</td>
<td>R</td>
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<td>3.26</td>
<td>9</td>
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<td>-30</td>
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<tr>
<td>Lobule VI of Vermis</td>
<td></td>
<td>63</td>
<td>4.15</td>
<td>3.51</td>
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<td>-69</td>
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<td>Lobule VIII of Cerebellar</td>
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<td>90</td>
<td>4.01</td>
<td>3.41</td>
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<td>-62</td>
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<td>Lobule VIII of Cerebellar</td>
<td>R</td>
<td>3.81</td>
<td>3.28</td>
<td>23</td>
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<td>-44</td>
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<tr>
<td>Hemispheric</td>
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<td></td>
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<td></td>
<td></td>
<td></td>
</tr>
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<td>3.93</td>
<td>3.36</td>
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<td>Putamen</td>
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<td>3.62</td>
<td>3.16</td>
<td>18</td>
<td>0</td>
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</tbody>
</table>

*Note.* * Indicates significance at the cluster level using \( p < .001 \). MNI = Montreal Neurological Institute. L = Left, R = Right.
**Appendix C**

*FKBP5 Methylation and Brain Morphology*

<table>
<thead>
<tr>
<th>Anatomical Region</th>
<th>L/R</th>
<th>Cluster Size (k)</th>
<th>t</th>
<th>z</th>
<th>MNI coordinates x</th>
<th>y</th>
<th>z</th>
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<td><strong>Positive Correlation</strong></td>
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<tr>
<td><em>FKBP5 methylation site 5</em></td>
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<tr>
<td>Inferior Temporal Gyrus</td>
<td>L</td>
<td>27</td>
<td>3.91</td>
<td>3.33</td>
<td>-63</td>
<td>-24</td>
<td>-18</td>
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<td></td>
<td></td>
<td></td>
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<td></td>
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<tr>
<td>Angular</td>
<td>L</td>
<td>27</td>
<td>4.63</td>
<td>3.77</td>
<td>-36</td>
<td>-51</td>
<td>30</td>
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<td><strong>Negative Correlation</strong></td>
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<td><em>FKBP5 methylation site 5</em></td>
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<tr>
<td>Precentral</td>
<td>R</td>
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<td>4.13</td>
<td>3.47</td>
<td>26</td>
<td>-27</td>
<td>63</td>
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<tr>
<td>Inferior Parietal Lobule</td>
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<td>26</td>
<td>4.12</td>
<td>3.46</td>
<td>-62</td>
<td>-26</td>
<td>47</td>
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<td>L</td>
<td>12</td>
<td>3.98</td>
<td>3.37</td>
<td>-32</td>
<td>29</td>
<td>38</td>
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<tr>
<td><em>FKBP5 methylation site 6</em></td>
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<td>Supramarginal Gyrus</td>
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<td>-23</td>
<td>47</td>
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<td>5.86</td>
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<td>65</td>
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<td>-11</td>
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<td>-38</td>
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<td>-20</td>
<td>81</td>
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</tbody>
</table>

Note. * Indicates significance at the cluster level using p < .001. MNI = Montreal Neurological Institute. L = Left, R = Right.
Appendix D

Childhood Experience of Care and Abuse Interview Sample Rating Forms

CECA RATING FORM: FAMILY ARRANGEMENTS

1. ARRANGEMENT NO.: _____
2. Age at Beginning of Arrangement: _______  3. Age at End of Arrangement: _______
4. ID for Family Structure: (0) Boyfriend/Girlfriend (1) Spouse (2) Child (3) Mother (4) Father
   (5) Surrogate Mother (6) Surrogate Father (7) Full Sibling (8) Half-Sibling
   (9) Ex-Partner (10) Alternative Caregiver (11) Confidant (12) Friend
   (13) Grandmother (14) Grandfather (15) Aunt (16) Uncle (17) Cousin
   (18) Family Friend (19) Stranger (20) Subject (21) Other [please specify]
5. Number of Full siblings: _____  6. Number of surrogate siblings: _____

Description of family arrangement:

(A) ANTIPATHY

Description – Maternal
7. ANTIPATHY: (1) Marked (2) Moderate (3) Some (4) Little/None

Description – Paternal
8. ANTIPATHY: (1) Marked (2) Moderate (3) Some (4) Little/None
9. OVERALL ANTIPATHY: (1) Marked (2) Moderate (3) Some (4) Little/None

B) INDIFFERENCE/NEGLECT

Description – Maternal
10. INDIFFERENCE: (1) Marked (2) Moderate (3) Some (4) Little/None

Description – Paternal
11. INDIFFERENCE: (1) Marked (2) Moderate (3) Some (4) Little/None
12. OVERALL INDIFFERENCE: (1) Marked (2) Moderate (3) Some (4) Little/None

CECA RATING FORM: ABUSE (PHYSICAL, SEXUAL, OR PSYCHOLOGICAL)

ID/Description
AGE AT START OF ABUSE: _____  AGE AT END OF ABUSE: _____
DURATION (in weeks) IF LESS THAN 1 YEAR: _____

WORST: (1) Marked (2) Moderate (3) Some (4) Little/None

TYPICAL: (1) Marked (2) Moderate (3) Some (4) Little/None

FREQUENCY: WORST: _______  TYPICAL: _______
1. Once only  5. Twice a year or more
2. Weekly or more  6. Once a year
3. Monthly or more  7. Less than once a year
4. Every 3 months or more

CHANGE IN CARE RECORD

<table>
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<tr>
<th>ID of Caregiver</th>
<th>Variable Involved</th>
<th>New Rating</th>
<th>Age at Change</th>
<th>Reason for Change</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Appendix E

Adolescent Assent Form

I understand that the aim of this study is to learn more about the cognitive, emotional, and biological characteristics of adolescents with depression.

