The role of hippocampal and amygdala volumes in mediating the effects of prenatal maternal stress on cognitive and behavioural outcomes in the child.

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Abstract

Problem Statement

Prenatal maternal stress (PNMS) has been associated with increased behavioural problems and cognitive deficits in exposed children. Interestingly, research has shown that the developing brain is sensitive to PNMS. In particular, animal studies suggest that exposure to PNMS alters hippocampal (HC) and amygdala (AG) volumes, two regions important for cognition and emotions. Changes in HC and AG volume have been associated with behavioural problems and cognitive deficits. Yet, no studies have assessed the mediating effect of the HC and AG on the association between the various components of PNMS (i.e., objective stress, subjective stress and cognitive appraisal) and behavioural and cognitive outcomes in humans.

Objectives

The objectives were (1) to determine the extent to which cognitive appraisal, objective and/or subjective PNMS affect HC and AG volume in 11½ year old children; (2) To determine the extent to which hippocampal subfield volumes are altered by PNMS, and determine the extent to which the effects of PNMS on the right versus left HC and AG differ; (3) to determine the extent to which timing moderates the effects of PNMS on HC and AG volumes; and (4) To determine the extent to which HC and AG volumes mediate the effects of PNMS on behavioural and cognitive outcomes at age 11½ in children exposed in utero to varying levels of disaster-related PNMS.

Methods

T1-weighted images using a Siemens 3T scanner were acquired and HC and AG volume measured using semi-automated segmentation from 69 11½ year-old children whose mothers were pregnant during the January 1998 Quebec Ice Storm, or became pregnant within three months of exposure to the storm. The mother's objective stress, subjective stress and cognitive appraisal of the storm were assessed in June 1998. Behavioural and cognitive assessments were also obtained at 11½ year-old assessments.

Results

In girls, higher levels of maternal objective stress predicted higher IQ and this was associated with an increase in both left and right HC volume. In boys and girls, higher levels of objective and subjective stress were associated with more externalizing problems, and this was in part associated with an increase in AG volume. In boys, the greatest impact was seen when PNMS occurred during late gestation. Other results suggested that earlier age of menarche was associated with larger HC volume at age 11 ½.

Discussion

Findings from the present study provide support for the premise that cognitive abilities and susceptibility to behavioural problems may, in part, be programmed in utero, and that this effect may be mediated through changes in anatomy of the HC and AG. The effect of PNMS on the local development of AG and HC volumes seems to be stronger in girls. This may be explained by sex-specific placental adaptation to stress exposure or increase susceptibility of female brain to its milieu given the more rapid neurodevelopment trajectory in females compared to males.

Résumé

Problématique

Les enfants exposés à un stress maternel prénatal (SMPN) ont été associés à une augmentation de problèmes comportementaux et de déficits cognitifs. Il est intéressant de noter que des études ont montré que le cerveau est sensible au SMPN. En particulier, des études faites chez les animaux suggèrent qu'être exposé au SMPN affecte les volumes de l'hippocampe (HC) et de l'amygdale (AG), deux régions importantes pour la cognition et les émotions. Des changements de volumes dans l'HC et l'AG ont été associés avec des problèmes comportementaux et des déficits cognitifs. Néanmoins, aucune étude n'a testé l'effet de médiation de l'HC et l'AG sur l'association entre les différentes composantes du SMPN (i.e., le stress objectif, le stress subjectif et l'évaluation cognitive) et les issues comportementales et cognitives chez l'humain.

Objectifs

Nos objectifs étaient de (1) déterminer dans quelle mesure l'évaluation cognitive, le stress objectif et / ou subjectif affecte l'HC et l'AG chez les enfants de 11½ ans ; (2) déterminer dans quelle mesure les volumes des sous-parties de l'HC sont altérées, et dans quelle mesure l'effet de SMPN sur l'HC et l'AG droite versus gauche diffère ; (3) déterminer dans quelle mesure le timing modère l'effet du SMPN sur les volumes de l'HC et l'AG ; et (4) déterminer dans quelle mesure les volumes de l'HC et l'AG expliquent l'effet de SMPN sur le comportement et la cognition chez des enfants de 11½ ans qui ont été exposés à une catastrophe naturelle pendant la période de gestation.

Méthode

69 enfants de 11½ dont les mères étaient enceintes pendant la tempête de glace du Québec en 1998 ou sont tombées enceintes jusqu'à trois mois après la tempête ont été recrutés. Des images T1 pondérées ont été acquises à l'aide d'un scanner 3T Siemens, et lesvolumes de l'HC et l'AG mesurés par segmentation manuelle. Le stress objectif, subjectif et l'évaluation cognitive ont été mesuré en juin 1998. Les évaluations comportementales et cognitives ont été effectuées à l'âge de 11½.

Résultats

Chez les filles, un niveau de stress objectif élevé chez la mère était associé à un QI plus élevé, et ceci était expliqué par une augmentation du volume de l'HC droite et gauche. Chez les garçons et les filles, un niveau de stress objectif et subjectif maternel élevé était associé avec plus de problèmes d'extériorisations et ceux-ci était en partie expliqué par une augmentation du volume de l'AG. Chez les garçons, l'effet était plus important lorsqu'ils étaient exposé au stress plus tard dans la gestation. D'autres résultats montre aussi que une ménarche plus prematuré etait associé avec un plus grand volume de l'HC.

Discussion

Les résultats de cette étude soutiennent l'idée que les capacités cognitives et la susceptibilité aux problèmes comportementaux sont, en partie, programmées in utero, et que cet effet peut être expliqué à travers des altérations dans l'anatomie de l'HC et l'AG. L'effet de SMPN sur le développement de l'HC et l'AG paraît être plus important chez les filles. Ceci pourrait être expliqué par l'adaptation du placenta en réponse au stress ou par une sensibilité du cerveau à son environnement plus forte chez les filles étant donné la croissance neurodeveloppemental plus rapide qu'elles ont comparé aux garçons.

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Preface

The scanning protocol was designed by Arnaud Charil. The children were escorted through scanning protocol at age 11 by Rowena Lung, Karine Ferron, Gila Foomani, Weirquan Zeng, Marjolaine Massé, Maria Papastergiou. The scans were obtained by André Cyr and Carollyne Hurst. Child behavioural and cognitive testing was done by Jenifer Chappell and Isabelle Bouchard. The automated segmentation was done by Raihaan Patel and myself.

The manual correction of hippocampal and amygdala volumes was done by myself. The statistical analysis was done by me with the help of Guillaume Elgbeili. The interpretation and writing was done by me.

Abbreviations used in thesis

AG	Amygdala
AGV	Amygdala volume
CA1, 2, 3, 4	cornu ammonis 1, 2, 3, 4
CBCL	Child Behaviour Checklist
CMS	Child Memory Scale
DG	Dentate Gyrus
GHQ	General Health Questionnaire
HC	Hippocampus
HCV	Hippocampus volume
IES-R	impact of event scale revised
LAG	left amygdala
LAGV	left amygdala volume
LCA1, 2, 3, 4	left cornu ammonis 1, 2, 3, 4
LHC	left hippocampus
LHCV	left hippocampal volume
MRI	magnetic resonance imaging
PNMS	Prenatal maternal stress
RAG	right amygdala
RAGV	right amygdala volume
RCA1, 2, 3, 4	right cornu ammonis 1, 2, 3, 4
RHC	right hippocampus
RHCV	right hippocampal volume
SES	socioeconomic status
TIC	total intracranial volume
WISC	Wechsler Intelligence Scale for Children

I. Introduction

A certain amount of life stress is unavoidable. But when sudden stressors occur to pregnant women, it influences the development of the unborn child, which may affect their cognitive, behavioural and physical features later in life. The negative consequences of prenatal maternal stress (PNMS) may be mediated by changes in foetal brain morphology. However, no study in humans has been able to relate the severity and timing of PNMS to effects on specific brain regions. There is, however, direct evidence from animal experimentation, and indirect evidence from correlational behavioural studies in humans.

