Maternal history of early adversity: Transgenerational risk transmission to offspring temperament development

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ABSTRACT

Epidemiological data and the perinatal programming hypothesis suggest that the effects of a maternal history of early adverse experiences may affect the next generation. Such effects are likely to occur interactively with offspring factors, such as genotype. The serotonin transporter polymorphism (5-HTTLPR) is a plausible candidate for the early emergence of individual differences in temperament, especially negative emotionality, in combination with prenatal adversity. The focus in this study was on the 5-HTTLPR gene in the child and the interactive effects of this polymorphism and early childhood experience of the mother on the negative emotionality/behavioural regulation of the offspring. Offspring negative emotionality/behavioural regulation was not affected by this gene x environment interaction and only maternal postnatal depression was predictive of offspring negative emotionality/behavioural regulation. Although depression is known to influence mother-reports of infant temperament, offspring negative emotionality/behavioural regulation ratings remained stable between 18 and 36 months. Negative emotionality/behavioural regulation was also predictive of psychosocial impairments at 60 months, as assessed by both mothers and fathers, thereby confirming the impact of maternal depression on offspring temperament, over and above any bias reflected in the parental reports.
RÉSUMÉ

Les études épidémiologiques suggèrent que les événements négatifs survenus chez la mère lors de son enfance et/ou de sa grossesse sont associés à des difficultés autant au niveau comportemental qu’émotionnel plus tard dans la vie de l’enfant. Les impacts de ces événements négatifs sont également influencés par le génotype des enfants. Il est fort probable que les polymorphismes du transporteur de sérotonine (5-HTTLPR) influencent, de concert avec l'historique d'événements négatifs vécus par les mères, l'émergence des premières différences individuelles au niveau du tempérament des enfants, en particulier l'émotivité négative. Cette étude visait à déterminer les impacts du gène 5-HTTLPR chez les enfants, combinés aux impacts des expériences négatives vécues par les mères antérieurement à la grossesse, sur l'émotivité négative/régulation du comportement des enfants. L'émotivité négative/régulation du comportement des enfants ne fut pas affectée par cette interaction gène x environnement et seule la dépression maternelle postnatale fut associée à l'émotivité négative/régulation du comportement des enfants. Même s'il a été établi que la dépression affecte l'exactitude de l'évaluation que les mères font du tempérament de leurs enfants, l'émotivité négative/régulation du comportement demeura stable entre 18 et 36 mois. De même, l'émotivité négative prédit les troubles psychosociaux des enfants à l'âge de 60 mois, tel qu'évalués tant par les mères que par les pères, confirmant, dès lors, que la dépression maternelle affecte bel et bien le tempérament des enfants.
ACKNOWLEDGMENTS

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1. INTRODUCTION

Experience, even during the prenatal period, associates with emotional and behavioural difficulties in later life. Indeed, the risk of developing chronic illnesses is robustly influenced by environmental conditions during early development (Hertzman, 1999). Poverty and physical or psychosocial stressors all affect later mental and physical health. Identifying such risk factors is critical for studies defining the pathways that lead to early psychopathology.

Although infant temperament has a strong hereditary component (Saudino, 2009), it is also affected by early life experiences (Lang, 2010) Differences in temperament have been found in infants as young as four months-old (Calkins et al., 1996).

The present thesis used a large longitudinal sample of mothers and their infants tested as part of the MAVAN cohort (see below) to test the hypothesis that early temperament in infants would reflect the interactive effects of genetic and environmental influences. Specifically, it predicted that infant temperament would be influenced by the interaction of infant genotype for the serotonin transporter gene (5-HTT) and early familial experience of the mother. It was also further predicted that the interactive effects of infant genotype and mothers’ early
experiences on temperament would be influenced by the affective state of the mother. We therefore predicted relations between mothers’ early adversity, maternal depression, infant genotype and infant temperament. Finally, the present thesis also assessed infant temperament negative emotionality/behavioural regulation in a novel way through the use of an already established mother-report questionnaire.

1.1 INFANT TEMPERAMENT

Temperament is a constitutional factor in children that is considered to be inherently stable. Assessment of infant temperament is of particular relevance since it allows for an early evaluation of behaviour prior to the full influence of familial environment (Rothbart & Derryberry, 1981). Temperament has also been examined as a potential moderator or mediator between environmental stress and psychopathology (Compas et al., 2004).

To date, several links between early temperament and psychopathology have been documented. For instance, high levels of withdrawal in children, which refers to the act of pulling oneself out of an unrewarding or uncertain situation (Bijttebier & Roeyers, 2009), are associated with depression (Holzwarth & Meyer, 2006), eating disorders (Loxton & Dawe, 2001), anxiety (Johnson et al., 2003),
and alcoholism (Sher & Trull, 1994). In contrast, low levels of withdrawal relate to psychopathy (Newman et al., 1997). Furthermore, links are found between high levels of approach, which refers to approach and seeking toward incentives or rewards (Bijttebier & Roeyers, 2009), and substance use and abuse (Franken & Muris, 2006), eating disorders (Loxton & Dawe, 2001), and manic episodes (Holzwarth & Meyer, 2006). Low levels of approach, in turn, are related to depression (Holzwarth & Meyer, 2006).

1.2 NEGATIVE EMOTIONALITY

Negative emotionality is one of the most robust depression endophenotype (Hayden et al., 2010). It is an early-emerging, stable, and heritable trait (Durbin et al., 2007) that encompasses high levels of sub-components such as fear, anger, and sadness (Hayden et al., 2010). Early life negative emotionality is thought to be linked to adolescent depression through the development of negative cognitive styles (rumination, for instance) that increase vulnerability to depression (Hyde et al., 2008). High levels of negative emotionality during infancy are related to high levels of distress in situations encompassing novel or frustrating situations as well as strong startle responses to new or aversive stimuli (Mezulis et al., 2010). In youth, high levels of negative emotionality relate to reactions of dislike, avoidance, and even distress in the context of novel situations as well as
to increased expression of negative emotions such as fear, distress, and frustration (Belsky et al., 1996). Furthermore, this temperament characteristic associates with childhood, adolescent, and adult depression (Anthony et al., 2002).

The link between infant temperament and later depression is also analyzed in terms of both low positive emotionality as well as high negative emotionality (Clark & Watson, 1999). Most child and adult models of temperament and personality encompass both positive and negative emotionality (Caspi & Shiner, 2006). Whereas low positive affectivity is specifically associated with depression, high negative emotionality is characterized as a non-specific risk factor for conduct disorders, antisocial personality, substance use, and more so for anxiety disorders (Clark, 2005). Although support for associations between negative emotionality and externalizing problems is documented both cross-sectionally (Cukrowicz et al., 2006) and longitudinally (Gjone & Stevenson, 1997), some studies do not show significant findings (John et al., 1994). Hence, despite a recent meta-analysis providing support for moderate associations between early negative emotionality and later externalizing disorders (Singh & Waldman, 2010), this relationship still requires further investigation.
Various methods are reported in the literature to assess negative emotionality. Several researchers use average scores of different subscales of temperament questionnaires, such as fear, frustration, and sadness (Mezulis et al., 2010; Neppl et al., 2010, Pluess et al., 2010), while others use single scales (such as emotionality or anger proneness in the Toddler Behaviour Assessment Questionnaire [Goldsmith, 1996]) (Neppl et al., 2010; Singh & Waldman, 2010). Still others code directly from laboratory assessments (Olino et al., 2010). The use of various measuring strategies means that differences in outcomes in the literature may derive from discrepancies in the way emotionality is assessed, rather than from variations in other factors that may vary across studies. Hence, in order to optimize the validity and the consistency of negative emotionality measures, a standard assessment method is desirable, and constitutes one of the goals of the present thesis.

1.3 SEROTONIN TRANSPORTER (5-HTT) GENE

Because of the known associations of serotonin to mood, mental health, stress reactivity, (Caspi et al., 2003), attention (Roiser et al., 2007), and maternal behaviour (Bakermans-Kranenburg & van IJzendoorn, 2008), polymorphisms in the serotonin transporter gene in the offspring, may function to modulate the
effects of maternal experience on the emotional adjustment of the offspring and, in the present context, the infants' temperament.

There is evidence for the importance of polymorphic variations in the serotonin transporter gene in the regulation of serotonin function (Hu et al., 2006). The serotonin transporter (5-HTT) gene codes for a protein located at the presynaptic terminal of 5-HT neurons. 5-HT signalling is terminated by the reuptake of 5-HT at brain synapses by the 5-HTT. Two functional alleles, long (L) and short (S) result from a 43 bp insertion/deletion in the promoter region of 5-HTT. In the S, as opposed to L, allele, there occurs a three-times lower in vitro basal transcription of 5-HTT mRNA (Heils et al., 1996) and presumably reduced levels of 5-HTT protein.