I understand that the study involves one (or more if I want to split the time up) visits to the Queen’s Psychology Building (Humphrey Hall) and one visit to the fMRI Facility in the Queen’s Centre for Neuroscience Studies, which is located in the lower level of the Cancer Research Institute. That means that I will need to come to the Queen’s University campus once, or twice, if I prefer to complete the study in two separate sessions. I understand that I have the option to complete the study in one 4-hour session, or in two 2-hour sessions, and that my appointment(s) can be made to fit into my (and my parent’s) schedule.

I understand that I will be asked some questions in person (interviews), as well as fill in some questionnaires dealing with some personal information. I will also be asked to do two tasks on the computer: for one, I will read words and decide whether they describe me; for the other, I will indicate the orientation of an arrow. I will also spit into a tube to give a saliva sample so that the researchers can take it to their laboratory and extract DNA (Deoxyribonucleic acid), which contains important genetic information.

I will get a brain scan, or a Magnetic Resonance Image (MRI) scan. The MRI scanner is a big machine that uses a strong magnet and radio waves to make images of the inside of the body. I will lie down on the scanner bed and the machine will scan around me. I won’t feel anything from the strong magnet and I won’t be harmed by the MRI scanner. The scan will take about 40 minutes. I will have an intercom in the MRI scanner and will be able to tell the MR operator to stop if I feel uncomfortable or tired at any time. I will need to wear clothes without metal and remove earrings, piercings etc. I’ll also go through a checklist beforehand to make sure I can have an MRI. Pregnant females shouldn’t have an MRI, so I may have to take a quick pregnancy test (it will be provided at no cost to me). If I cannot have or do not want to have an MRI, then I can still participate in the rest of the study.

I understand that one interview will be audio-taped. I understand that these tapes will be private, and heard only by Dr. Booij and her helpers. I know that I will be assigned a number, and that my name will not be connected to any of the information, including the audiotape of my interview.

I understand that I may skip any question that I do not want to answer. I understand that I may get out of the study at any time without giving a reason, and that nobody will be mad at me if I do. My parent can also decide, at any time, that we don’t want to be in this study any longer.

I understand that after I complete the interviews, the questionnaires, the computer tasks, the fMRI scan, and provide my saliva sample, I will get a $50 gift certificate to either Bestbuy, Lululemon, or Chapters/Indigo to thank me for my participation (I get to choose the gift certificate from these three options). I will also get a black and white picture of my brain, if I want it. My family will also receive $10 for parking and transportation costs.

I understand that if I am a First Nations person, or an indigenous person who has contact with spiritual elders I may want to talk with them before I proceed with being part of this experiment. Elders have reservations about genetic procedures.

I understand that I may contact Dr. Booij, or any of her helpers, at any time during the study, if I have questions. Dr. Booij is a professor at Queen’s University, and her helpers are
students at Queen’s University. Dr. Booij can be reached at (613) 533-6000 ext 78158. I also understand that I may contact the Head of the Psychology Department (Dr. Wendy Craig: (613) 533-6014) or the Chair of the Queen’s University Health Sciences and Affiliated Teaching Hospitals Research Ethics Board (Dr. Albert Clark, (613) 533-6081) if I have any concerns about the project that I do not want to discuss with Dr. Booij or her helpers.

Circling “I agree” below means that I would like to participate in the study.

I agree  I do not agree

Signature: _________________________________    Date: ________________
Appendix F

LETTER OF INFORMATION & CONSENT FORM

ATTENTION:

If 16 years of age and older: Adolescent is hereafter “The Participant” and can give his/her own consent to participate.

If under 16 years of age: Adolescent is hereafter “The Participant” and parent or guardian must consent to their adolescent’s participation. The participant also signs an Assent Form to agree to participate.

BACKGROUND INFORMATION: (Overview of study)

We are inviting adolescents to participate in a research study conducted by researchers at Queen’s University (Dr. L. Booij (PI), Ms. L. Schumann, Dr. K. Harkness, and Dr. I. Johnsrude), in collaboration with Hotel Dieu Hospital (Dr. S. Khalid-Khan, Ms. B. Blaney, Dr. J. Smythe, and Ms. Anita Peter), and Trinity College in Dublin, Ireland (Dr. Thomas Frodl). The purpose of this study is to examine cognitive, emotional, and biological characteristics of adolescents with depression. Ms. Schumann or another member of the research team will read through this consent form with you, describe procedures in detail and answer any questions you may have. Participation in the study involves interviews, questionnaires, computer tasks, and a saliva sample, which will be completed or collected during one or two visits to the Queen’s Psychology Building (Humphrey Hall) and the fMRI Facility in the Queen’s Centre for Neuroscience Studies, which is located in the lower level of the Cancer Research Institute. The total amount of time for participation will be approximately 4 hours.