Retrospective studies in humans, and experimental research with animals, suggest that psychological stressors during pregnancy can influence the behavioural and cognitive outcomes of the offspring. However, there are important gaps in the literature that obscure the nature of the underlying mechanism. Human research on PNMS is hampered by methodological and ethical constraints. For example, stressful events cannot be randomly assigned to pregnant women by an experimenter, so researchers rely on correlational studies. Retrospective and prospective studies of stressful life events during pregnancy in humans are unable to disentangle the effects between the objective severity of the exposure to the event, and their subjective response to the event. Even prospective studies of these naturally occurring life events or of anxiety during pregnancy cannot determine whether problems in the offspring are due to genetic transmission of factors such as maternal temperament, alterations in the intrauterine environment, or to post-natal effects such as parenting style. While animal research provides an excellent means of directly assessing the effects of prenatal stress on brain development and functioning, there are limitations to animal studies. For example, rodents are not born at the same stage of development as humans are, and critical differences exist between animal and human central nervous system. Moreover, we cannot disentangle objective exposure, cognitive factors and distress in animals. Given these potential confounds, extrapolation from controlled animal studies to humans is particularly challenging.

Natural and man-made disasters act as “natural experiments”, randomizing the distribution of stress exposure. This enables the removal of the genetic influence, and the differentiation between objective exposure, cognitive factors and distress in humans. The Quebec ice storm in 1998 qualifies as such an event. It resulted in electrical power failures for more than 3 million individuals for anywhere from 6 hours to more than 5 weeks. As

such, a large number of pregnant women in various stages of pregnancy were randomly exposed to varying degrees of storm-related hardship. Previous findings from Project Ice Storm have shown that both objective stress and subjective distress predict cognitive and behavioural outcomes in the children. Little is known about the biological mechanisms of these effects, however. Thus, the goal of our current project is to take advantage of this unique opportunity to extend our understanding of the effects of PNMS by determining (1) the extent to which PNMS affects brain morphology, specifically hippocampal (HC) and amygdala (AG) size, and (2) the extent to which changes in HC and AG volumes, in turn, affect behavioural and cognitive development of children exposed to the ice storm in utero.

II. Literature review

A. How maternal stress affects foetal development

Stress involves the occurrence of environmental stressors and the response of an individual to these stressors. How the individual will respond to the stressor has been termed cognitive appraisal and occurs when the individual considers two factors (Lazarus and Folkman, 1984). The first is the demand brought on by the stimulus to the individual, and the second is the assessment of resources required to deal with the stimulus, i.e. to cope with the situation. There are two stages to the cognitive appraisal response. Primary appraisal is assessing whether there is a stress or not. If resources exceed the demands of the situation no stress is perceived – the stimulus acts as a challenge. If however the demands of the situation exceed the available resources, a threat is perceived. This is then followed by secondary cognitive appraisal, which is the assessment and selection of available strategies (cognitive, behavioural, emotional) to deal with the situation at hand. It is well established that the perception of a situation as stressful results in the secretion of glucocorticoids, such as cortisol (in humans) or corticosterone (in rodents) from the adrenal glands into the bloodstream (Harris and Seckl, 2011). Cortisol is probably one link between prenatal stress and offspring outcomes (Benediktsson et al., 1997). Being exposed to a stressor during pregnancy causes maternal cortisol levels to rise and, at high concentrations, maternal cortisol can cross the placental barrier and reach the foetus. A linear relationship exists between maternal and foetal cortisol levels, thus an increase in maternal cortisol can cause the foetus to be exposed to high amounts of cortisol. In the foetus, cortisol can exert direct effects on gene transcription leading to long-term

impairments in cognitive, behavioural and physical development of the children (Harris and Seckl, 2011). A study in rats has shown that corticosterone is one important way by which prenatal stress enacts long-term neurobiological changes in rats (Bingham et al., 2013). Moreover, mediation by obstetrical complications could be another mechanism by which PNMS influences brain development (Paarlberg et al. 1999). Many brain regions including the hippocampus, amygdala, prefrontal cortex, cerebellum and hypothalamus have been shown to be sensitive to prenatal stress (Charil, 2010).

B. Prenatal maternal stress (PNMS) affects behaviour and cognition in the offspring

Various developmental outcomes in the offspring have been reported in rodents or non- human primates following exposure to various different types of "prenatal stressors" including maternal restraint, administration of synthetic glucocorticoids (such as dexamethasone), saline injection and acoustical startle. Human research also uses different types of "prenatal stressors" including maternal psychological functioning, non-independent life events and independent life events.

Research has shown that maternal stress during pregnancy may have long-lasting adverse consequences on the behavioural development of the offspring. In rats, PNMS has been associated with increased levels of anxiety (Weinstock, 2007, 2011) and decreased behavioural adaptation to postnatal stressful conditions in offspring (Takahashi et al., 1992). Further studies in monkeys suggest that PNMS is linked to changes in social behaviour such as higher frequencies of abnormal behavioural stereotypies (Clarke and Schneider, 1993) and decreased exploration of novel or intimidating environments (Schneider, 1992).

In humans, it has long been a common belief that the emotional state of the mother may affect the child she is carrying (Ferreira, 1965). Most studies investigating the effects of the mother's emotional state on child outcomes have used measures of maternal self-report psychological functioning, such as pregnancy specific anxiety, psychosocial stress or perceived stress. For example, pregnancy-specific anxiety has been associated with increased affective disorders and more specifically increased depression levels in the offspring during adolescence (Van Den Bergh et al., 2008, Van Lieshout and Boylan, 2010). Moreover, various measures of maternal self-report psychological functioning have been

associated with temperament variation and difficult behaviour in infants (Huizink et al., 2002, Buitelaar et al., 2003, Gutteling et al., 2005, Sandman et al., 2012), as well as more externalizing problems in toddlers (Wadhwa et al., 2001, Gutteling et al., 2005, Rice et al., 2007, Chuang et al., 2011, Liu, 2011) and increased attention deficit and hyperactivity disorder symptomatology (Grizenko et al 2015).

Another type of stressor used in some studies is life events. The occurrence of these events is usually not randomly distributed and is likely influenced by the mother's own propensity to cause these stressful events through either behavioural or genetic means, either of which could have its own effect on child outcome. Non-independent maternal life events in pregnancy (such as living in family discord) have been associated with affective disorders in the child and adolescent aged 4 to 19 (Ward, 1991), and with more externalizing problems in the child aged 2 and 5 (Robinson et al., 2008).

In contrast, independent stressors are more likely to affect pregnant women in a random fashion. Thus, they enable the removal of the confounding influence of genetics and behavioural modeling. Watson et al. and Huizink et al. have investigated the effects of PNMS from natural disasters on child emotional outcomes. Watson et al. reported that maternal exposure to an earthquake during pregnancy predicted greater depression in the offspring at 18 years of age (Watson et al., 1999). Huizink et al. reported that children exposed to more maternal displacement from the Chernobyl disaster during pregnancy had more depression symptoms at age 14 years than those who were not exposed (Huizink et al., 2007). These results were unchanged when adjusted for maternal symptoms of depression, maternal symptoms of childhood conduct disorder, maternal symptoms of adult antisocial personality disorder, maternal alcohol (ab)use and maternal smoking during pregnancy. A limitation of those natural disaster studies is that they compare exposed to non-exposed groups, and do not assess the effect of various levels of stress.