The presence of the S allele is related to numerous health outcomes. For instance, individuals carrying an S allele and presenting with a history of early adversity are at greater risk for adulthood depression (Caspi et al., 2003; Uher and McGuffiy, 2008). This short allele is associated with increased negative emotion, such as heightened anxiety (Sen et al., 2004), elevated neuroticism (Lesch et al., 1996), harm avoidance (Munafò et al., 2005), and fear conditioning (Lonsdorf et al., 2009). Neuroimaging studies reveal greater amygdala neuronal
activity when observing fearful stimuli for carriers of at least one copy of the S allele (Hariri et al., 2002). In contrast, individuals with at least one copy of the S allele show improved cognitive performance on various tasks when compared with participants homozygous for the L allele (Borg et al., 2008; Roiser et al., 2007).

However, the role of 5-HTTLPR in predicting depression in gene x environment interactions is rather controversial. For instance, Risch and colleagues (2009) conducted a meta-analysis in which they attempted to replicate the findings reported by Caspi et al. (2003). Although an association between stressful life events and depression was found, the meta-analysis of 14 studies failed to reveal neither significant 5-HTTLPR genotype effect nor genotype by stressful life events interaction effect on depression. Likewise, a systematic review and meta-analysis of studies based on the Caspi model revealed results similar to those reported by Risch et al., (2009) (Munafo et al., 2009). Based on their results, the group of researchers argued that most of the published studies that have claimed a replication of Caspi et al.’s findings were underpowered and prematurely published. Yet, authors who published evidence of such gene x environment interactions remain convinced of their findings, thereby underpinning the controversial nature of this literature.
1.4 TRANSGENERATIONAL RISK TRANSMISSION

A plurality of mental disorders is known to be familial. For instance, risk for depression and anxiety is known to be genetically influenced (Eley, 1999). Not only are these disorders themselves heritable, but so are risky personality profiles and cognitive factors such as neuroticism, behavioural inhibition, and low self-esteem (Burt et al., 2005). Depression risk transmission has also an additive effect as the risk for this psychopathology increases as the number of affected relatives increases (Todd et al., 1993). Parenting also plays a role whereby infants of mothers suffering from social phobia, for instance, show increased stranger avoidance and fearful responses (Murray et al., 2008). Moreover, increased negativity due to depression amplifies the demands of parenthood, often leading to increased amount of critical comments directed at children (Webster-Stratton & Hammond, 1988). Direct mother-infant interactions are also affected by maternal depression with depressed mothers expressing less positive affect and responding slowly and inconsistently to their children’s demands compared to non-depressed counterparts (Burt et al., 2005). Furthermore, maternal depression associates with both negative and disengaged behaviours towards offspring (Lovejoy et al., 2000).
Transgenerational risk transmission also applies to maternal history of early adverse experiences. The Avon Longitudinal Study of Parents and Children, (Collishaw et al., 2007) provides support for the assessment of the paradigm of transgenerational risk transmission of maternal history of early adversity. Indeed, this longitudinal study targeted mainly maternal history of abuse, maternal mental health, parent-child relationship, family type, early family life events, interim child life events, and offspring adjustment. Researchers found that the more severely the mothers rated the impact of their own childhood maltreatment, the worse was the adjustment of their offspring. Moreover, exposure to several types of abuse was related to greater offspring problems than exposure to only one type. Adjustment problems in offspring persisted over a longer duration when mothers reported severe abuse compared to less severe adversity. Collishaw et al. (2007) also found that exposure to psychosocial adversity in families of maltreated mothers tended to be cumulative over time. Specifically, offspring of maltreated mothers showed an increased risk of experiencing frightening and shocking events and physical assaults. Changes in their families were also more frequent, comprising separations from parents and caretakers, and the acquisition of new parent figures. Finally, maltreated mothers’ offspring also faced a wider range of stressful life events such as moving in another neighbourhood, changing school, losing contact with friends, etc.
There are many ways that mothers’ early experiences could be translated into an experience that could impact the child such as alterations in mother-child interactions and effects of her mood and her communication of negative affect.

1.5 EARLY ADVERSITY AND LONG-TERM CONSEQUENCES

Childhood maltreatment is defined as “any acts of commission or omission by a parent or other caregiver that result in harm, potential for harm, or threat of harm to a child (usually interpreted as up to 18 years of age), even if harm is not the intended result” (Leeb et al., 2008). Researchers have investigated the long-term consequences of childhood trauma related to education, mental health, physical health, and violence or criminal behaviour. Among potential consequences are an increased likelihood of suffering from depression, anxiety or both due to the exposition to early adverse events (Heim & Nemeroff, 2001). Child maltreatment is also associated with drug and alcohol abuse, risky sexual behaviour, obesity and criminal behaviour; all of which persist into adulthood (Gilbert et al., 2009). Despite receiving the least scientific and public attention, in the long-term, neglect is at least equally as detrimental and harmful as are physical or sexual abuse (Gilbert et al., 2009). Indeed, family quarrels, prolonged emotional neglect, and incoherent and/or harsh authority affect intellectual development (Ammerman et al., 1998) and growth (Montgomery et al., 1997). Likewise, cold
and/or distant parent-child interactions are related to increased risk for suffering from chronic illnesses later in life (Canetti et al., 1997; Russak & Schwartz, 1997).

Internalizing behaviours such as anxiety and depression as well as externalizing behaviours such as aggression are increased by childhood maltreatment (Lansford et al., 2002). No link has been found between childhood trauma and personality disorders and a potential association with psychosis does not seem straightforward (Read et al., 2005). Both physical and sexual abuse are linked to a doubling of the risk of attempted suicide for people in their 20s, more so in the case of repeated maltreatment (Fergusson et al., 2008). Early adverse events also increase the risk for alcohol use and abuse in both adolescence and adulthood, especially for females (Andrews et al., 2004). Individuals who have suffered from abuse and neglect during their childhood are at increased risk of displaying aggressive behaviours and of perpetrating violent and/or criminal acts (Widom, 1989).

Physical/health outcomes are associated with childhood maltreatment to some extent (Felitti et al., 1998). Indeed, physical abuse, neglect, and sexual abuse are strongly related to obesity (Thomas et al., 2008). Sexual abuse and eating
disorders (such as anorexia nervosa and bulimia nervosa) are also linked, although more research is needed, especially in cases of abuse different from sexual abuse (Brewerton, 2007). Moreover, repeated or prolonged child adversities are associated with health outcomes such as ischaemic heart disease, cancer, chronic lung disease, skeletal fractures and liver disease (Felitti et al., 1998). Child maltreatment is also related to teenage pregnancy (Lansford et al., 2007).

1.6 EARLY ADVERSITY, PARENTING, AND INFANT TEMPERAMENT

Women maltreated during childhood might also have difficulty parenting their own offspring. This effect is documented more thoroughly in the case of sexual abuse, although it is reported with all subtypes of maltreatment. Indeed, prior child sexual abuse is linked to impaired parenting skills (Ruscio, 2000). Those impaired skills include higher levels of child neglect, diminished confidence in one’s parenting skills, negative self-appraisal as a parent, greater use of physical punishment, and lack of emotional control in parenting situations (Roberts et al., 2004). A strong will to avoid motherhood due to childhood negative experiences is also reported (Roberts et al., 2004). Sexually abused women are more permissive and less authoritarian than control counterparts (Ruscio, 2001). Above-average permissive parenting is associated with a history of childhood
traumatic stressors (Ruscio, 2001). Poor parenting among women who suffered from childhood sexual abuse could be due to an internalized model of their youth family dynamics (Roberts et al., 2004) as well as increased awareness of consequences of adults’ power over children (Ruscio, 2001).

1.7 EARLY ADVERSITY, MATERNAL DEPRESSION, AND INFANT TEMPERAMENT

Maternal well-being and dysphoria can impact infant development through maternal behaviour. For instance, children of women who suffer from depression and/or anxiety tend to display social, emotional, and behavioural impairments. These disturbances can persist throughout infancy, adolescence, and even adulthood (Rubin et al., 1991). Children of depressed parents are also more likely to exhibit externalizing problems, increased peer aggression, and more fearful behaviour during free play (Cicchetti & Toth, 1995).

Individuals with a history of childhood maltreatment have a moderately increased risk of developing a depression in adolescence and adulthood. More specifically, approximately 25 – 30% of victims meet criteria for major depression by their late 20s (using criteria from the Diagnostic and Statistical Manual of Mental Disorders [DSM]) (Fergusson et al. 2008). For a majority of those individuals, the onset of
depression first occurs during childhood, which emphasizes the need for early intervention with these victims (Thornberry et al., 2001).