DETAILS OF THE STUDY

1. Aim of the study

As stated above, the aim of this study is to examine cognitive, emotional, and biological characteristics of adolescents with depression. The participant will be asked to report on
aspects of his/her personal history, how he/she deals with life stressors, as well as to perform
two computerized tasks. The assessment also consists of a Functional Magnetic Resonance
Imaging or fMRI scan (lasting approximately 45-60 minutes, including set up time). The
scanner uses a strong magnetic field to create detailed images of brain structure and
function. Additionally, we would like to collect a saliva sample for genetic research
(deoxyribonucleic acid; DNA; information necessary for the proper development and
function of living organisms). Using the results, we can investigate the relationship between
depressive symptoms and the structure and function of certain brain regions as well as the
expression of DNA (so called DNA methylation).

2. **Description of visit(s) and tests to be performed as part of the study**

*What does participation involve?*

If the participant agrees to participate, the research will consist of one or two visits
(depending on the participant’s scheduling preferences) to Queen’s University
campus. Total time is four hours. Ms. Lyndall Schumann (project coordinator, PhD
student) will conduct two interviews with participants (approximately 2 hours).
Participants will provide information about their background and answer questions
about the presence or absence of adverse experiences in childhood. The interviews
will be audiotaped for later analysis and stored with other confidential information.
Participants will also complete nine short questionnaires, two computer tasks, and
will provide a saliva sample (participants will spit into a tube; see Page 4 of this
form). These tasks will take approximately 1 hour in total.

The study also includes a functional magnetic resonance imaging (fMRI) scan in the
Queen’s Centre for Neuroscience Studies fMRI Facility (approximately 1 hour).
During the scan, the participant’s brain will be imaged while he/she is lying in a 3
tesla MRI scanner. At the completion of the session(s), the participant will receive
a $50 gift certificate to a store (one of three options: Bestbuy, Chapters/Indigo or
Lululemon).

**MRI Scan**
a) Depending on the participant’s scheduling preferences, the fMRI scan may be completed immediately following the interview or during a separate appointment time. Participants will be led by a member of the research team from the Queen’s Psychology building to the Queen’s Centre for Neuroscience Studies fMRI Facility (5 minute walk). Participants who choose to complete the measures in two appointments will meet a member of the research team at the fMRI Facility. There, the participant will fill out a checklist and questionnaire to make sure he/she is eligible for an fMRI scan. This questionnaire will take about 5 minutes. Participants must have no history of chronic neurological disorders, accidents involving prolonged loss of consciousness, or long-term psychoactive medication use. Participants are also not eligible for the MRI scan if they have orthodontic braces or appliances that cannot be removed at the time of the scan. If participants are pregnant or are trying to conceive they will not be eligible. If there is any uncertainty regarding whether or not a participant is pregnant and she wants to participate in the study, we will provide a pregnancy test that must be done prior to the experiment.

b) Prior to the scan, the participant will be familiarized with a 15-minute task that he/she will be asked to do in the scanner, which will consist of looking at pictures with different emotions (positive, negative, or neutral) and answering “yes” or “no” to questions by pressing one of two buttons.

c) Participants will be asked to remove or change out of any clothes that contain metal that will be near the area being imaged, and they will be asked to remove their shoes. Please try to wear clothing containing no metal, or to bring a change of clothing. Metal in zippers, snaps, and the wire and metal clasps in some bras can interfere with the imaging. Many shoes contain metal as well. For imaging the brain, the snaps and zippers in jeans or other pants are far enough from the area being imaged that they do not cause a problem.

d) Participants will be given a set of earplugs to wear in the scanner and then asked to lie on their back on the well-padded bed of the magnet. Pillows will be placed under their legs for comfort and a blanket will be placed over their legs if the
participant wishes. A head coil will be placed over the participant’s head. This is fitted with a mirror so that the participant can see out of the magnet towards his/her head or feet. The participant and the bed will then slide into a long tube (the magnet).

e) The participant will need to keep still while the images are taken. To help him or her, we will make the participant as comfortable as possible and we will pack soft foam around his or her head if needed.

f) The MR system has a two-way intercom for communication. Because scanning can be quite noisy, the participant will also be given an alarm bulb so that he/she can call the researcher or operator during the scan if something is wrong or he/she needs to come out.

g) During the scan, the participant will first have two sessions of 10-minutes each where he/she will only be required to rest and lie still with eyes closed. The first part (called anatomical MRI) gives us a clear picture of his/her brain, and the second (called diffusion tensor imaging) tells us about the connections between different parts of the brain. Next, there will be a 6-minute session where we will ask participants to lie still with his/her eyes open while we measure his/her brain activity at rest. Finally, we will ask participants to perform the 15-minute task, which will consist of looking at pictures with different emotions and orientations (horizontal or vertical). Participants are asked to respond “yes” or “no” to questions regarding the emotional content and orientation of the picture by pressing one of two buttons. Overall, the amount of time spent in the scanner should amount to about 40 minutes. The whole scanning session, including set up time, should last between 45 minutes and one hour.

3. Exclusions/Contraindications

The following are contraindications for a magnetic resonance imaging study:

- Pacemaker
- Aneurysm Clip
- Heart/Vascular Clip
- Prosthetic Valve
- Metal Prosthesis
- Claustrophobia
- Metal fragments in body
- Tattoos and piercings (piercings must be removed prior to scanning)
- Pregnancy
- Transdermal Patches (must be removed prior to scanning. The participant is advised to bring an additional patch to reapply post scanning)

Participants will also be excluded from the study if they are:
- Have a history of a psychotic disorder
- Are actively suicidal
- Are currently abusing drugs or alcohol

and
- Participants may not be able to have the MRI if they have orthodontic braces (unless they can be removed by the participant at the time of the scan).