A large literature has shown that PNMS in rodents affects cognitive development in the offspring. In fact, it has been shown that exposure to PNMS is associated with poor performance in various learning paradigms and memory tasks (Takahashi et al., 1992, Lemaire et al., 2000, Ishiwata et al., 2005, Gi et al., 2006, Yang et al., 2006, Zagron and Weinstock, 2006, Weinstock, 2007, 2011). For example, Lemaire et al. used a place navigation task in which the rats had to locate a small platform submerged in a pool of water

made opaque by the addition of milk. The authors found that prenatal stress in rats induced lifespan reduction of neurogenesis in the hippocampus and produced impairment in hippocampal-related spatial tasks (Lemaire et al., 2000). However, one study showed that exposure to PNMS for one day predicted a faster learning rate in the offspring (Cannizzaro et al., 2006). It is possible that mild/short PNMS predicts better outcomes while severe or long-lasting stress predicts worse outcomes.

Similarly in humans, measures of maternal self-reported psychological functioning have been associated with negative cognitive outcomes in the child. A study by Niederhofer and Reiter showed a significant correlation between prenatal maternal psychological stress, perinatal temperament of the child, and his/her school marks at the age of 6 years (Niederhofer and Reiter, 2004). Similarly, Brouwers et al. found that prenatal maternal anxiety predicts lower mental development in infants (Brouwers et al., 2001).

Other studies have investigated the association between non-independent maternal stressful life events during pregnancy and child cognitive abilities. Bergman et al. (2007) showed that the number of stress events accounted for 17% of the variance in cognitive ability in 14 to 19 month-old infants. Using the mother's perceived impact of events or the number of events resulted in similar outcomes (Bergman et al., 2007).

In summary, the human literature provides strong evidence that maternal psychological functioning and stressful life events during foetal development predict poorer behaviour and cognitive abilities in the male and female offspring. Nonetheless, more literature is needed using a random stressor (i.e., natural disaster) to support the causal conclusion about the effect of PNMS on child cognitive outcomes.

C. PNMS reduces hippocampal volume (HCV) in the offspring

It is widely believed that the hippocampus is highly involved in learning, memory and emotion processes. The HC plays a major role in the regulation of cortisol production (Hellhammer et al., 2009). Exposure to stressors leads to the activation of the hypothalamic-pituitary-adrenal (HPA) axis triggering a rise in cortisol levels in the body and the brain. In response, the HC exerts a negative feedback on the HPA axis reducing the production of cortisol (Joels, 2008). Interestingly, increases in one's own cortisol secretion

during the day has been associated with one having a smaller HCV (Frodl and O'Keane, 2013).

Interestingly, if changes in cortisol level occur in the mother during pregnancy, the offspring's HC may be affected. In fact, numerous studies in animals suggest that exposure to more PNMS causes smaller HCV in the offspring. For example, Uno and his colleagues (1994) conducted research on the effects of PNMS on the HC in non-human primates. They simulated the effects of prenatal stress by administering intramuscular injections of dexamethasone to pregnant females. Their studies showed that more dexamethasone was associated with a severe reduction in the number of neurons in the offspring's HC, resulting in a 30% reduction in HCV, with the total brain volume unchanged (Uno et al., 1994). Magnetic resonance imaging (MRI) studies have indicated that this decrease in HCV was unchanged for two years post-natally (Uno et al., 1994). Coe and his colleagues found similar outcomes using a more natural acoustic startle stressor in Rhesus monkeys (Coe et al., 2003). Furthermore, it was found that prenatally stressed female rodents, but not males had a significant reduction in the volume of granule or pyramidal cell layers and a reduction in those cell numbers compared to their non-prenatally stressed counterparts (Schmitz et al., 2002) suggesting a stress by sex interaction.

The decrease in HCV associated with PNMS may be explained by impairment in hippocampal cell development. A large body of literature has shown that PNMS in rodents causes lower cell density, neurogenesis and cell proliferation in the HC (Lemaire et al., 2000, Zhu et al., 2004, Kawamura et al., 2006, Lemaire et al., 2006, Behan et al., 2011, Belnoue et al., 2013). Interestingly, (Hayashi et al., 1998) showed that PNMS in rats also induces synaptic loss in the offspring HC. The authors further suggest that this may be associated with reported changes in behaviour and learning ability in prenatally stressed offspring.

There is evidence that the negative effect of PNMS on HCV and cell count in animals lasts onto adulthood. Coe et al. found that 3-year-old monkeys exposed to PNMS still displayed smaller HCV compared to controls (Coe et al., 2003). Belnoue et al. reported smaller HCV in adult mice (Belnoue et al., 2013) and Lemaire et al showed that PNMS affects cell proliferation between adolescence and old age in mice (Lemaire et al., 2006).

Very few studies in humans have investigated the effects of PNMS on the HC. Self-

reported prenatal maternal anxiety has been associated with smaller grey matter volume in the medial temporal lobe of children at the age of 6-9 (Buss et al., 2010). Another study has shown that self-reported prenatal maternal anxiety was not associated with HCV at birth but was associated with slower HC growth over the first six months of life, (Qiu et al., 2013). In contrast, a large pool of research suggests that child *post-natal* stress, especially childhood maltreatment, correlates with higher cortisol levels and smaller HCV in the child (Frodl and O'Keane, 2013). Thus, the existing human literature suggests that stress affects the HC but more studies are needed to support causal conclusions about any effects of PNMS on HCV in humans.

Although the left and right HC may have different functions, no studies have investigated the differential effect of PNMS on the right or left HC in either humans or animals. A large body of research has investigated the respective functions of the right and left HC suggesting that they have different roles. For example the right HC may be more involved in spatial memory (Maguire et al., 1997, Maguire et al., 2000, Spiers et al., 2001, Burgess et al., 2002) and the left HC in verbal memory (Frisk and Milner, 1990, Seidenberg et al., 1993, Burgess et al., 2002). However, no clear consensus has been made so far and other studies suggest that the roles are confounded by education and handedness (true right versus left- handedness). Investigating the sensitivity of right and left HC to stress may emphasize their functional differences. To my knowledge, one study so far has compared the effects of PNMS on right and left HC, and found that the effect of PNMS on HC growth was stronger in the right HC (Qiu et al., 2013).

Recently, there has been increased interest in measuring the subfields of the human hippocampal formation using MRI (Geuze et al., 2005) given the differential role of HC subfields in several neuropsychiatric diseases. However, progress in this area has been somewhat impeded by a significant amount of disagreement in the anatomical definition of the subfields. Although terminology varies among authors, the terms most frequently used for hippocampal subfields are cornu ammonis (CA) 1, 2, 3, 4; dentate gyrus; and the subicullum (Duvernoy, 2005)**.** Although their respective functions are not clear, the existing literature suggests that the dentate gyrus is the main region of neurogenesis in the HC (Duvernoy, 2005). In fact, studies in the rhesus monkey (Coe et al., 2003) and in rodents (Lemaire et al., 2000, Belnoue et al., 2013) have shown that PNMS causes decreased

neurogenesis and cell proliferation in the dentate gyrus. No studies so far have investigated the effects of PNMS on HC subfields in humans.

D. The association between HCV and cognition

Some animal studies have shown that decreased HCV is associated with learning and memory deficits in animals. Luo et al (2014) found that rats exposed to chronic unpredictable mild stress postnatally showed a dynamic and gradual decrease in HCV over time of the procedure, and this was correlated with learning and memory deficits (Luo et al., 2014). Further studies in rats support these findings (Yin et al., 2011, Cominski et al., 2014).

The human literature regarding the link between HCV and cognitive deficits is inconsistent. A large number of studies have investigated the association between HC size and memory in healthy humans across the life span. A meta-analysis by Van Petten summarizes the current state of the literature well (Van Petten, 2004). The author suggests that there is no support for the "bigger is better" hypothesis. In fact, results across studies include both positive and negative correlations between HCV and memory. Instead, it seems that the association between HCV and memory is dependent on age: this meta-analysis supports a developmental hypothesis, which suggests that until early adulthood HCV and memory are negatively correlated, and the aging hypothesis, which suggests that in adulthood, HCV and episodic memory are positively correlated. However, the conclusions from a review and meta-analysis of results from 33 studies were that empirical support for a causal relationship is weak (Van Petten, 2004). Although in the review and meta-analysis by Van Petten (2004), results from 33 studies were used, only one study reported findings in children between 7 and 16 years old. This study supported the developmental hypothesis as the results showed that frontal and mesial temporal lobe gray matter thinning was predictive of greater verbal memory functioning (Sowell et al., 2001).