1.8 MATERNAL CARE AND CHILD DEPRESSION

The association between childhood trauma and later depression also appears to be moderated by parental care. Indeed, poor parental care has been found to increase the risk of developing adulthood depression in victims of childhood sexual abuse. For instance, in a study conducted with women aged 25-36, Hill et al. (2001) found that the risk of developing depression associated with early-life adversity was considerably increased by poor parental care (neglect and institutional care). In addition, researchers found that childhood sexual abuse and poor parental care were each associated with long-term patterns of difficult intimate relationships which were, in turn, associated with major depressive disorder between ages 21-30, for the victims.

Apart from being an outcome of early adversity, adult depression is also related to detrimental outcomes on offspring. Indeed, maternal depression is a risk factor for later childhood difficulties such as behavioural problems, and psychopathology (Martins & Gaffan, 2000). During the postnatal period, maternal depression associates with delayed cognitive development and insecure
attachment patterns in early life especially in young boys (Martins & Gaffan, 2000). Depressive symptoms such as low mood, apathy, helplessness, irritability, hostility, over-indulgence, or excessive concern toward the child might prevent the mother from relating in a favourably receptive, responsive, and available manner with her baby. This might, in turn, impede the development of secure attachment between mother and infant, which is known to be a risk factor for behavioural and emotional problems for the child (Martins & Gaffan, 2000). Maternal well-being may impact on child development (Cicchetti & Toth, 1995) through various factors such as parenting, genotype, and the child’s own characteristics. Maternal depression and/or anxiety, for instance, are often associated with offspring social and emotional impairments throughout childhood, adolescence, and even adulthood (Cicchetti & Toth, 1995). Depression can prevent a mother from adequately fulfilling her child’s needs, thereby impeding the child’s normal course of development (Cicchetti & Toth, 1995). Child temperament, then, can play a major role as to how maternal mood will affect child development. A greater understanding of this risk transmission model and underlying mechanisms would allow for specific, early, and hence more efficient, prevention and intervention methods.
1.9 THE MAVAN COHORT

The current thesis has been developed as part of the Maternal Adversity, Vulnerability, and Neurodevelopment (MAVAN) project. The MAVAN is a multidisciplinary cohort study of mother-children dyads that aims to assess the effects of environmental adversity, more specifically the impact of maternal well-being and behaviour on infant development. It also explores the impact of early and ongoing stress on interactions between mother and child and on subsequent child development. Thus, the MAVAN study links neurocognitive/behavioural function with structural neurodevelopment and genetic vulnerability in humans, in infants and children whose mothers did or did not experience adversity in their early lives.

1.10 SUMMARY

We used a novel method to assess negative emotionality/behavioural regulation based on mother ratings of infant temperament. After comparing this method to parental reports of children psychosocial adjustment at a later point in time, we used it to investigate the main and interactive effects of maternal history of early adversity and offspring 5-HTTLPR genotype on child development.
2. GENERAL METHODS

2.1 PARTICIPANTS

Participants of the MAVAN project were recruited from hospitals affiliated with
McGill University, l'Université de Montréal, and McMaster University. Currently,
two populations of a total of 600 subjects are part of the project. The first
population is comprised of about 340 infants born at normal weight (controls,
3000-3750 g at birth) and enriched with infants presenting with intrauterine
growth retardation (IUGR, 2200-2750 g at birth). The other population consists of
about 250 infants whose mothers have been referred or self-referred to the
Women’s Health Concerns Clinic, St. Joseph’s Healthcare, Hamilton (Ontario) for
symptoms of depression and/or anxiety during their first or early second trimester
of pregnancy. This high-risk sample is also balanced with normal controls.
Recruitment occurred mostly at the time of routine ultrasound examinations (16-
20 weeks). A minority of participants were also recruited in prenatal care clinics
or at time of prenatal blood drawing (typically 8-12 weeks). The study’s
populations represent a broad range of socioeconomic status as well as French
and English speakers.

All women aged greater than 18, in the second or third trimester of pregnancy at
the time of recruitment, able to read, write, and speak English or French, with
singleton gestation, and able to provide written, informed consent were included.

Exclusion criteria were comprised of history of or current bipolar disorder, or psychosis, acute suicidal, infanticidal or homicidal thoughts, current or within the past 6 months drug or alcohol dependence, concurrent acute medical condition (other than hypertension, asthma, or diabetes) which required or might required treatment (e.g. thyroid abnormality), placenta previa, history of incompetent cervix diagnosed in a previous pregnancy, impending delivery, or a foetus/infant affected by a major anomaly. Only babies born at 37 weeks or later were included and children presenting with serious developmental delays according to the Bayley (Bayley, 1969) (administered at 12, 24, and 36 months) were removed from the study.

For the present thesis, a subset ranging between 43 and 196 parent-child dyads were studied. The sample size varied depending on the time point under assessment.

2.2 PROCEDURE

Mothers were first assessed during their pregnancy and were then followed with their child at 3, 6, 12, 18, 24, 36, 48, 60, and 72 months (so far). At every time point, both home visits and laboratory sessions were conducted. Written,
informed consent was obtained for all participants. For all time points, a research assistant visited the mother’s home to administer several questionnaires. At the beginning of the visit, women were explained the nature of the visits and the laboratory sessions. Upon acceptance, questionnaires were given and explained.

The Childhood Trauma Questionnaire (CTQ; Berstein et al., 1994) was administered prenatally and at 24 months. Both the Parental Bonding Instrument (PBI; Parker et al., 1979) and the Edinburgh Ante/Postnatal Depression Scale (EPDS; Cox et al., 1987) were administered at 6 months. The Early Childhood Behaviour Questionnaire (ECBQ; Putnam et al., 2006) was administered at both 18 and 36 months. Finally, the Strengths and Difficulties Questionnaire (Goodman, 1997) were administered at 48 months and 60 months, respectively.

2.3 MEASURES

2.3.1 Maternal history of early adversity

Maternal history of early adversity was assessed with a combination of the Childhood Trauma Questionnaire (CTQ; Berstein et al., 1994) and the Parental Bonding Instrument (PBI; Parker et al., 1979). The CTQ focuses on five dimensions of trauma: sexual abuse, physical abuse, emotional abuse, physical neglect, and emotional neglect. Other traumatic events that may have been
experienced during childhood, such as a major illness or the death of a parent, are not covered by the questionnaire. Based on self-reports, the CTQ is comprised of 28 items that are rated on a 5-point Likert scale ranging from “never true” to “very often true.” Sample items include “When I was growing up, I didn’t have enough to eat,” “When I was growing up, people in my family looked out for each other,” and “When I was growing up, someone molested me.” The PBI is a 48-item questionnaire that assesses parental educational style. This self-report measure is rated on a 4-point Likert scale ranging from “very true” to “very untrue” and yields 4 subscores, namely, “mother care”, “mother overprotection”, “father care”, and “father overprotection”. Sample items, asking to characterize the participant’s relationship with both of her parents, include “Spoke to me in a warm and friendly voice”, “Did not want me to grow up”, and “Did not praise me”. The CTQ was administered to mothers both prenatally and when their infants were aged 24 months whereas the PBI was administered at 6 months postpartum.

2.3.2 Infant genotype

Infants were genotyped at 36 months. DNA was collected using buccal swabs. Our focus was on the serotonin transporter gene. We compared two groups: those carrying either one or two copies of the short allele (SLA, SLG, SS) to
those homozygous for the long allele (LALA), based on the evidence that there are two functional variants of the L allele (LA and LG) (Hu et al., 2006; Nakamura et al., 2000). Buccal cell swabs and genotyping information is provided in chapter 4.

2.3.3 Negative emotionality/behavioural regulation

Infant negative emotionality/behavioural regulation was measured using the Early Childhood Behaviour Questionnaire (ECBQ; Putnam et al., 2006) when infants were aged 18 and 36 months. The ECBQ is a mother-report questionnaire that is comprised of 201 items grouped in 18 subscales and is based on a 7-point Likert scale ranging from “never” to “always”.

Table 2.1. Early Childhood Behaviour Questionnaire Description (ECBQ; Putnam et al., 2006).