4. An explanation of the special research techniques that will be used

a) fMRI

The MRI scanning procedure is very much like other medical imaging used in hospitals, but participants will not be exposed to x-rays. This MRI machine uses a strong magnet and radio waves to make images of the interior of the body. Participants will not feel either. The MRI being used in this study is a 3 Tesla MRI that is twice that used for most clinical imaging, although 3 tesla systems are becoming more common in hospitals. The levels of magnetism and radio waves used in the MRI have not been shown to cause harmful effects. However, the MR scanner uses a very strong magnet that will attract metal. Therefore ALL metallic objects must be removed from a participant’s person before he/she approaches the scanner. Those with a cardiac pacemaker or a metallic clip in their body (e.g., an aneurysm clip in your brain or an I.U.D.) should not participate in any MRI study. In addition, credit cards and other cards with magnetic strips should also be removed as these will be damaged. (These items will be kept safe for the participant).

Participants will be in voice contact with the operator, and the operator will be able to see them via a camera. Participants may ask the operator to stop the experiment
at any time. Participants should ask to stop the experiment if they feel tired, claustrophobic, or uncomfortable.

b) DNA analysis from saliva sample

We want to learn more about the relationship between depressive symptoms, the brain, and DNA. Simply, DNA methylation is a process that happens to DNA, where a chemical component (a methyl group) is added to DNA, which can affect the functioning of the gene by turning its activity on or off. DNA methylation is influenced by the environment, for example by stressors happening early in life. To assess DNA methylation, we will require participants to donate 3-6 ml of saliva (approximately one tea spoon) into a tube. Participants should not to eat or drink anything 30 minutes before the saliva sample is collected. Samples will be securely stored in Dr. Booij’s laboratory until further analysis. DNA analyses will be performed in the laboratory of Dr. Xudong Liu, in the Queen’s University department of Psychiatry, as well as the laboratory of Dr. Moshe Szf, in the McGill University department of Pharmacology and Therapeutics. Participant samples will be identified by participant code only, and Dr. Liu and Dr. Szf and their teams will not have access to any personal information (e.g., names, etc.) of any participants. DNA can be used for future related studies conducted by Dr. Booij and her team 10 years after the start of this study if the participant (or parent if participant is under the age of 16 at the time that this consent form is signed) gives special permission to do so. All DNA will be destroyed after 10 years (September 2023).
5. Advantages
There are no direct advantages from participation in this research project. However, the knowledge acquired will contribute to scientific advancements. It is our hope that a greater understanding of the cognitive, emotional, and biological characteristics of adolescents with depression will lead to the identification of adolescents at risk of developing depression and improve methods to prevent and treat chronic recurrent depression.

6. Risks
There are no known risks involved with Magnetic Resonance Imaging when all the necessary steps of precaution are undertaken. However, the MR scanner uses a very strong magnet that will attract metal. Therefore ALL metallic or magnetic objects must be removed from a participant’s person before he/she approaches the scanner. Parents/guardians will be invited to sit outside the resonance magnetic imaging zone during the scanning. Verification of any contraindication will be strictly reinforced by the technologist on duty in the brain-imaging center.

The requirements imposed on the use of Magnetic Resonance Imaging may cause certain discomfort due to the need to remain still during the length of the examination and the noise generated by the scanner when images are being acquired. Participants will be provided with earplugs to minimize noise. Participants may also feel a certain sense of stress or a sense of claustrophobia. Should participants experience discomfort during the scan, they may withdraw at any time. This option will be explained to each participant.

Although this is not a diagnostic scan and any images obtained are for research purposes only, it is possible that the MR scan may disclose an unknown abnormality. In this event, a medical imaging specialist will review the images and a report will be sent to the participant’s physician. The researchers directly involved with this procedure do not have the credentials to diagnose medical conditions.
7. Confidentiality

All data gathered as a part of this project is confidential. There is the risk of loss of confidentiality for participants only if during any assessment they (a) disclose involvement in the abuse of children or elderly individuals, (b) disclose being the victim of current abuse (if under age 16), or (c) disclose threat to seriously harm self or others. If an adolescent discloses current abuse or severe neglect the interviewer will inform Child and Family Services and the adolescent’s treatment provider.

All information is identified using a participant code and no name will be attached to confidential information. Only the researchers directly related to this study will have access to the data files and the subject codes. As outlined above, saliva samples will only be identified using participant codes, and those researchers performing DNA analyses will not have access to participant names or any other personal information. Participants will not be identified in any publication or reports.

8. Voluntary nature of study/Freedom to withdraw or participate

Participation in this study is voluntary. Participants may withdraw from this study at any time and their withdrawal will not affect their current or future medical care. Participants are not obliged to answer any questions that they find objectionable or which make them feel uncomfortable. If participants withdraw, no more information will be collected from them. When a participant indicates that he/she wishes to withdraw, the investigator will ask if the information already collected can be used. If the participant does not provide consent for the use of this information, it will be destroyed.