The developments of higher-field scanners and novel segmentation approaches have enabled studies to examine the relationship between hippocampal subfield volumes and cognitive performance (Shing et al., 2011, Engvig et al., 2012). So far no consensus has been reached regarding the role of the hippocampal subfields in memory, thus I will not enter the debate on their potential roles in memory. Nevertheless, one line of research that seems

consistent suggests that CA1 is the first region to degenerate in Alzheimer's disease (West et al., 1994).

In summary, animal studies suggest that smaller HCV is associated with cognitive deficits. However, in humans, no consensus has been reached in children or young adolescents, and the role of HC subfields in cognitive functioning has yet to be defined.

E. Altered amygdala volume (AGV) is linked to behavioural problems

Another limbic system structure may also be involved in the stress system; it is generally agreed that the amygdala (AG) is involved in several forms of emotional regulation and emotional learning and memory (Phelps and LeDoux, 2005, Quirk and Beer, 2006). Yang et al investigated how natural variation in AGV associates with specific fear and stress-related phenotypes in rodents (Yang et al., 2008). The study showed that the mice with small basolateral amygdaloid (BLA) complex (consisting of the lateral, basal and accessory-basal nuclei of the amygdala) presented significantly stronger conditioned fear responses to stress to both auditory tone and contextual stimuli compared to the larger BLA group. The small BLA group also showed significantly greater cortisol response to stress than the larger BLA group. However, BLA volume did not predict clear differences in measures of anxiety-like behaviour or depression-related behaviour. Similarly, in Rhesus monkeys Haley and colleagues found that monkeys with smaller AGV had higher anxiety compared to those with higher AGV (Haley et al., 2012). Howell et al (2014) explored the link between juvenile abuse, AGV and behavioural outcome in the Rhesus monkey (Howell et al., 2014). They found that higher abuse rates during infancy predicted larger AGV, which in turn was positively correlated with exploration rates (Howell et al., 2014). In conclusion, animal studies seem to suggest that smaller AGV is associated with greater anxiety, possibly reflecting stronger emotional learning.

In humans, the research is inconsistent regarding the direction of the association between AGV and behavioural problems. Major depression disorder and generalized anxiety disorder have been associated with both smaller (Sheline et al., 1998, Milham et al., 2005, Rosso et al., 2005) and larger (De Bellis et al., 2000, MacMillan et al., 2003, Schienle et al., 2011) AGV.

At a non-clinical level, smaller AGV has been associated with more depressive

symptoms (Yap et al., 2008) and more internalizing problems (McQueeny et al., 2011) in healthy children. In contrast, one study by Buss et al (2012) found that larger right AG predicted more internalizing problems (Buss et al., 2012); and Koolschijn and colleagues suggest that AGV is not associated with internalizing problems in the child (Koolschijn et al., 2013). Many studies have also associated smaller AGV or lower AG grey matter with more externalizing problems (Sterzer et al., 2007, Fairchild et al., 2011, Matthies et al., 2012, Bobes et al., 2013, Gopal et al., 2013, Pardini et al., 2014). Most studies have focused on men only (Sterzer et al., 2007, Fairchild et al., 2011, Matthies et al., 2012, Bobes et al., 2013, Gopal et al., 2013, Pardini et al., 2014), while one study has shown similar findings in women (Matthies et al., 2012). To date, a single study has included both males and females, and the authors report that in boys only, externalizing problems during preschool were associated with smaller AGV at 15 years of age (Caldwell et al., 2015).

In summary, there is a trend but no consensus suggesting that smaller AGV in both males and females may be more strongly associated with behavioural problems than larger AGV.

F. PNMS alters AGV in offspring

Few studies have investigated the effects of PNMS on AGV in animals. Kraszpulski et al (2006) studied the effect of prenatal maternal stress on the offspring's amygdala nuclei volumes in rats and found that the exposed offspring had basolateral, central and lateral nuclei volumes that were 20-25% smaller at postnatal day 25 compared to controls.

Interestingly though, the decrease in volume seemed to resolve by postnatal day 45 (adulthood) (Kraszpulski et al., 2006). Another study done in rats has shown that the volume of the lateral AG nucleus in prenatally stressed offspring is not decreased, but is rather increased by 30% between postnatal days 80 and 120 compared to controls (Salm et al., 2004). There was no change in volumes of the other nuclei observed at this time. These two studies used the same protocol (type of stressor, timing and duration of exposure) and differ only in terms of the time of postnatal AG nuclei volume assessment. Although Kraszpulski et al. and Salm et al. find diverging results, their results are not incompatible. Kraszpulski et al. suggest that a developmental lag caused by PNMS may explain the smaller nuclei volumes observed in the early postnatal period; the later increase in volume is likely

due to a surge in neuronal growth (Salm et al., 2004). Interestingly, it seems that PNMS does not alter the developmental trajectory of all AG nuclei in the same way. These findings emphasize the importance of taking into account assessment times when interpreting findings from animal studies as well as investigating sub-regions of a particular structure when possible. Further research is needed in this area to determine the extent to which PNMS affects total AGV.

To our knowledge, no studies have investigated the effects of PNMS per se on AGV in humans. Teicher et al. reviewed the existing literature on the neurological manifestations of early stress on various brain regions including the AG, and from the existing literature they proposed that exposure to maternal cortisol is a crucial factor in organizing the AG along a stress-responsive pathway (Teicher et al., 2003). Later, Buss et al. investigated the associations between maternal cortisol levels during pregnancy and AGV in seven-year-old children (Buss et al., 2012). The authors found that greater maternal cortisol levels at 15 weeks gestation predicted more affective problems in girls, and this association was, in part, mediated by larger right AGV (Buss et al., 2012). Because cortisol is a biomarker of stress, it might be possible to anticipate the effects of PNMS on AGV based on the effects of maternal cortisol on child AGV. Nonetheless, further research is needed in humans using a randomized stressor to show a causal effect of PNMS on AGV. No studies have reported a different sensitivity of the left and right AG to PNMS. Although some studies suggest that the right and left AG have different roles in the regulation of emotions and memory, no clear consensus has been reached (Markowitsch, 1998, Demaree et al., 2005).

G. Sexual dimorphism of brain and development

The existing literature suggests that males and females respond to PNMS differently (Mueller and Bale, 2008, Gabory et al., 2009). Although the mechanisms underlying these differences are unknown, they are likely the result of the activity of various sex hormones. More specifically for the brain, animal studies have shown that specific regions of the normal female and male brains differ in cell numbers, neuronal morphology, structure volumes and synaptic connections (Weinstock, 2007, Schwarz and McCarthy, 2008). Thus, during critical periods of brain development, male and female brain may be affected by sex

hormones in different ways. For example, some studies suggest that in males, a rise in cortisol levels leads to a sooner-than-normal peak in testosterone which may translate into neurodevelopmental, cognitive and behavioural changes observed later in adulthood (Coe et al., 2002, Schwarz and McCarthy, 2008). Other studies suggest that males and females have different placental response to the same maternal environment (Montano et al., 1993, Charil et al., 2010, Dickinson et al., 2012). In the past literature, there is support for both a greater male sensitivity to PNMS (Charil et al., 2010, Dickinson et al., 2012), and for greater female sensitivity (Montano et al., 1993, Schmitz et al., 2002, Zhu et al., 2004, Behan et al., 2011, Buss et al., 2012).

In conclusion, one way to compare the effects of PNMS on males and females would be to look at a stress by sex interaction. Moreover, to limit the confounding effect of sexual dimorphism, males and females could also be studied separately.