<table>
<thead>
<tr>
<th>Scale</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Activity level/energy</td>
<td>Extent and rate of locomotion; rate and strength of gross motivity.</td>
</tr>
<tr>
<td>Attentional focusing</td>
<td>Sustained attention to and/or with an object and resistance to distraction.</td>
</tr>
<tr>
<td>Attentional shifting</td>
<td>Capacity to shift focus of attention from one activity/task to another.</td>
</tr>
</tbody>
</table>
**Table 2.1. Early Childhood Behaviour Questionnaire Description (ECBQ; Putnam et al., 2006) (continued).**

<table>
<thead>
<tr>
<th>Scale</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cuddliness</td>
<td>Child’s facial appearance regarded as an indication of positive mood/feeling in and molding of the body to being held by a caregiver.</td>
</tr>
<tr>
<td>Fear</td>
<td>Shock to unanticipated situation; negative affect including apprehension, concern, or anxiety related to foreseen pain or distress and/or possibly threatening event.</td>
</tr>
<tr>
<td>Frustration</td>
<td>Negative affect due to discontinuity of activity in progress or to goal interruption.</td>
</tr>
<tr>
<td>High-intensity pleasure</td>
<td>Positive emotion, affect or reaction elicited by a situation involving stimulus of high intensity, rate, complexity, novelty, and incongruity.</td>
</tr>
<tr>
<td>Impulsivity</td>
<td>Rapidness of response onset.</td>
</tr>
<tr>
<td>Inhibitory control</td>
<td>Ability to stop, moderate, or refrain from instructed behaviour.</td>
</tr>
<tr>
<td>Low-intensity pleasure</td>
<td>Positive emotion, affect or reaction elicited by a situation involving stimulus of low intensity, rate, complexity, novelty, and incongruity.</td>
</tr>
</tbody>
</table>
Table 2.1. Early Childhood Behaviour Questionnaire Description (ECBQ; Putnam et al., 2006) (continued).

<table>
<thead>
<tr>
<th>Scale</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Motor activation</td>
<td>Repetitive small-motor movements and fidgeting.</td>
</tr>
<tr>
<td>Perceptual sensitivity</td>
<td>Ability to discern feeble, low intensity stimuli from the external environment.</td>
</tr>
<tr>
<td>Positive anticipation</td>
<td>Excitement about foreseen enjoyable and positive activities.</td>
</tr>
<tr>
<td>Sadness</td>
<td>Lowered affect or tearfulness elicited by exposure to personal or others’ suffering, dismay, object loss, disapprobation.</td>
</tr>
<tr>
<td>Shyness</td>
<td>Reaction of inertness or constrained approach and/or uneasiness in social situations involving originality or incertitude.</td>
</tr>
<tr>
<td>Sociability</td>
<td>Seeking and enjoying social interactions.</td>
</tr>
<tr>
<td>Soothability</td>
<td>Recovery rate from global arousal, excitement, or peak distress.</td>
</tr>
</tbody>
</table>

2.3.4 Early psychosocial adjustment

Children early psychopathology was assessed at 60 months with the Strengths and Difficulties Questionnaire (SDQ; Goodman, 1997). The SDQ is a brief
behavioural screening that takes into account mother, father, and teacher-reports. It is comprised of 25 items divided into 5 scales: emotional symptoms (5 items), conduct problems (5 items), hyperactivity/inattention (5 items), peer relationship problems (5 items), and prosocial behaviour (5 items). A total difficulties score is also obtained by summing all scores from all subscales except the prosocial one. Respondents are asked to rate each item on a 3-point scale ranging from “not true” to “certainly true”. Sample items include “considerate of other people’s feelings”, “easily distracted, concentration wanders”, and “often lies or cheats”.

2.3.5 Maternal depression

Current depressive state of mothers was assessed with the Edinburgh Ante/Postnatal Depression Scale (EPDS), a self-report, 10-item measure (Cox et al., 1987). This measure was developed to identify depression in postnatal samples and asks retrospective questions for a 2-week period. This questionnaire includes questions such as “I have been able to laugh and see the funny side of things: as much as I always could, not quite so much now, definitely not so much now, or not at all;” and “I have felt sad or miserable: yes, most of the time; yes, quite often; not very much; no, not at all.” For the purpose of this study, EDPS data collected at 6 months postpartum was used.
2.4 STATISTICS

Data were statistically analyzed with PASW Statistics 18.0 for Windows (Rel. 18.0.0 2009). \( P < .05 \) was used as the statistical significance criterion.
3. MATERNAL HISTORY OF EARLY ADVERSITY

We assessed maternal history of early adversity with a combination of two self-report questionnaires administered prenatally and when infants were aged 6 and 24 months.

3.1 MEASURES

The Childhood Trauma Questionnaire (CTQ; Berstein et al., 1994) was given to mothers to fill out during a home visit both prenatally and when their children were aged 24 months. All five subscales (emotional neglect, emotional abuse, physical neglect, physical abuse, and sexual abuse) were used in our analyses. The Parental Bonding Instrument (PBI; Parker et al., 1979) was also given to mothers to complete by themselves during a home visit when their infants were aged 6 months. Of the four subscales the instrument is comprised of, only maternal care was kept in the analytical models since it was the only one related to the construct under study.

3.2 RESULTS

3.2.1 Principal component analysis

To reduce our measures of maternal history of early adversity (CTQ and PBI), we used a principal component analysis to derive one factor. Dimensions that loaded
highly on this early adversity factor included physical neglect, physical abuse, emotional neglect, emotional abuse, sexual abuse, and mother care and explained 52% of the shared variance in the 6 input variables (eigenvalue was 3.134). However, because mothers who did not successfully complete both questionnaires (CTQ and PBI) were systematically excluded from the analyses, we imputed values of the derived components. Using stochastic regression single imputation in PASW Statistics 18.0, we entered the initial 9 CTQ and PBI variables as predictors and maternal history of early adversity as the outcome variable. Obtained scores were also normalized and centered for all further analyses.
4. GENOTYPE

4.1 MEASURES

Infants’ DNA was collected during a home visit and a laboratory session at 36 months. Mothers were instructed that their child was not allowed to drink and/or eat prior to sampling. Children’s inner cheeks were brushed with four Omniswabs (cotton brushes) to collect a small sample of cheek cells. Each inner cheek was brushed twelve times with two Omniswabs (i.e. six brushes per Omniswab). Upon sampling completion, all the Omniswabs were placed within one conic tube. Required information to ensure accurate genotyping (e.g. birth date, sampling date, previous mental health diagnostic, genetic family history, mother tongue, religion, etc.) was also gathered. DNA extraction and genotyping for 5-HTTLPR was performed at the Center for Addiction and Mental Health (CAMH) in Toronto (Ontario, Canada), which has extensive experience in genetics and molecular biology. Standard techniques were used for DNA extract and PCR were employed for polymorphism detection. Detection was performed using Single-Stranded Conformational Polymorphism (SSCP) technique (Barr et al., 2000), which is a PCR-RFLP detection method (Petronis et al., 1999). Direct DNA sequencing was performed using ABI Prism 310 Genetic Analyzer.
Although not taken into account in every study on 5HTTLPR (e.g. Uher & McGuffin, 2008), there is evidence that two functional variants of the L allele (LA and LG) result from a single nucleotide polymorphism (A→G, rs25531), upstream of the 5-HTTLPR region (Hu et al., 2006; Nakamura et al., 2000). The LALA genotype is associated with a greater binding potential in human putamen (Praschak-Rieder et al., 2005) and midbrain (Reimold et al., 2007) as well as with higher mRNA expression in vitro (Hu et al., 2006). Given this evidence, we used rs25531 along with 5-HTTLPR polymorphism to optimize our genotypic information for 5-HTT. Moreover, since LG and S alleles have been found to be functionally similar, (Hu et al., 2006), we grouped them. For the purposes of this discussion we refer to the S allele as meaning S and LG. To determine the impact of carrying at least one copy of the short allele, we compared infants homozygous for the long allele (LALA) to all the others (SLA and SS). One-hundred and sixty-four infants presented with LALA genotype and 82 carried one or two copies of the short allele.
5. NEGATIVE EMOTIONALITY/BEHAVIOURAL REGULATION

Early negative emotionality has been related to later mood problems such as adolescent depression (Hyde et al., 2008). With the aim of investigating the risk pathway leading to the development of early psychopathology, we targeted negative emotionality/behavioural regulation as a main infant outcome measure. Because there is no standard, commonly used measuring method for negative emotionality that is reported in the literature, we decided to conduct a principal component analysis on the Early Childhood Behaviour Questionnaire subscales (ECBQ; Putnam et al., 2006) at both 18 and 36 months and we derived a negative emotionality/behavioural regulation factor.