9. Withdrawal of subject by principal investigator

The study director may decide to withdraw a participant from this study if:

1) He/she does not meet the criteria for the study, including those mentioned in the Magnetic Resonance Screening Form.
2) He/she meets any of the exclusionary criteria listed above.
3) He/she is unable to perform the tasks requested.
4) He/she misses sessions or does not come to sessions on time.

10. Liability

In the event that a participant is injured as a result of the study procedures, medical care will be provided to him or her until resolution of the medical problem. By signing this consent form, participants do not waive their legal rights nor release the investigator(s) and sponsors from their legal and professional responsibilities.

11. Compensation

As previously outlined, participants will each receive a $50 gift certificate after completing the interviews, the questionnaires, the computer tasks, the fMRI scan, and providing a saliva sample. Participant families will be compensated up to $10 for travel expenses and parking. In addition, participants will receive a black and white picture of their brain.

12. First Nations Elders

If you are a First Nations person, or an indigenous person who has contact with spiritual elders you may want to talk with them before you proceed with being part of this experiment. Elders have reservations about genetic procedures.
SUBJECT STATEMENT AND SIGNATURE

I have read and understand the consent form for this study. I have had the purposes, procedures and technical language of this study explained to me. I have been given sufficient time to consider the above information and to seek advice if I chose to do so. I have had the opportunity to ask questions which have been answered to my satisfaction. I have named Dr. ________________ at ________________ as the physician to be contacted for follow-up purposes. I am voluntarily signing this form. I will receive a copy of this consent form for my information. If at any time I have further questions, problems or adverse events, I can contact:

Dr. Linda Booij (P.I.)
Assistant Professor
Queen's University
Kingston, Ontario
K7L 3N6
Phone:  613-533-2881

Ms. Lyndall Schumann, M.Sc.
PhD Student, Clinical Psychology
Queen's University
Kingston, Ontario
K7L 3N6

If I have questions regarding my rights as a research participant I can contact

Dr. Albert Clark, Chair, Queen’s University Research Ethics Board at 613-533-6081
or
Dr. Wendy Craig, Head of Psychology Department, Queen’s University at 613-533-6014.
By signing this consent form, I am indicating that I agree to participate in:

☐ The imaging portion of the study (the fMRI)
☐ Collection of DNA using saliva
☐ Interviews and cognitive tasks

☐ AND I agree that DNA will be stored under a code and can be used for future related studies done by the P.I. (Dr. Booij) and her team. Any new use of DNA will undergo REB review/approval first.
☐ AND I agree that some information (about my diagnosis and medication) will be passed from my psychiatrist to the researchers

______________________________________________  __________________
Signature of Participants (16 years of age or older) Date

______________________________________________  __________________
Signature of Parent of Child Under 16 Years of Age Date

______________________________________________  __________________
Signature of Person Conducting Consent Discussion Date

STATEMENT OF INVESTIGATOR

I, or one of my colleagues, have carefully explained to the subject the nature of the above research study. I certify that, to the best of my knowledge, the subject understands clearly the nature of the study and demands, benefits, and risks involved to participants in this study.

______________________________________________  __________________
Signature of Principal Investigator Date
Appendix G
Adolescent Assent Form

I understand that the study involves one (or more if I want to split the time up) visits to the Queen’s Psychology Building (Humphrey Hall) and one visit to the fMRI Facility in the Queen’s Centre for Neuroscience Studies, which is located in the lower level of the Cancer Research Institute. That means that I will need to come to the Queen’s University campus once, or twice, if I prefer to complete the study in two separate sessions. I understand that I have the option to complete the study in one 4-hour session, or in two 2-hour sessions, and that my appointment(s) can be made to fit into my (and my parent’s) schedule.

I understand that I will be asked some questions in person (interviews), as well as to fill in some questionnaires dealing with some personal information. I will also be asked to do two tasks on the computer: for one, I will read words and decide whether they describe me; for the other, I will indicate the orientation of an arrow. I will also spit into a tube to give a saliva sample so that the researchers can take it to their laboratory and extract DNA (Deoxyribonucleic acid), which contains important genetic information.

I will get a brain scan, or a Magnetic Resonance Image (MRI) scan. The MRI scanner is a big machine that uses a strong magnet and radio waves to make images of the inside of the body. I will lie down on the scanner bed and the machine will scan around me. I won’t feel anything from the strong magnet and I won’t be harmed by the MRI scanner. The scan will take about 40 minutes. I will have an intercom in the MRI scanner and will be able to tell the MR operator to stop if I feel uncomfortable or tired at any time. I will need to wear clothes without metal and remove earrings, piercings etc. I’ll also go through a checklist beforehand to make sure I can have an MRI. Pregnant females shouldn’t have an MRI, so I may have to take a quick pregnancy test (it will be provided at no cost to me). If I cannot have or do not want to have an MRI, then I can still participate in the rest of the study.

I understand that one interview will be audio-taped. I understand that these tapes will be private, and heard only by Dr. Booij and her helpers. I know that I will be assigned a number, and that my name will not be connected to any of the information, including the audiotape of my interview.

I understand that I may skip any question that I do not want to answer. I understand that I may get out of the study at any time without giving a reason, and that nobody will be mad at me if I do. My parent can also decide, at any time, that we don’t want to be in this study any longer.