Interestingly, due to the different rates of neurodevelopment in males and females, some studies suggest the importance of timing of exposure to the stressor in the response to PNMS (Ellman et al., 2008, Buss et al., 2009, Buss et al., 2012)).

H. Timing moderates the effect of PNMS on HC and AG volumes and on behavioural and cognitive outcomes

The HC develops during the foetal period. In humans, at 9 weeks, the HC already contains four different cell layers. At 15-19 weeks, individual subfields can be distinguished, and by week 34 all HC subfields show a mature cytoarchitecture (Arnold and Trojanowski, 1996). A study conducted in non-human primates suggests that the effects of PNMS on HCV are stronger during early gestation than in later gestation (Coe et al., 2003). These findings are supported by a study done in humans, which showed that psychological markers of stress during pregnancy, especially early in gestation, predicted disrupted emotional regulation and impaired cognitive performance during infancy, as well as decreased brain volume in areas associated with learning and memory in children (Sandman et al., 2012).

In terms of development of the AG structure, it is established in humans that the amygdaloid complex is formed by 15 weeks gestations (Nikolic and Kostovic, 1986). In accordance with this, one study investigated the association between early and late maternal cortisol levels and subsequent child AGV, and found that there was an increase in

child AGV in response to high maternal cortisol exposure during early but not late prenatal period of life (Buss et al., 2012). Thus, this study highlights early gestation as a developmental window of susceptibility to stress-related environmental conditions on the developing AG

Thus is seems that both the HC and AG might be more sensitive to disturbances in earlier gestation compared to later.

III. Problem Statement

A. State of current literature

Prenatal maternal psychological stress has been associated with increased behavioural problems, such as internalizing and externalizing problems, as well as cognitive deficits in children. Interestingly, research has also shown that the brain is sensitive to PNMS. In particular, a few animal studies suggest that exposure to PNMS especially in females, and especially during early gestation, alters HC and AG volumes, two structures important for cognition and emotions. In fact, in both animals and humans altered HC and AG volume have been associated with behavioural problems and cognitive deficits. Yet, to date, there have been no studies of prenatal maternal stress that can determine the extent, longevity, nature and mechanisms of the effects in the human HC and AG. In animal research, random assignment to various stress conditions provide an excellent manner of directly assessing the effects of PNMS on HC/AG development. However, animal studies have significant limitations: Firstly, rodents are not born at the same stage of development as humans – compared to humans their brains are substantially less developed at birth. Secondly, animal studies are unable to assess cognitive appraisals that occur in humans between a stressor and the response to a stressor. Thirdly, studies in animals have used a multitude of different stressors and exposed the animals to these stressors for varying lengths of time.

Furthermore, there are critical differences between animal and human brains and central nervous systems. Thus, the effect of PNMS on HC/AG development in humans cannot be fully anticipated using animal models.

B. Human studies have limitations

The human studies that do assess 'stress' during the pregnancy are unable to tease apart the various aspects of the process such as the mother's objective exposure, cognitive appraisal, subjective reaction and subsequent hormonal response. Psychosocial stressors are frequently not independent of the woman's own propensity to cause and experience stress. These stressors also seldom have discrete onsets, and are often not randomly distributed. One way to disentangle the objective and subjective stress and cognitive components is to use a natural disaster that affects pregnant women in a random fashion. Studies so far that have used a natural disaster as a stressor have compared exposed group to non-exposed and thus were unable to assess a dose-response relationship.

C. Project Ice storm

Project Ice Storm has been following a cohort of children whose mothers were pregnant during the worst natural disaster in Canadian history - the January 1998 Quebec ice storm. The ice storm in the Canadian province of Quebec resulted in power outages ranging from a few hours to more than 6 weeks for more than 3 million individuals during the coldest month of the year. The Insurance Bureau of Canada has listed the ice storm as the most costly natural disaster in Canadian history. The 224 women in our initial sample spent up to 15 days in temporary shelters, and an average of 15 days (0-42) without electricity in their homes during the crisis, when daily low temperatures dropped to between -10 and - 20ºC. Project Ice Storm families have completed the PNMS assessment in June 1998 and since then have participated in up to more than a dozen waves of assessments to date.

Questionnaires have been completed almost annually, and face-to-face assessments of children were done at ages 2, 5 ½, 8 ½, 11 ½, 13 ½, and 15 ½ years. Poject Ice Storm findings so far demonstrate moderate-to-large effects of PNMS from the ice storm on the development of these children, a fact that is especially impressive given that most of them came from affluent families. Here are a few past findings relevant to this study: Children at ages 4, 5 ½, 6 ½, 8 ½ and 9 ½ showed more internalizing and externalizing problems the higher the mothers' objective exposure and subjective distress were (King et al., 2012). At age 6, sub-clinical autistic symptoms correlated significantly with both the degree of objective stress exposure and with subjective distress (Walder et al., 2014). Combined, these two aspects of PNMS explained about 23% of the variance in subclinical autistic symptoms

rated by mothers (Walder et al., 2014) (King et al 2009a).

Children at ages 2, 5 ½ and 8 ½ showed lower IQ and language scores the higher the mothers' objective stress exposure was (Laplante et al., 2004, Laplante et al., 2008). At age 11 ½, the Project Ice Storm team found that higher objective stress was associated with lower IQ in boys, and we saw a non-significant trend towards better IQ in girls (non published Project Ice Storm results). Lastly, fingerprint development was studied. Fingerprints develop during weeks 14-22 of gestation, interestingly a developmental period that overlaps partly with that of the HC. Thus, minor physical anomalies may be potential indicators of disruptions in brain development (Valen, 1962). It was found that PNMS during the period of fingerprint development predicted greater dermatoglyphic asymmetry in their children, especially in the face of greater maternal subjective distress (King et al., 2009). The results of these fingerprint analyses highlighted the need to look directly at brain development in the ice storm cohort.

The ice storm provides a unique opportunity to determine whether a direct relationship exists between PNMS and HC, AG volume, and to determine to what extent variance in HC or AG volume is explained more by cognitive appraisal, objective and/or subjective PNMS. The varying intensity in which women were affected during the natural disaster allows me to look at a dose-response relationship between the level of prenatal stress and HC, AG volumes.

D. Objectives and hypothesis

My objectives are

(1) To determine the extent to which cognitive appraisal, objective and/or subjective PNMS affect HC and AG volume in 11 ½ years old children from the ice storm cohort.
Hypothesis: Higher prenatal maternal objective and / or subjective stress as well as negative cognitive appraisal will predict smaller HC size and a larger or smaller AGV in 11 ½ year-old adolescents, when controlling for birth weight, timing of the storm in utero and sex.

(2-a) To determine the extent to which cognitive appraisal, objective and/or subjective PNMS affect HC subfield volumes in 11 ½ years old children from the ice storm cohort.

Exploratory research question 2-a: To what extent does cognitive appraisal, objective and/or subjective PNMS affect the HC subfield volumes in 11 ½ years old children from the ice storm cohort?

(2-b) To determine the extent to which the effects of PNMS on the right versus left HC and AG differ.

Exploratory research question 2-b: To what extent does PNMS affects the right versus left HC and AG?

(3) To determine the extent to which timing of the ice storm in utero moderates the effects of PNMS on HC and AG volumes.

Hypothesis: The effects of PNMS on HC and AG volumes will be stronger when stress occurs during early gestation.

(4-a) To determine the extent to which the effects of PNMS on cognitive outcomes at age 11½ are mediated by changes in HC and AG volumes in 11½ year-old children exposed to a natural disaster during gestation.

Hypothesis 4-a: Lastly it is hypothesized that smaller HC mediates negative effects of PNMS on IQ and memory in the children at 11½ years of age.

(4-b) To determine the extent to which the effects of PNMS on behavioural outcomes at age 11½ are mediated by changes in AG volumes in 11½ year-old children exposed to a natural disaster during gestation.

Hypothesis 4-b: It is hypothesized that a change in AGV (smaller or larger) mediates the negative effects of PNMS on internalizing and externalizing problems in the children at 11½ years of age.