5.1 RESULTS

5.1.1 Principal component analysis

We inputted the ECBQ 18 subscales both at 18 and 36 months to conduct a principal component analysis. After excluding all items that loaded with a coefficient absolute value below 0.40, we retained one negative emotionality/behavioural regulation factor that included discomfort, fear, frustration, and sadness which are core negative emotionality/behavioural regulation components. At 18 months the negative emotionality/behavioural regulation factor accounted for 21% of the shared variance in the nine remaining
input variables (eigenvalue = 3.9). This factor was comprised of positive ratings of discomfort, fear, frustration, sadness, activity level, and motor activation and with negative ratings of inhibitory control and soothability of infant temperament. At 36 months, the negative emotionality/behavioural regulation factor explained 21% of the shared variance in the ten remaining input variables (eigenvalue = 3.8). This factor was comprised of positive ratings of discomfort, fear, frustration, activity level, motor activation, and sadness, and of negative ratings of attentional focusing, cuddliness, inhibitory control, and soothability of infant temperament. Obtained scores were also normalized and centered for all further analyses.

Although Putnam and colleagues (2006) reported negative affectivity factor loadings for the ECBQ, we preferred to use our own factor since it includes items that tap at cognitive and motor aspects not accounted for by Putnam et al.’s factor (e.g. inhibitory control, attentional focusing, activity level) and that are closer to the definition of temperament postulated by Rothbart and Posner (2006). Indeed, they view temperament as encompassing domains relating to emotionality, motor activity, and attention and we believe negative emotionality/behavioural regulation should be examined along the same dimensions. However, because aspects such as attentional focusing and cuddliness, for instance, are not typically conceptually related to negative
emotionality per se, we labelled our factor “negative emotionality/behavioural regulation”.

5.1.2 Measurement stability
To assess the stability of our negative emotionality/behavioural regulation measure, we first performed Pearson correlations between negative emotionality/behavioural regulation scores at 18 months and scores at 36 months. Negative emotionality/behavioural regulation scores at 18 and 36 months were strongly associated, $r = 0.646, p < 0.01$ (n = 196).

To further assess stability, we divided negative emotionality/behavioural regulation scores into three groups, namely those comprising children scoring in the low, mid, and high score range. We defined ranges by ranking the scores in an ascending order and then we divided them into thirds (i.e. values under 33.33 percentile, values between 33.33th and 66.66th percentiles, and values above 66.66 percentiles).

We conducted two series of ANOVAs followed by Tukey’s HSD post-hoc tests. Results revealed that 36-month continuous scores were predicted by 18-month grouped scores, $F(2,196) = 53.236, p < 0.01$. Tukey’s HSD post-hoc test
confirmed that low scores at 18 months remained in a lower range at 36 months, mid scores at 18 months remained in a mid range at 36 months and high scores at 18 months remained in a higher range at 36 months, all ps < 0.01.
Figure 5.1 Negative emotionality/behavioural regulation scores at 36 months as a function of negative emotionality/behavioural regulation scores at 18 months, divided as lows, mids, and highs.
Likewise, 18-month continuous scores were predicted by 36-month grouped scores, $F(2, 196) = 40.458$, $p < 0.01$. Again, Tukey's HSD post-hoc test confirmed that low scores at 36 months were in a lower range at 18 months, mid scores at 36 months were in a mid range at 18 months and high scores at 36 months remained in a higher range at 18 months, all $p < 0.01$. 
Figure 5.2 Negative emotionality scores at 18 months as a function of negative emotionality scores at 36 months, divided as lows, mids, and highs.
5.1.3 Predictive validity

To determine the predictive validity of the temperament negative emotionality/behavioural regulation measure, we performed Pearson correlations between negative emotionality/behavioural regulation scores at both 18 and 36 months and psychosocial adjustment parental ratings as assessed with the Strengths and Difficulties Questionnaire (SDQ; Goodman, 1997) at 60 months.

Negative emotionality/behavioural regulation scores at 18 months were positively associated with mother reports of total difficulties ($r = 0.416, p < 0.01$), hyperactivity ($r = 0.290, p < 0.05$), and peer problems ($r = 0.539, p < 0.01$) ($n = 59$). Negative emotionality/behavioural regulation scores at 18 months were positively associated with father reports of total difficulties ($r = 0.360, p < 0.05$), emotional symptoms ($r = 0.308, p < 0.05$), and negatively associated with prosocial scores ($r = -0.456, p < 0.01$). Moreover, trend associations were noted with hyperactivity scores ($r = 0.252, p = 0.088$) and with peer problems scores ($r = 0.281, p = 0.056$) ($n = 47$).

Negative emotionality/behavioural regulation scores at 36 months were positively associated with mother reports of total difficulties ($r = 0.416, p < 0.01$), emotional symptoms ($r = 0.296, p < 0.05$), and peer problems ($r = 0.465, p < 0.01$). Trend
association was noted with hyperactivity scores ($r = 0.225, p = 0.095$) ($n = 56$).

Negative emotionality/behavioural regulation scores at 36 months were negatively associated with father reports of prosocial scores ($r = -0.474, p < 0.01$) ($n = 43$).

### 5.1.4 General linear model

With the aim of investigating the influence of maternal history of early adversity and offspring 5-HTTLPR genotype, alone or in interaction, on infant negative emotionality/behavioural regulation, we customized a general linear model. Provided evidence for later gender differences in temperament (e.g. De Boo & Spiering, 2010), we controlled for offspring gender. Sample of origin was also controlled for (i.e. Montreal or Hamilton). 5-HTTLPR genotypes were divided in two groups: LALA versus all others.

At 18 months, the interaction effect of maternal history of early adversity and offspring 5-HTTLPR genotype on infant negative emotionality/behavioural regulation was not significant, $F(1, 140) = 1.873, p = 0.173$. Likewise, main effects of maternal adversity and offspring genotype on offspring negative emotionality/behavioural regulation were not significant, $F(1, 140) = 0.563, p = 0.454$ and $F(1, 140) = 0.076, p = 0.783$ ($n = 140$).
**Figure 5.3** Interaction effect of maternal history of early adversity and offspring 5-HTTLPR genotype on infant negative emotionality/behavioural regulation at 18 months.
At 36 months, the interaction effect of maternal history of early adversity and offspring 5-HTTLPR genotype on infant negative emotionality/behavioural regulation was not significant, $F(1, 114) = 0.021, \, p = 0.884$. Likewise, main effects of maternal adversity and offspring genotype on infant negative emotionality/behavioural regulation were not significant, $F(1, 114) = 1.213, \, p = 0.273$ and $F(1, 114) = 0.001, \, p = 0.982$ (n = 114).
Figure 5.4 Interaction effect of maternal history of early adversity and offspring 5-HTTLPR genotype on infant negative emotionality/behavioural regulation at 36 months.
6. MATERNAL DEPRESSION

Maternal well-being can impact considerably infant development either directly or through various factors. Individuals with a history of childhood maltreatment have a moderately increased risk of developing a depression in adolescence and adulthood. More specifically, approximately 25 – 30% of victims meet criteria for major depression by their late 20s (using criteria from the Diagnostic and Statistical Manual of Mental Disorders [DSM]) (Fergusson et al. 2008). For a majority of those individuals, the onset of depression first occurs during childhood, which emphasizes the need for early intervention with these victims (Thornberry et al., 2001). Maternal depression is a risk factor for later childhood difficulties such as behavioural problems, and psychopathology (Martins & Gaffan, 2000). During the postnatal period, maternal depression is associated with delayed cognitive development and insecure attachment patterns in early life especially in young boys (Martins & Gaffan, 2000). Depressive symptoms such as low mood, apathy, helplessness, irritability, hostility, over-indulgence, or excessive concern toward the child might prevent the mother from relating in a favourably receptive, responsive, and available manner with her baby. This might, in turn, impede the development of secure attachment between mother and infant, which is known to be a risk factor for behavioural and emotional problems for the child (Martins & Gaffan, 2000).
Thus, we investigated the impacts of non-clinical levels of maternal depression using the Edinburgh Postnatal Depression Scale (EPDS) at 6 months postpartum in our transgenerational transmission of risk model.

6.1 RESULTS

Keeping the same customized general linear model we used previously, we added maternal depression as assessed 6 months postpartum.

At 18 months, the interaction effect of maternal history of early adversity and offspring 5-HTTLPR genotype on infant negative emotionality/behavioural regulation was not significant, $F(1, 140) = 1.484, p = 0.225$. Likewise, main effects of maternal adversity and offspring genotype on infant negative emotionality/behavioural regulation were not significant, $F(1, 140) = 0.583, p = 0.447$ and $F(1, 140) = 0.139, p = 0.710$. However, maternal depression main effect on infant negative emotionality/behavioural regulation was highly significant, $F(1, 140) = 13.413, p < 0.01$ (n = 140).