I understand that after I complete the interviews, the questionnaires, the computer tasks, the fMRI scan, and provide my saliva sample, I will get a $50 gift certificate to either Bestbuy, Lululemon, or Chapters/Indigo to thank me for my participation (I get to choose the gift certificate from these three options). I will also get a black and white picture of my brain, if I want it. My family will also receive $10 for parking and transportation costs.
I understand that if I am a First Nations person, or an indigenous person who has contact with spiritual elders I may want to talk with them before I proceed with being part of this experiment. Elders have reservations about genetic procedures.

I understand that I may contact Dr. Booij, or any of her helpers, at any time during the study, if I have questions. Dr. Booij is a professor at Queen’s University, and her helpers are students at Queen’s University. Dr. Booij can be reached at (613) 533 6000 ext 78158. I also understand that I may contact the Head of the Psychology Department (Dr. Rick Benninger: (613) 533-2492) or the Chair of the Queen’s University Health Sciences and Affiliated Teaching Hospitals Research Ethics Board (Dr. Albert Clark, (613) 533-6081) if I have any concerns about the project that I do not want to discuss with Dr. Booij or her helpers.

Circling “I agree” below means that I would like to participate in the study.

I agree  I do not agree

Signature: ____________________________________    Date: __________________
Appendix H

LETTER OF INFORMATION & CONSENT FORM

ATTENTION:

If 16 years of age and older: Adolescent is hereafter “The Participant” and can give his/her own consent to participate.

If under 16 years of age: Adolescent is hereafter “The Participant” and parent or guardian must consent to their adolescent’s participation. The participant also signs an Assent Form to agree to participate.

We are inviting adolescents to participate in a research study conducted by researchers at Queen’s University (Dr. L. Booij (PI), Ms. L. Schumann, Dr. K. Harkness, and Dr. I. Johnsrude), in collaboration with Hotel Dieu Hospital (Dr. S. Khalid-Khan, Ms. B. Blaney, Dr. J. Smythe, and Ms. Anita Peter) and Trinity College in Dublin, Ireland (Dr. Thomas Frodl). The purpose of this study is to examine cognitive, emotional, and biological characteristics of adolescents with depression. Ms. Schumann or another member of the research team will read through this consent form with you, describe procedures in detail and answer any questions you may have. Participation in the study involves interviews, questionnaires, computer tasks, and a saliva sample, which will be completed or collected during one or two visits to the Queen’s Psychology Building (Humphrey Hall) and the fMRI Facility in the Queen’s Centre for Neuroscience Studies, which is located in the lower level of the Cancer Research Institute. The total amount of time for participation will be approximately 4 hours.

DETAILS OF THE STUDY

13. Aim of the study

As stated above, the aim of this study is to examine cognitive, emotional, and biological characteristics of adolescents with depression. The participant will be asked to report on aspects of his/her personal history, how he/she deals with life stressors, as well as to perform two computerized tasks. The assessment also consists of a Functional Magnetic Resonance Imaging or fMRI scan (lasting approximately 45-60 minutes, including set up time). The
scanner uses a strong magnetic field to create detailed images of brain structure and function. Additionally, we would like to collect a saliva sample for genetic research (deoxyribonucleic acid; DNA; information necessary for the proper development and function of living organisms). Using the results, we can investigate the relationship between depressive symptoms and the structure and function of certain brain regions as well as the expression of DNA (so called DNA methylation).

14. Description of visit(s) and tests to be performed as part of the study

What does participation involve?

If the participant agrees to participate, the research will consist of one or two visits (depending on the participant’s scheduling preferences) to Queen’s University campus. Total time is four hours. Ms. Lyndall Schumann (project coordinator, PhD student) will conduct two interviews with participants (approximately 2 hours). Participants will provide information about their background and answer questions about the presence or absence of adverse experiences in childhood. The interviews will be audiotaped for later analysis and stored with other confidential information.

Participants will also complete nine short questionnaires, two computer tasks, and will provide a saliva sample (participants will spit into a tube; see Page 4 of this form). These tasks will take approximately 1 hour in total.

The study also includes a functional magnetic resonance imaging (fMRI) scan in the Queen’s Centre for Neuroscience Studies fMRI Facility (approximately 1 hour). During the scan, the participant’s brain will be imaged while he/she is lying in a 3 Tesla MRI scanner. At the completion of the session(s), the participant will receive a $50 gift certificate to a store (one of three options: Bestbuy, Chapters/Indigo or Lululemon).

MRI Scan

h) Depending on the participant’s scheduling preferences, the fMRI scan may be completed immediately following the interview or during a separate appointment time. Participants will be led by a member of the research team from the Queen’s
Psychology building to the Queen’s Centre for Neuroscience Studies fMRI Facility (5 minute walk). Participants who choose to complete the measures in two appointments will meet a member of the research team at the fMRI Facility. There, the participant will fill out a checklist and questionnaire to make sure he/she is eligible for an fMRI scan. This questionnaire will take about 5 minutes. Participants must have no history of chronic neurological disorders, accidents involving prolonged loss of consciousness, or long-term psychoactive medication use. Participants are also not eligible for the MRI scan if they have orthodontic braces or appliances that cannot be removed at the time of the scan. If participants are pregnant or are trying to conceive they will not be eligible. If there is any uncertainty regarding whether or not a participant is pregnant and she wants to participate in the study, we will provide a pregnancy test that must be done prior to the experiment.

i) Prior to the scan, the participant will be familiarized with a 15-minute task that he/she will be asked to do in the scanner, which will consist of looking at pictures with different emotions (positive, negative, or neutral) and answering “yes” or “no” to questions by pressing one of two buttons.