V. Methods

A. Participants

In order to identify women who were pregnant during the ice storm, obstetricians affiliated with four hospitals in the Monteregie, a region southeast of Montreal that was most affected by the ice storm, were contacted soon after the crisis. The physicians identified 1,440 women who met the inclusion criteria: being pregnant during the ice storm or became pregnant within 3 months of the ice storm, white French Canadians, and 18 years old or older. Their clinics then forwarded the first questionnaire "reaction to the storm" to these patients on June 1, 1998. A total of 224 women responded to the

questionnaire, of whom 178 gave consent to be contacted for further follow-up. The women were highly educated and had a higher socioeconomic status than the regional average.

The present study included 68 of these women whose children underwent a cognitive and behavioural assessment at the ages of 11 ½ , and were scanned with MRI at 11 ½ years of

age. Of the 68 children, 34 were male and 34 were female. 31% (21/68) of the participants were exposed to the storm during preconception, 22% (15/68) during the first trimester, 23% (16/68) during the second trimester and another 22% during the third trimester.

B. Instruments

1. Assessment of Objective PNMS, Subjective PNMS and Cognitive Appraisal

Objective prenatal maternal stress (PNMS) was assessed using a questionnaire developed by Suzanne King and mailed on June 1, 1998 using the mother's responses to questions associated with four categories of exposure that were used in other disaster studies: threat, loss, scope and change (Bromet and Dew, 1995). Because each natural disaster presents unique experiences to the exposed population, questions pertaining to each of the four categories must be tailor-made (table 1, see appendix). Each dimension was scored on a scale of 0 – 8, ranging from no exposure to high exposure. A total objective stress score, referred to as STORM32, was calculated by summing scores from all four dimensions. Because there was no theoretical basis to believe that any one of the four dimensions of exposure is more predictive than the other dimensions, and based on McFarlane's study of Australian firefighters (McFarlane, 1988), each dimension was weighted equally to obtain the total score of our scale.

Mothers' subjective distress was assessed in the June 1, 1998 questionnaire using the Impact of Event Scale-Revised (IES-R; (Weiss and Marmar, 1997). This scale is one of the most widely-used measures in the disaster literature for the assessment of distress following trauma. This is a 22-item scale, which describes symptoms from three categories relevant to post-traumatic stress disorder: Hyper-arousal, avoidance and intrusive thoughts or images. Scale items were written to reflect the mothers' symptoms relative to the ice storm crisis. Participants respond on a 5-point (0-4) Likert scale, from 'not at all' to

'extremely', the extent to which the behavior describes how they felt over the preceding seven days. I used the Total score in all analyses.

Mother's cognitive appraisal of the ice storm crisis was assessed in the June 1, 1998 questionnaire. To assess the mothers' cognitive appraisal about the ice storm, the following item was included in the STORM32 questionnaire: "Overall, what were the consequences of the ice storm on you and your family?"; response options were on five-point scale of "Very negative" (1), "negative" (2), "neutral" (3), "Positive" (4), and "Very positive" (5). This item was recoded into "negative" (0) or "neutral/positive" (1).

2. Maternal measures

The Life Experience Survey (LES) (Sarason et al., 1978) was used to control for whether the mothers experienced any other major life events during their pregnancies and was included in the second postal questionnaire sent 6 months after each women's due date. The LES lists 57 life changes, such as death of a spouse or a promotion at work. Respondents indicate, first, whether the event occurred or not, and then rate the impact of the event (if it occurred) on a 7-point Likert scale ranging from "Extremely Negative" to "Extremely Positive". Women indicated events that occurred between 6 months before the baby's conception to the birth of their child. Respondents were asked to indicate the month and year of events.

Mothers' current psychological well-being was assessed using the General Health questionnaire (GHQ-28) (Goldberg, 1972). The GHQ-28 is one of the most frequently used scales in epidemiological and disaster studies. Each of its 28 items describes a psychological or somatic symptom, and subjects indicate on a 4-point Likert scale how much they have experienced it in the preceding 2 weeks. There are subscales for Anxiety, Depression, Somatic Complaints, and Dysfunction. In the present study, each item was rescored as either 0 (a rating of 0 or 1) or 1 (a rating of 2 or 3), according to the Goldberg method (Goldberg, 1972), resulting in a minimum possible score of 0 and a maximum possible score of 28.

3. Child measures

Child internalizing and externalizing problems were assessed using the Child Behaviour Checklist (CBCL,(Achenbach et al., 1983)) at 11 ½ years of age. The checklist was

completed by the mother, and it aims to detect emotional and behavioural problems in the adolescent. The CBCL consists of 113 questions, scored on a three point Likert scale (0=absent, 1=occurs sometimes, 2=occurs often). The time frame for item responses is the past six months. The CBCL provides eight subscales from which internalizing (anxious/depressed, withdrawn/depressed, and somatic complaints) and externalizing (rule-breaking behaviour and aggressive behaviour) problem scales can be obtained.

The children's memory performance was measured using four subtests of the francophone version of the Children's Memory Scale (CMS,(Cohen, 1997)) at the 11 ½ year face to face assessment. The CMS provides information concerning the children's immediate and delayed visual and verbal memory abilities. To evaluate visual memory abilities, the Dot Location subtest, which evaluates the capacity to memorize the spatial location of dots on a grid after 3 learning trials, and the Faces subtest, which evaluates the capacity to remember and to recognize a series of faces, are combined. To evaluate verbal memory abilities, the Stories subtest, which evaluates the capacity to remember meaningful verbal information, and the Word Pairs subtest, which evaluates the capacity to learn a list of paired words following 3 learning trials are combined.

At 11 ½, children's IQ was measured with the Wechsler Intelligence Scale for Children (WISC-IV,(Wechsler, 2003)). The WISC-IV IQ was derived from three subscales: Vocabulary, information and block design. The three subscales were shown to have high reliability (0.939) and high validity (0.903) compared to the complete WISC-IV (Sattler and Dumont, 2004).

Puberty was assessed using the Puberty Development Scale (PDS) 132 which includes 5 questions for boys or girls to assess (1) growth in height; (2) armpit and leg hair; (3) skin changes like pimples; and 2 items for boys (voice deepening and facial hair) and 2 items for girls (breast growth and menstruation) (Petersen et al., 1988).

In neuroimaging research there is little agreement as to weather raw volumes or normalized volumes to total intracranial volume (TIC) should be used for volumetric brain imaging studies (Voevodskaya et al., 2014). Therefore, in this study, analyses will be done and presented using three outcome markers: local volume (HC, AG), TIC and normalized volumes (HC/TIC, AG/TIC). It is the various combinations of the three markers that will allow I to draw a conclusion regarding the effect size and specificity. As shown in table 2, I can conclude that as long as the local/TIC ratio is significant, I can assume that the effect

seen is due to change in local HC or AG volume. However, this effect will be more strongly related to local volume change if both the local/TIC and TIC are significant. This effect will be somewhat less strongly associated with local volume change if both HC/TIC and HC are significant.

4. Timing of in utero exposure

Timing of in utero ice storm exposure was determined as the number of days between estimated date of conception and January 9th, 1998, the date at which the storm peaked; higher storm exposure days indicate storm exposure later in pregnancy. To calculate estimated date of conception, 280 days (40 weeks) was subtracted from the women's due date, which was first calculated using baby's gestational age and date at delivery.

5. Control variables

Maternal characteristics: Demographic information, including socioeconomic status, was obtained in the June 1 1998 questionnaire. The socioeconomic status (SES) of the participants was measured in June 1998 by the Hollingshead (1973) scale for estimating SES by combining information about maternal and paternal education levels, and maternal and paternal occupations. Notice that the lower the score, the higher the SES.