At 36 months, the interaction effect of maternal history of early adversity and offspring 5-HTTLPR genotype on infant negative emotionality/behavioural regulation was not significant, $F(1, 114) = 0.155, p = 0.695$. Likewise, main
effects of maternal adversity and offspring genotype on infant negative emotionality/behavioural regulation were not significant, $F(1, 114) = 1.404, \ p = 0.239$ and $F(1, 114) = 0.003, \ p = 0.957$. However, again, maternal depression main effect on infant negative emotionality/behavioural regulation was highly significant, $F(1, 114) = 30.921, \ p < 0.01$ (n = 114).

Provided the effect of maternal depression on infant negative emotionality/behavioural regulation at both 18 and 36 months, we further investigated the interactive effect of maternal depression and infant 5-HTTLPR genotype on infant negative emotionality/behavioural regulation at 18 and 36 months. At 18 months, the interaction effect of maternal depression 6 months postpartum and offspring 5-HTTLPR genotype on infant negative emotionality/behavioural regulation was not significant, $F(1,140) = 0.594, \ p = 0.442$ (n = 140). Likewise, at 36 months, the interaction effect of maternal depression 6 months postpartum and offspring 5-HTTLPR genotype on infant negative emotionality/behavioural regulation was not significant, $F(1,114) = 0.525, \ p = 0.470$ (n = 114).
Figure 6.1 Interaction effect of maternal depression 6 months postpartum and offspring 5-HTTLPR genotype on infant negative emotionality/behavioural regulation at 18 months.
Figure 6.2 Interaction effect of maternal depression 6 months postpartum and offspring 5-HTTLPR genotype on infant negative emotionality/behavioural regulation at 36 months.
7. GENERAL DISCUSSION

Our assessment of infant negative emotionality/behavioural regulation based on the ECBQ was shown to be stable for infants aged 18 to 36 months. Furthermore, evidence for the predictive validity of this novel assessment method was reported, as it was associated with the behavioural assessment of the children at 60 months performed with a validated behavioural screening questionnaire.

Contrary to our predictions, maternal history of early adversity and offspring 5-HTTLPR genotype did not have any effect on offspring negative emotionality/behavioural regulation, either alone or in interaction. Maternal depression at 6 months postpartum revealed to be the only predictor of infant negative emotionality/behavioural regulation at both 18 and 36 months. This conclusion is accounted for by the fact that maternal depression assessed 6 months postpartum had a significant and strong effect on infant temperament at 18 and 36 months, when included in the interactive model of maternal history of early adversity and offspring 5-HTTLPR. However, we believe that children negative emotionality/behavioural regulation might be affected by an interaction of both pre and postnatal environment (namely maternal early adverse
experiences and maternal postnatal depression) despite our findings and that further investigations are required. Detailed results are discussed below.

7.1 PREDICTIVE VALIDITY OF THE NEGATIVE EMOTIONALITY/BEHAVIOURAL REGULATION ASSESSMENT

7.1.1 Negative emotionality assessment in the literature

Negative emotionality is a robust depression endophenotype (Hayden et al., 2010) that has been widely examined in the past ten years. Yet, no measure of negative emotionality has emerged as a gold standard. Outcome disparities in the literature, then, are potentially due to the lack of uniformity in the way negative emotionality is assessed. As such, a valid and consistent assessment method is desirable. With the aim of using a standard assessment procedure, we derived a negative emotionality/behavioural regulation factor based on a validated mother report questionnaire, the Early Childhood Behaviour Questionnaire (ECBQ; Putnam et al., 2006).

7.1.2 Construction of the assessment

The process of deriving a new measure of children negative emotionality/behavioural regulation from the ECBQ revealed further temperament
constituents than those typically reported in the literature for negative emotionality (i.e. discomfort, frustration, fear, and sadness) and different from the negative affectivity factor derived from the ECBQ by Putnam and colleagues (2006). Indeed, within our derived negative emotionality/behavioural regulation factor at 18 months, not only were discomfort, frustration, fear, and sadness part of the derived factor, but so were activity level, inhibitory control, motor activation, and soothability. Provided that these additional components encompass motor, emotional, and cognitive components, we labelled our factor negative emotionality/behavioural regulation and we believe that it targets a broader range of negative emotionality/behavioural regulation elements than what has been examined thus far. Following this result, it appears to be worth investigating more behavioural, physical, and cognitive aspects of negative emotionality/behavioural regulation including motor actions, behavioural inhibition, and impulse control. In fact, it might be worth considering negative emotionality beyond traditional temperament traits, to include behavioural regulation, and to start viewing this construct as a multi-faceted one, beyond emotions. This view of temperament is consistent with a definition formulated by Rothbart and Posner (2006) who viewed temperament as “constitutionally based individual differences in reactivity and self-regulation, as observed in the domains of emotionality, motor activity, and attention.” However, the fact that motor activity and attention domains were
included within our negative emotionality/behavioural regulation factor might speak to the need of not considering these domains as mutually exclusive.

Furthermore, the derived factor at 36 months included all the same components than those derived at 18 months as well as two additional ones, namely attentional focusing and cuddliness. Although these two temperament subcomponents only arose from our factor analysis at 36 months but not at 18 months, they share common features with the subcomponents that were part of the derived factor. This leads us to conclude that negative emotionality/behavioural regulation factors derived at both 18 and 36 months are very similar and highly consistent with one another.

Hence, this assessment method of child negative emotionality/behavioural regulation appears to be more homogeneous and focused than the ECBQ which yields 18 different scale scores that tap various aspects and a wider range of early childhood temperament and behaviour. The argument, here, is not made against the use of the ECBQ which is a valuable assessment tool for global temperament traits. However, our negative emotionality/behavioural regulation assessment method allows for a more specific and precise way of targeting this subcomponent of temperament. Meanwhile, it is more complete than most of the
measures reported in the literature for negative emotionality thus far that only
focus on low affect-related aspects of temperament.

7.1.3 Validity and psychosocial adjustment

Comparing our assessment method with the Strengths and Difficulties
Questionnaire (SDQ; Goodman, 1997) that was administered at 60 months
supports its validity. Indeed, four of the five SDQ scales (namely, emotional
symptoms, hyperactivity, peer problems, and prosocial scales) were associated
with temperament negative emotionality/behavioural regulation both at 18 and 36
months. Hence, mother-ratings were consistent over time and our factor
succeeded at capturing this consistency. Finally, father-ratings of their offspring
psychosocial adjustment at 60 months were also related to mother-reports of
infant temperament negative emotionality/behavioural regulation thereby
providing good support for the validity of this measure both over time and across
raters. Associations between our negative emotionality/behavioural regulation
factors and the SDQ scores make also a good argument in favour of the validity
of the assessment method we developed provided that the validity of the SDQ as
a screening measure has been demonstrated (e.g. Goodman, 1997; Holtmann &
al., 2010; Stone et al., 2010).
Besides, this study is consistent with the links that are documented between temperament and early psychopathology/behavioural problems. For instance, early negative emotionality/behavioural regulation relates to later higher levels of internalizing and externalizing problems (Lengua & Long, 2002). Likewise, high levels of withdrawal predict vulnerability to depression (Holzwarth & Meyer, 2006), eating disorders (Loxton & Dawe, 2001), anxiety (Johnson et al., 2003), and alcoholism (Sher & Trull, 1994). High levels of approach associate with manic episodes (Holzwarth & Meyer, 2006) and eating disorders (Loxton & Dawe, 2001), whereas low levels of approach relate to depression (Holzwarth & Meyer, 2006). Hence, our findings that negative emotionality/behavioural regulation scores at both 18 and 36 months were related to psychosocial/behavioural impairments at 60 months further support the pathway relating certain temperament profiles to early psychopathology/behavioural problems.

7.1.4 Stability of infant negative emotionality/behavioural regulation

Strong correlations between the negative emotionality/behavioural regulation measure at both 18 and 36 months is the first of a three-fold argument to support its stability. Indeed, mothers consistently reported their infants’ temperament throughout this time period which was well statistically demonstrated and
captured. Moreover, dividing negative emotionality/behavioural regulation scores into low-range score, mid-range score, and high-range score groups, as defined by two cut-off values associated with the 33.33th and the 66.66th percentiles, confirmed its stability. As such, infants who displayed low levels of negative emotionality/behavioural regulation at 18 months remained in the lower range at 36 months and those in the mid range and in the high range followed the same consistent pattern. In other words, negative emotionality/behavioural regulation levels at 18 months predicted negative emotionality/behavioural regulation scores at 36 months and the opposite was also true.

Finally, comparing negative emotionality/behavioural regulation scores at both 18 and 36 months with psychosocial adjustment scores (as assessed with the SDQ) at 60 months further supported stability of the assessment over time.