j) Participants will be asked to remove or change out of any clothes that contain metal that will be near the area being imaged, and they will be asked to remove their shoes. Please try to wear clothing containing no metal, or to bring a change of clothing. Metal in zippers, snaps, and the wire and metal clasps in some bras can interfere with the imaging. Many shoes contain metal as well. For imaging the brain, the snaps and zippers in jeans or other pants are far enough from the area being imaged that they do not cause a problem.

k) Participants will be given a set of earplugs to wear in the scanner and then asked to lie on their back on the well-padded bed of the magnet. Pillows will be placed under their legs for comfort and a blanket will be placed over their legs if the participant wishes. A head coil will be placed over the participant’s head. This is fitted with a mirror so that the participant can see out of the magnet towards
his/her head or feet. The participant and the bed will then slide into a long tube (the magnet).

1) The participant will need to keep still while the images are taken. To help him or her, we will make the participant as comfortable as possible and we will pack soft foam around his or her head if needed.

m) The MR system has a two-way intercom for communication. Because scanning can be quite noisy, the participant will also be given an alarm bulb so that he/she can call the researcher or operator during the scan if something is wrong or he/she needs to come out.

n) During the scan, the participant will first have two sessions of 10-minutes each where he/she will only be required to rest and lie still with eyes closed. The first part (called anatomical MRI) gives us a clear picture of his/her brain, and the second (called diffusion tensor imaging) tells us about the connections between different parts of the brain. Next, there will be a 6-minute session where we will ask participants to lie still with his/her eyes open while we measure his/her brain activity at rest. Finally, we will ask participants to perform the 15-minute task, which will consist of looking at pictures with different emotions and orientations (horizontal or vertical). Participants are asked to respond “yes” or “no” to questions regarding the emotional content and orientation of the picture by pressing one of two buttons. Overall, the amount of time spent in the scanner should amount to about 40 minutes. The whole scanning session, including set up time, should last between 45 minutes and one hour.

15. Exclusions/Contraindications

The following are contraindications for a magnetic resonance imaging study:

- Pacemaker
- Aneurysm Clip
- Heart/Vascular Clip
- Prosthetic Valve
- Metal Prosthesis
- Claustrophobia
- Metal fragments in body
- Tattoos and piercings (piercings must be removed prior to scanning)
- Pregnancy
- Transdermal Patches (must be removed prior to scanning. The participant is advised to bring an additional patch to reapply post scanning)

Participants will also be excluded from the study if they:
- Have a history of a psychiatric disorder
- Are actively suicidal
- Are currently abusing drugs or alcohol

and
- Participants may not be able to have the MRI if they have orthodontic braces (unless they can be removed by the participant at the time of the scan), but can participate in the rest of the study.

16. An explanation of the special research techniques that will be used

a) fMRI
The MRI scanning procedure is very much like other medical imaging used in hospitals, but participants will not be exposed to x-rays. This MRI machine uses a strong magnet and radio waves to make images of the interior of the body. Participants will not feel either. The MRI being used in this study is a 3 Tesla MRI that is twice that used for most clinical imaging, although 3 tesla systems are becoming more common in hospitals. The levels of magnetism and radio waves used in the MRI have not been shown to cause harmful effects. However, the MR scanner uses a very strong magnet that will attract metal. Therefore ALL metallic objects must be removed from a participant’s person before he/she approaches the scanner. Those with a cardiac pacemaker or a metallic clip in their body (e.g., an aneurysm clip in your brain or an I.U.D.) should not participate in any MRI study. In addition, credit cards and other cards with magnetic strips should also be removed as these will be damaged. (These items will be kept safe for the participant).

Participants will be in voice contact with the operator, and the operator will be able to see them via a camera. Participants may ask the operator to stop the experiment at any time. Participants should ask to stop the experiment if they feel tired, claustrophobic, or uncomfortable.
b) DNA analysis from saliva sample

We want to learn more about the relationship between depressive symptoms, the brain, and DNA. Simply, DNA methylation is a process that happens to DNA, where a chemical component (a methyl group) is added to DNA, which can affect the functioning of the gene by turning its activity on or off. DNA methylation is influenced by the environment, for example by stressors happening early in life. To assess DNA methylation, we will require participants to donate 3-6 ml of saliva (approximately one tea spoon) into a tube. Participants should not to eat or drink anything 30 minutes before the saliva sample is collected. Samples will be securely stored in Dr. Booij’s laboratory until further analysis. DNA analyses will be performed in the laboratory of Dr. Xudong Liu, in the Queen’s University department of Psychiatry. Participant samples will be identified by participant code only, and Dr. Liu and his team will not have access to any personal information (e.g., names, etc.) of any participants. DNA can be used for future related studies conducted by Dr. Booij and her team 10 years after the start of this study if the participant (or parent if participant is under the age of 16 at the time that this consent form is signed) gives special permission to do so. All DNA will be destroyed after 10 years (September 2023).

17. Advantages

There are no direct advantages from participation in this research project. However, the knowledge acquired will contribute to scientific advancements. It is our hope that a greater understanding of the cognitive, emotional, and biological characteristics of adolescents with depression will lead to the identification of adolescents at risk of developing depression and improve methods to prevent and treat chronic recurrent depression.