Smoking and alcohol habits in pregnancy were assessed at the 6-month after due date questionnaire in order to capture the entire pregnancy and were assessed by asking about the number of cigarettes smoked per day, and the number of drinks consumed per week. These three variables, as well as the mother's current psychological well-being at 11½ years postpartum (GHQ-28) were used as covariates if associated with brain, behavioural and/or cognitive outcome, otherwise they will not be included in the analysis.

Child characteristics: I controlled for child handedness as it was shown to influence brain structure (Good et al., 2001). Handedness was determined by indicating which hand the child used to complete the visual-motor integration task. The outcome for child handedness could be right handed or left handed. I also controlled for timing of exposure to the storm in utero regardless of their association with the brain, behavioural and cognitive outcomes.

C. MRI image acquisition

Anatomical magnetic resonance imaging (MRI) was perfomed at the Unité de Neuroimagerie Fonctionelle (UNF) du Centre de Recherche de l'Institut Universitaire de Gériatrie de Montréal (CRIUGM). Brain imaging was done with a 3.0T Siemens MAGNETOM Trio TIM Syngo (Siemens, Erlangen, Germany), with a 12-channel head coil. For each participant, we obtained a 3D, T1-weighted Magnetization Prepared Rapid Gradient Echo [MPRAGE] sequence (TR/TE/TI = 2300/2.98/900 ms) (voxel size = 1 x 1 x 1 mm^3; sagittal acquisition; 176 slices; 256 x 256 mm grid). Scans were obtained from 65 (33 male and 32 female participants), however we had only 60 usable right hippocampal volumes (RHCV) scans and 59 usable left hippocampal volumes (LHCV) scans due to too much artefact caused by movement of the children in the scanner**.** The raw images have undergone automated correction for intensity non-uniformity and normalization for signal intensity (Talairach and Tournoux, 1988). Analyses were carried out on the local HC and AG volumes, TIC volume, as well as on the normalized HC (HC/TIC) and AG (AG/TIC) volumes.

D. MRI image analysis

1. HC, AG and TIC segmentation

I automatically segmented the HC and AG using the MAGeT pipeline (Pipitone et al., 2014). For the HC subfields, the cornu ammonis (CA) 1, CA2/CA3, CA4/dentate gyrus, stratum radiatum/stratum moleculare, and subiculum were all labeled as separate structures and subfield volumes were acquired directly from the MAGeT output. After that, all labels were merged to create one label for the whole HC and the whole AG in each hemisphere, and hippocampal and amygdala volumes were then manually corrected using a validated manual segmentation protocol (Pruessner et al., 2000). Total Intracranial volume (TIC) was obtained using the Brain Extraction based on nonlocal Segmentation Techniques (BEaST) method, which is based on nonlocal segmentation in a multi-resolution framework (Eskildsen et al., 2012). After the BEaST masks were automatically created, the labels underwent quality control and manual corrections.

E. Statistical analysis

First, descriptive analyses (mean, range, standard deviation) were conducted on outcome, predictor and control variables. Pearson zero-order correlations, as well as

Spearman's rho non-parametric testing between predictor and outcome variables were conducted to determine which covariates were to be included in a specific model.
To test hypothesis 1 which claimed that higher prenatal maternal objective and / or subjective stress as well as negative cognitive appraisal will predict smaller HC size and a larger or smaller AGV in 11 ½ year-old adolescents, when controlling for birth weight, timing of the storm in utero and sex, Person's and partial correlation coefficients were computed between PNMS and brain volume variables including local right and left HCV and AGV, TIC, and normalized right and left HCV and AGV. For the partial correlations, all covariates that significantly correlated with a given brain variable were added as control variables in that specific model. This method of selectively adding covariates to the models allowed for the conservation of power considering the limitations imposed by our sample size.

To test exploratory research question 2-a which asked to what extent does cognitive appraisal, objective and/or subjective PNMS affect the HC subfield volumes in 11 ½ years old children from the ice storm cohort, Person's and partial correlation coefficients were computed between PNMS and brain volume variables including local right and left HC subfields, TIC and normalized right and left HC subfields. The covariates were selectively added in the same manner as done in hypothesis 1.

For exploratory research question 2-b which asked to what extent does PNMS affects the right versus left HC and AG I wanted to compare the Pearson's and partial correlation coefficients obtained in hypothesis 1 between PNMS and normalized right and left HC. The two correlations being compared are not independent because they share a PNMS variable and were computed with the same sample. Thus, they were compared using the Steiger's Z-test for "correlated correlations". The same analysis was done on the normalized AG to compare the effects of PNMS on right versus left AG.

For the third hypothesis which claimed that the effects of PNMS on HC and AG volumes will be stronger when stress occurs during early gestation, to assess the moderating effects of timing of exposure on the relationship between PNMS and brain volume variables including right and left HCV and AGV, TIC and normalized right and left HCV and AGV, multiple regression analyses were carried out, with either Objective stress x Timing of exposure, Subjective stress x Timing of exposure or Cognitive appraisal x Timing of exposure, as the interaction terms, entered in separate analyses. Again, the covariates

that significantly correlated with a given brain variable were added as control variables in its regression model.

Hypothesis 4-a which claimed that that smaller HC mediates negative effects of PNMS on IQ and memory, and hypothesis 4-b which claimed that a change in AGV (smaller or larger) mediates the negative effects of PNMS on internalizing and externalizing problems in the children at 11½ years of age were tested using simple mediation analyses. Because the indirect effect is more likely to be significant if the 2 paths forming it are significant, we carried out partial correlation analysis between brain (local right and left HCV and AGV, TIC and normalized right and left HCV and AGV) and behavioural/cognitive outcomes. The mediation was only tested when both paths of the indirect effect (PNMS to brain, and brain to behaviour/cognition) were significant.

For hypotheses 3 and 4, the SPSS PROCESS macro (Hayes, 2013) was used. All analyses were conducted using SPSS 20.0.

VI. Results

I removed 5 participants born preterm (before 37 weeks) with a low birth weight as it has been shown to affect the brain (Buss et al., 2007). As mentioned in the method section, the two variables, timing of exposure in utero and handedness, were entered in all statistical models regardless of their significant associations with the various dependent variables.

Descriptive statistics for outcome, predictor, and control variables are presented for boys and girls separately in Table 3.

Hypothesis 1: Higher prenatal maternal objective and / or subjective stress as well as negative cognitive appraisal will predict smaller HC size and a larger or smaller AGV in 11 ½ year old adolescents, when controlling for birth weight, timing of the storm in utero and sex.

All the results for hypothesis 1 are presented in Table 4. In boys, Pearson's correlation coefficients showed that more objective stress was associated with significantly smaller TIC volumes, and a similar trend was seen with subjective stress. Cognitive appraisal was not associated with TIC volume. We then looked at the Pearson's correlation and Spearman's rho coefficients between potential covariates and TIC volumes and found no significant associations. To answer hypothesis 1, we looked at the partial correlation

coefficients between PNMS and TIC controlling only for timing of in utero exposure and handedness since no other covariates were associated with TIC. We found that higher objective and subjective stress levels predicted smaller TIC volumes, and we can notice that this association was stronger, and significant for both objective and subjective stress, after adding timing and handedness as covariates into the model.

The same analyses were done with normalized right and left HCV. Pearson correlation coefficients showed no association between PNMS and normalized right (RHC/TIC) and left HCV (LHC/TIC). Higher maternal smoking during pregnancy was associated with smaller RHC/TIC and LHV/TIC but no other covariates were associated with either measure. To answer hypothesis 1 we added timing, handedness and smoking as covariates and found that more subjective stress was significantly associated with larger RHC/TIC. Objective stress and cognitive appraisal were not associated with RHC/TIC. PNMS did not predict changes in LHC/TIC.

Lastly to increase our understanding of the effect size and specificity seen in the HC we looked at the association between predictor variables and right and left HCV, without adjusting for TIC, and found no significant Pearson's correlation and Spearman's rho coefficients. When adding timing and handedness as covariates, PNMS still did not predict changes in either RHC or LHC.