Thus, not only does our proposed assessment of infant negative emotionality/behavioural regulation appears to be valid, stable, and consistent, but our results also support an early emergence and stable, enduring effect of temperament negative emotionality/behavioural regulation.
7.2 GENE X ENVIRONMENT INTERACTION IMPACTS ON INFANT NEGATIVE EMOTIONALITY/BEHAVIOURAL REGULATION

The hypothesis that maternal history of early adversity and offspring 5-HTTLPR genotype, alone or in interaction, would impact on offspring temperament was not supported. Indeed, temperament negative emotionality/behavioural regulation scores were solely impacted by maternal depression, assessed 6 months postpartum. Although the gene x environment interaction under investigation was not significant, further hypotheses could be made to examine maternal adversity as a continuum in the gene x environment equation of transgenerational risk transmission. Indeed, both pre and postnatal environments should be considered as they all might have an impact on offspring development and as it might be the cumulative effect of the two (i.e. prenatal adversity and postnatal adversity) that affects offspring development. Since detrimental consequences of maternal depression on mothering are documented (e.g. Fleming et al., 1988; Malphurs et al., 1996), it may be the presence of adversity both prenatally and postpartum that, along with “risky” genes, predict offspring adjustment difficulties. Perinatal programming resulting from adverse early experiences might not be as harmful if it is not followed by adverse or impoverished postnatal environment. It would be worth considering, hence, both pre and postnatal environments in the gene x environment equation, especially provided the numerous outcomes of parenting.
Indeed, inadequate parenting affects emotional as well as cognitive development (e.g. Landry et al., 2006). Parental care also predicts psychopathology development or resistance (Bifulco et al., 1991). Harsh as well as neglectful parenting associates with increased risk for mental illness (Holmes & Robins, 1988), obesity (Lissau & Sorensen, 1994), and impaired intellectual development (Trickett & McBride-Chang, 1995). Distant, cold parent-infant interaction also increases the risk for later chronic illnesses (Russak & Schwartz, 1997). Thus, maternal care and sensitivity appear as key proxy measures of postnatal environment to assess.

Other types of adversity such as stressors and life hassles would also be interesting to assess. Besides, the obtained non-significant results when looking at the main and interactive effects of maternal history of early adversity could be due to the fact that adversity levels were rather low in our community sample.

Besides, candidate genes other than the serotonin transporter polymorphism are worth investigating in this gene x environment interaction model. As much as the environment component appears as a complex, multidimensional variable to consider, transgenerational risk transmission may be due to more than one susceptibility gene. The brain-derived neurotrophic factor (BDNF) val66met
polymorphism is one possible candidate worth further investigation as it associates with parental depression and marital strain to predict the emergence of infant negative emotionality (Hayden et al., 2010). Likewise, the dopamine D4 receptor (DRD4) is another candidate that would need to be considered, as it predicts, along with 5-HTTLPR polymorphisms, temperament components such as negative emotionality and distress to limitations in 2-month-old infants (Auerbach, 1999). Tryptophan hydroxylase-2 (TPH2) genes and 5-HTTLPR polymorphisms also have additive effects on response to emotional stimuli (Canli et al., 2008), a relationship that might extend to the emergence of negative emotionality/behavioural regulation. Hence, the proposed gene x environment interaction model needs to be broadened with a plurality of candidate genes along with both pre and postnatal environments.

7.3 PARENTAL REPORT OF INFANT TEMPERAMENT

There are two main approaches to assessing infant temperament: parent-reports and laboratory assessments, each of which has strengths and weaknesses. Parent-reports allow gathering information about the child in various contexts and at several points in time, which makes it easier to disentangle typical from atypical behaviours. Parental reports are also very easy and inexpensive to use. However, shared method variance can be a major issue, as parent-reports can
be altered by parents expectations, experience (Kagan, 1998), and mood. This is even more problematic when studying the contribution of maternal adversity to infant temperament. Indeed, not only do parents contribute to both variables of study, but the fact that depressed parents can provide biased reports of child temperament is well documented (Leerkes & Crockenberg, 2003; Whiffen, 1990). Hence, observational measures allow for more objectivity and control to assess infant temperament. However, such measures are limited to a single observation point in time (Olino et al., 2010).

Despite these known effects of maternal depression on reports of infant temperament, offspring negative emotionality/behavioural regulation ratings remained stable between 18 and 36 months in the current study. Furthermore, negative emotionality/behavioural regulation at 18 and 36 months was predictive of psychosocial impairments at 60 months as assessed by both mothers and fathers, thereby providing evidence for the impact of maternal mood on offspring temperament over and above any bias reflected in the parental reports. Corresponding father ratings of psychosocial impairments at 60 months are key as they make an argument against shared method variance as does the fact that negative emotionality/behavioural regulation ratings remained consistent and stable over a 3.5 year period.
7.4 CONCLUSION

We provide support for the use of a standard assessment method for infant negative emotionality/behavioural regulation. Factor analysis of mother-reported infant temperament questionnaire captured a larger scope of temperament components and demonstrated good validity and stability. Maternal history of early adversity and offspring 5HTTLPR genotype, alone or in interaction, did not predict infant negative emotionality/behavioural regulation. However, we provided evidence that maternal depression affects infant negative emotionality/behavioural regulation at both 18 and 36 months of age.
8. REFERENCES


9. APPENDICES

9.1 APPENDIX A: MATERNAL HISTORY OF EARLY ADVERSITY FACTOR ANALYSIS

Table A1:

<table>
<thead>
<tr>
<th></th>
<th>Initial</th>
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<tbody>
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<td>CTQ Physical Neglect</td>
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<td>,584</td>
</tr>
<tr>
<td>CTQ Physical Abuse</td>
<td>1,000</td>
<td>,561</td>
</tr>
<tr>
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<td>1,000</td>
<td>,528</td>
</tr>
<tr>
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<td>1,000</td>
<td>,715</td>
</tr>
<tr>
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<td>1,000</td>
<td>,521</td>
</tr>
<tr>
<td>PBI Mother Care</td>
<td>1,000</td>
<td>,226</td>
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</table>

Extraction Method: Principal Component Analysis.
Table A2:

<table>
<thead>
<tr>
<th>Component</th>
<th>Initial Eigenvalues</th>
<th>Extraction Sums of Squared Loadings</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total</td>
<td>% of Variance</td>
</tr>
<tr>
<td>1</td>
<td>3,134</td>
<td>52,241</td>
</tr>
</tbody>
</table>

Extraction Method: Principal Component Analysis.
Table A3:

**Component Matrix**

<table>
<thead>
<tr>
<th>Component</th>
<th>Component</th>
</tr>
</thead>
<tbody>
<tr>
<td>CTQ Physical Neglect</td>
<td>.764</td>
</tr>
<tr>
<td>CTQ Physical Abuse</td>
<td>.749</td>
</tr>
<tr>
<td>CTQ Emotional Neglect</td>
<td>.727</td>
</tr>
<tr>
<td>CTQ Emotional Abuse</td>
<td>.845</td>
</tr>
<tr>
<td>CTQ Sexual Abuse</td>
<td>.722</td>
</tr>
<tr>
<td>PBI Mother Care</td>
<td>-.475</td>
</tr>
</tbody>
</table>

Extraction Method: Principal Component Analysis.

a. 1 components extracted.
### 9.2. APPENDIX B: INFANT NEGATIVE EMOTIONALITY/BEHAVIOURAL REGULATION 18 MONTHS FACTOR ANALYSIS

Table B1:

<table>
<thead>
<tr>
<th>Communality</th>
<th>Initial</th>
<th>Extraction</th>
</tr>
</thead>
<tbody>
<tr>
<td>ECBQ_18_Distress</td>
<td>1,000</td>
<td>.682</td>
</tr>
<tr>
<td>ECBQ_18_Fear</td>
<td>1,000</td>
<td>.598</td>
</tr>
<tr>
<td>ECBQ_18_Frustration</td>
<td>1,000</td>
<td>.644</td>
</tr>
<tr>
<td>ECBQ_18_Sadness</td>
<td>1,000</td>
<td>.577</td>
</tr>
<tr>
<td>ECBQ_18_Activity_Level</td>
<td>1,000</td>
<td>.742</td>
</tr>
<tr>
<td>ECBQ_18_Attentional_Focusing</td>
<td>1,000</td>
<td>.590</td>
</tr>
<tr>
<td>ECBQ_18_Attentional_Shifting</td>
<td>1,000</td>
<td>.576</td>
</tr>
<tr>
<td>ECBQ_18_Cuddliness</td>
<td>1,000</td>
<td>.541</td>
</tr>
<tr>
<td>ECBQ_18_High_Intensity_Pleasure</td>
<td>1,000</td>
<td>.688</td>
</tr>
<tr>
<td>ECBQ_18_Impulsivity</td>
<td>1,000</td>
<td>.508</td>
</tr>
<tr>
<td>ECBQ_18_Inhibitory_Control</td>
<td>1,000</td>
<td>.578</td>
</tr>
<tr>
<td>ECBQ_18_Low_Intensity_Pleasure</td>
<td>1,000</td>
<td>.687</td>
</tr>
<tr>
<td>ECBQ_18_Motor_Activation</td>
<td>1,000</td>
<td>.601</td>
</tr>
<tr>
<td>ECBQ_18_Perceptual_Sensitivity</td>
<td>1,000</td>
<td>.694</td>
</tr>
<tr>
<td>ECBQ_18_Positive_Anticipation</td>
<td>1,000</td>
<td>.516</td>
</tr>
<tr>
<td>ECBQ_18_Shyness</td>
<td>1,000</td>
<td>.478</td>
</tr>
<tr>
<td>ECBQ_18_Sociability</td>
<td>1,000</td>
<td>.578</td>
</tr>
<tr>
<td>ECBQ_18_Soothability</td>
<td>1,000</td>
<td>.610</td>
</tr>
</tbody>
</table>