18. Risks

There are no known risks involved with Magnetic Resonance Imaging when all the necessary steps of precaution are undertaken. However, the MR scanner uses a very strong magnet that will attract metal. Therefore ALL metallic or magnetic objects must be removed.
from a participant’s person before he/she approaches the scanner. Parents/guardians will be invited to sit outside the resonance magnetic imaging zone during the scanning. Verification of any contraindication will be strictly reinforced by the technologist on duty in the brain-imaging center.

The requirements imposed on the use of Magnetic Resonance Imaging may cause certain discomfort due to the need to remain still during the length of the examination and the noise generated by the scanner when images are being acquired. Participants will be provided with earplugs to minimize noise. Participants may also feel a certain sense of stress or a sense of claustrophobia. Should participants experience discomfort during the scan, they may withdraw at any time. This option will be explained to each participant.

Although this is not a diagnostic scan and any images obtained are for research purposes only, it is possible that the MR scan may disclose an unknown abnormality. In this event, a medical imaging specialist will review the images and a report will be sent to the participant’s physician. The researchers directly involved with this procedure do not have the credentials to diagnose medical conditions.

19. Confidentiality

All data gathered as a part of this project is confidential. There is the risk of loss of confidentiality for participants only if during any assessment they (a) disclose involvement in the abuse of children or elderly individuals, (b) disclose being the victim of current abuse (if under age 16), or (c) disclose threat to seriously harm self or others. If an adolescent discloses current abuse or severe neglect the interviewer will inform Child and Family Services and the adolescent’s treatment provider.

All information is identified using a participant code and no name will be attached to confidential information. Only the researchers directly related to this study will have access to the data files and the subject codes. As outlined above, saliva samples will only be identified using participant codes, and those researchers
performing DNA analyses will not have access to participant names or any other personal information. Participants will not be identified in any publication or reports.

20. Voluntary nature of study/Freedom to withdraw or participate

Participation in this study is voluntary. Participants may withdraw from this study at any time and their withdrawal will not affect their current or future medical care. Participants are not obliged to answer any questions that they find objectionable or which make them feel uncomfortable. If participants withdraw, no more information will be collected from them. When a participant indicates that he/she wishes to withdraw, the investigator will ask if the information already collected can be used. If the participant does not provide consent for the use of this information, it will be destroyed.

21. Withdrawal of subject by principal investigator

The study director may decide to withdraw a participant from this study if:

5) He/she does not meet the criteria for the study, including those mentioned in the Magnetic Resonance Screening Form.
6) He/she meets any of the exclusionary criteria listed above.
7) He/she is unable to perform the tasks requested.
8) He/she misses sessions or does not come to sessions on time.

22. Liability

In the event that a participant is injured as a result of the study procedures, medical care will be provided to him or her until resolution of the medical problem. By signing this consent form, participants do not waive their legal rights nor release the investigator(s) and sponsors from their legal and professional responsibilities.
23. Compensation

As previously outlined, participants will each receive a $50 gift certificate after completing the interviews, the questionnaires, the computer tasks, the fMRI scan, and providing a saliva sample. Participant families will be compensated up to $10 for travel expenses and parking. In addition, participants will receive a black and white picture of their brain.

24. First Nations Elders

If you are a First Nations person, or an indigenous person who has contact with spiritual elders you may want to talk with them before you proceed with being part of this experiment. Elders have reservations about genetic procedures.
SUBJECT STATEMENT AND SIGNATURE

I have read and understand the consent form for this study. I have had the purposes, procedures and technical language of this study explained to me. I have been given sufficient time to consider the above information and to seek advice if I chose to do so. I have had the opportunity to ask questions which have been answered to my satisfaction. I have named Dr. ________________ at ________________ as the physician to be contacted for follow-up purposes. I am voluntarily signing this form. I will receive a copy of this consent form for my information. If at any time I have further questions, problems or adverse events, I can contact:

Dr. Linda Booij (P.I.)
Assistant Professor
Queen's University
Kingston, Ontario
K7L 3N6
Phone: 613-533-2881

Ms. Lyndall Schumann, M.Sc.
PhD Student, Clinical Psychology
Queen's University
Kingston, Ontario
K7L 3N6

If I have questions regarding my rights as a research participant I can contact

Dr. Albert Clark, Chair, Queen’s University Research Ethics Board at 613-533-6081
or
Dr. Rick Beninger, Head of Psychology Department, Queen’s University at 613-533-2492.
By signing this consent form, I am indicating that I agree to participate in:

☐ The imaging portion of the study (the fMRI)

☐ Collection of DNA using saliva

☐ Interviews and cognitive tasks

☐ AND I agree that DNA will be stored under a code and can be used for future related studies done by the P.I. (Dr. Booij) and her team. Any new use of DNA will undergo REB review/approval first.

☐ AND I agree that some information (about my diagnosis and medication) will be passed from my psychiatrist to the researchers

______________________________________________
Signature of Participants (16 years of age or older)            Date

______________________________________________
Signature of Parent of Child Under 16 Years of Age           Date

______________________________________________
Signature of Person Conducting Consent Discussion            Date

STATEMENT OF INVESTIGATOR

I, or one of my colleagues, have carefully explained to the subject the nature of the above research study. I certify that, to the best of my knowledge, the subject understands clearly the nature of the study and demands, benefits, and risks involved to participants in this study.

______________________________________________
Signature of Principal Investigator            Date