Extraction Method: Principal Component Analysis.
Table B2:

<table>
<thead>
<tr>
<th>Component</th>
<th>% of Variance</th>
<th>Cumulative %</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>21,463</td>
<td>21,463</td>
</tr>
</tbody>
</table>

Extraction Method: Principal Component Analysis.
Table B3:

**Component Matrix**

<table>
<thead>
<tr>
<th>Component</th>
<th>Component 1</th>
</tr>
</thead>
<tbody>
<tr>
<td>ECBQ_18_Discomfort</td>
<td>0.632</td>
</tr>
<tr>
<td>ECBQ_18_Fear</td>
<td>0.651</td>
</tr>
<tr>
<td>ECBQ_18_Frustration</td>
<td>0.729</td>
</tr>
<tr>
<td>ECBQ_18_Sadness</td>
<td>0.715</td>
</tr>
<tr>
<td>ECBQ_18_Activity_Level</td>
<td>0.491</td>
</tr>
<tr>
<td>ECBQ_18_Attentional_Focusing</td>
<td></td>
</tr>
<tr>
<td>ECBQ_18_Attentional_Shifting</td>
<td></td>
</tr>
<tr>
<td>ECBQ_18_Cuddliness</td>
<td></td>
</tr>
<tr>
<td>ECBQ_18_High_Intensity_Pleasure</td>
<td></td>
</tr>
<tr>
<td>ECBQ_18_Impulsivity</td>
<td></td>
</tr>
<tr>
<td>ECBQ_18_Inhibitory_Control</td>
<td>-0.515</td>
</tr>
<tr>
<td>ECBQ_18_Low_Intensity_Pleasure</td>
<td></td>
</tr>
<tr>
<td>ECBQ_18_Motor_Activation</td>
<td>0.687</td>
</tr>
<tr>
<td>ECBQ_18_Perceptual_Sensitivity</td>
<td></td>
</tr>
<tr>
<td>ECBQ_18_Positive_Anticipation</td>
<td></td>
</tr>
<tr>
<td>ECBQ_18_Shyness</td>
<td></td>
</tr>
<tr>
<td>ECBQ_18_Sociability</td>
<td></td>
</tr>
<tr>
<td>ECBQ_18_Soothability</td>
<td>-0.577</td>
</tr>
</tbody>
</table>

Extraction Method: Principal Component Analysis.
a. 5 components extracted.
9.3. APPENDIX C: INFANT NEGATIVE EMOTIONALITY/BEHAVIOURAL REGULATION 36 MONTHS FACTOR ANALYSIS

Table C1:

<table>
<thead>
<tr>
<th>Communalities</th>
<th>Initial</th>
<th>Extraction</th>
</tr>
</thead>
<tbody>
<tr>
<td>ECBQ_36_Discomfort</td>
<td>1,000</td>
<td>.323</td>
</tr>
<tr>
<td>ECBQ_36_Fear</td>
<td>1,000</td>
<td>.177</td>
</tr>
<tr>
<td>ECBQ_36_Frustration</td>
<td>1,000</td>
<td>.540</td>
</tr>
<tr>
<td>ECBQ_36_Activity_Level</td>
<td>1,000</td>
<td>.336</td>
</tr>
<tr>
<td>ECBQ_36_Attentional_Focusing</td>
<td>1,000</td>
<td>.170</td>
</tr>
<tr>
<td>ECBQ_36_Attentional_Shifting</td>
<td>1,000</td>
<td>.130</td>
</tr>
<tr>
<td>ECBQ_36_Cuddliness</td>
<td>1,000</td>
<td>.178</td>
</tr>
<tr>
<td>ECBQ_36_High_Pleasure</td>
<td>1,000</td>
<td>.126</td>
</tr>
<tr>
<td>ECBQ_36_Impulsivity</td>
<td>1,000</td>
<td>.002</td>
</tr>
<tr>
<td>ECBQ_36_Inhibitory_Control</td>
<td>1,000</td>
<td>.466</td>
</tr>
<tr>
<td>ECBQ_36_Low_Intensity_Pleasure</td>
<td>1,000</td>
<td>.100</td>
</tr>
<tr>
<td>ECBQ_36_Motor_Activation</td>
<td>1,000</td>
<td>.306</td>
</tr>
<tr>
<td>ECBQ_36_Perceptual_Sensitivity</td>
<td>1,000</td>
<td>.058</td>
</tr>
<tr>
<td>ECBQ_36_Positive_Anticipation</td>
<td>1,000</td>
<td>.044</td>
</tr>
<tr>
<td>ECBQ_36_Sadness</td>
<td>1,000</td>
<td>.337</td>
</tr>
<tr>
<td>ECBQ_36_Shyness</td>
<td>1,000</td>
<td>.054</td>
</tr>
<tr>
<td>ECBQ_36_Sociability</td>
<td>1,000</td>
<td>.069</td>
</tr>
<tr>
<td>ECBQ_36_Soothability</td>
<td>1,000</td>
<td>.400</td>
</tr>
</tbody>
</table>

Extraction Method: Principal Component Analysis.
Table C2:

<table>
<thead>
<tr>
<th>Component</th>
<th>Initial Eigenvalues</th>
<th>Extraction Sums of Squared Loadings</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total</td>
<td>% of Variance</td>
</tr>
<tr>
<td>1</td>
<td>3,816</td>
<td>21,203</td>
</tr>
</tbody>
</table>

Extraction Method: Principal Component Analysis.
Table C3:

Component Matrix

<table>
<thead>
<tr>
<th>Component</th>
<th>Component</th>
</tr>
</thead>
<tbody>
<tr>
<td>ECBQ_36_Discomfort</td>
<td>0.568</td>
</tr>
<tr>
<td>ECBQ_36_Fear</td>
<td>0.421</td>
</tr>
<tr>
<td>ECBQ_36_Frustration</td>
<td>0.735</td>
</tr>
<tr>
<td>ECBQ_36_Activity_Level</td>
<td>0.580</td>
</tr>
<tr>
<td>ECBQ_36_Attentional_Focusing</td>
<td>-0.412</td>
</tr>
<tr>
<td>ECBQ_36_Attentional_Shifting</td>
<td></td>
</tr>
<tr>
<td>ECBQ_36_Cuddliness</td>
<td>-0.422</td>
</tr>
<tr>
<td>ECBQ_36_High_Pleasure</td>
<td></td>
</tr>
<tr>
<td>ECBQ_36_Impulsivity</td>
<td></td>
</tr>
<tr>
<td>ECBQ_36_Inhibitory_Control</td>
<td>-0.683</td>
</tr>
<tr>
<td>ECBQ_36_Low_Intensity_Pleasure</td>
<td></td>
</tr>
<tr>
<td>ECBQ_36_Motor_Activation</td>
<td>0.553</td>
</tr>
<tr>
<td>ECBQ_36_Perceptual_Sensitivity</td>
<td></td>
</tr>
<tr>
<td>ECBQ_36_Positive_Anticipation</td>
<td></td>
</tr>
<tr>
<td>ECBQ_36_Sadness</td>
<td>0.581</td>
</tr>
<tr>
<td>ECBQ_36_Shyliness</td>
<td></td>
</tr>
<tr>
<td>ECBQ_36_Sociability</td>
<td></td>
</tr>
<tr>
<td>ECBQ_36_Soothability</td>
<td>-0.633</td>
</tr>
</tbody>
</table>

Extraction Method: Principal Component Analysis.

a. 1 component extracted.