Thus, overall, in boys, more severe objective exposure to the ice storm predicted smaller overall intracranial volumes, but with no particular effect on HC. Also, more subjective stress predicted smaller TIC volume and larger RHC/TIC, with no association with local RHC, so we can conclude that the effect seen was very much specific to the RHC (Table2); in other words, more severe maternal subjective distress after the ice storm predicted smaller overall intracranial volumes, but not smaller HC per se, creating the situation in which the greater the subjective PNMS the greater RHC as a proportion of total brain.

When looking at the association between PNMS and RAG/TIC and LAG/TIC in boys, Pearson's correlation coefficients were not significant. Spearman's rho coefficients showed that higher SES was associated with larger RAG/TIC and LAG/TIC, and maternal psychological functioning was associated with larger LAG/TIC. Thus, to answer hypothesis 1 we looked at the association between PNMS and RAG/TIC controlling for timing, handedness and SES and found no significant association between PNMS and RAG/TIC. For

LAG/TIC we added timing, handedness, SES and maternal psychological functioning into the model and again found no association between PNMS and LAG/TIC. LAGV.

Lastly, when looking at the association between predictor variables and right and left AGV we found no significant Pearson's correlation and Spearman's rho coefficients. When adding timing and handedness as covariates, PNMS still did not predict AGV. Thus, in boys, there was a global effect of PNMS on TIC but no local effect of PNMS on AGV.

In girls, there was no significant Pearson's correlation or Spearman's Rho coefficients between predictor variables and TIC. To answer hypothesis 1, we looked at the partial correlation coefficients between PNMS and TIC controlling only for timing and handedness since no other covariates were associated with TIC. We found no association between PNMS and TIC.

The same analyses were done with RHC/TIC and LHC/TIC. Pearson correlation coefficients showed no significant association between PNMS and RHC/TIC and LHC/TIC, although there was a trend ($p < .10$) for greater objective stress to predict larger RHC/TIC. When looking at the association between potential covariates and HC/TIC we found that earlier age of menarche was associated with larger LHC/TIC. However this variable will not be entered in the model as a covariate as we only have this data for 23 of the 32 participants. To answer hypothesis 1 we only added timing and handedness as covariates and found that more objective stress was significantly associated with larger RHC/TIC and LHC/TIC. Subjective stress and cognitive appraisal were not associated with RHC/TIC.

Lastly, to increase our understanding of the effect size and specificity seen in the HC we looked at the association between predictor variables and right and left HCV and found no significant Pearson's correlation or Spearman's rho coefficients. When adding timing and handedness as covariates, PNMS still did not significantly predict changes in HCV however, there was a trend suggesting that more objective stress predicted larger RHC volume ($p < 0.10$).

Thus, in girls, more objective stress predicted larger RHC/TIC and LHC/TIC and non-significantly larger RHC. We can conclude that the effect seen is specific to the HC (Table 2).

When looking at the association between PNMS and RAG/TIC and LAG/TIC in girls, Pearson's correlation coefficients were not significant. However, Pearson's correlation coefficient indicated that exposure to the storm later in pregnancy predicted larger

RAG/TIC. To answer hypothesis 1 we looked at the association between PNMS and RAG/TIC and LAG/TIC controlling only for timing and handedness. We found no significant association between PNMS and RAG/TIC and LAG/TIC. However, we did see a non-significant trend ($p < .10$) for more objective stress to predict larger RAG/TIC and LAG/TIC. Interestingly, adding the covariates into the model has strengthened this association between objective stress and right/left AGV.

Lastly, to increase our understanding of the effect size and specificity seen in the AG we looked at the association between predictor variables and right and left AGV and found no significant Pearson's correlation or Spearman's rho coefficients. When adding timing and handedness as covariates, PNMS still did not predict changes in AGV.

Thus, in girls, we saw a non-significant trend where more objective stress predicted larger RAG/TIC and LAG/TIC and this effect was specific to the AG rather than to the whole brain (Table 2).

Exploratory research question: (2a) To what extent does cognitive appraisal, objective and/or subjective PNMS affect the HC subfield volumes in 11 ½ year old children from the ice storm cohort?

All the results for exploratory research question 2-a are presented in Table 5. In boys, Pearson's correlation coefficients showed that PNMS was not associated with RCA1/TIC and LCA1/TIC. When looking at the association between potential covariates and CA1/TIC we found that greater maternal smoking during pregnancy predicted smaller RCA1/TIC and LCA1/TIC. Additionally, higher SES predicted larger LCA1/TIC and this was seen as a trend for the RCA1/TIC. To answer hypothesis 1, we looked at the partial correlation coefficients between PNMS and RCA1/TIC and LCA1/TIC controlling for timing, handedness, smoking and SES. We found that higher subjective stress levels predicted larger RCA1/TIC. Although this association was not significant for the LCA1/TIC, it was strengthened after adding the covariates into the model ($p = 0.121$). Objective stress and cognitive appraisal were not associated with CA1 volume.

To increase our understanding of the effect size and specificity seen in the CA1 subfields we looked at the association between predictor variables and right and left CA1. Pearson's correlation coefficients were not significant between PNMS and right and left CA1 but greater smoking predicted smaller right and left CA1. When controlling for timing,

handedness and smoking, PNMS still didn't predict changes in right and left CA1 volume.

Thus, in boys we found that greater subjective stress predicted smaller TIC and larger RCA1/TIC so we can conclude that the effect seen is very much specific to the RCA1 subfield with a mild signal in LCA1 (Table 2).

We carried out the same analyses for the CA4DG subfield. We found no significant Pearson's correlation coefficients between PNMS and RCA4DG/TIC or LCA4DG/TIC.

Greater maternal smoking predicted smaller RCA4DG/TIC or LCA4DG/TIC. When controlling for timing, handedness, and smoking, there were no significant association between PNMS and RCA4DG/TIC.

We then looked at the association between PNMS and right and left CA4DG and found no significant Pearson's correlation coefficients. Greater maternal smoking predicted smaller LCA4DG volume and this was observed as a trend for the RCA4DG. When adding timing, handedness and smoking as covariates we found a non-significant trend where more subjective stress was associated with smaller left CA4DG ($p < 0.1$). This effect is not specific to that subfield and is likely due to changes in TIC (Table 2).

Thus in boys, PNMS did not predict changes in CA4DG volume.

Lastly we studied the CA23 subfield. There was no significant Pearson's correlation and Spearman's rho coefficients between predictor variables and RCA23/TIC and LCA23/TIC subfields. When adding timing and handedness as covariates PNMS still did not predict changes in CA23/TIC. Similarly no significant Pearson's correlation, Spearman's rho and partial coefficients were found between predictor variables and right and left CA23 subfield. Thus, there was no effect of PNMS on the local CA23 subfield.

In girls, we carried out the same analyses on CA1, CA4DG and CA23 subfields. Pearson's correlation coefficient showed that more objective stress predicted larger RCA1/TIC and this was a trend in LCA1/TIC. Another trend suggested that negative cognitive appraisal predicted smaller LCA1/TIC. No potential covariates were significantly associated with RCA1/TIC and LCA1/TIC volume. When controlling for timing and handedness, we saw that the significant association between objective stress and RCA1/TIC was strengthened whereas the trend seen in the LCA1/TIC was lost. Additionally, the trend seen between cognitive appraisal and LCA1/TIC was slightly strengthened but remained non-significant.

To increase our understanding of the effect size and specificity seen in the CA1 subfield we looked at the association between predictor variables and right and left CA1. We found no significant Pearson's correlation and Spearman's rho coefficients. When adding timing and handedness as covariates, PNMS, still, was not associated with right and left CA1 volume.

Thus, in girls, higher objective stress predicted larger RCA1 volume and this effect was highly specific to this region. Moreover, at a non-significant level, negative cognitive appraisal seems to predict larger LCA1 volume

When studying the association between PNMS and CA4DG/TIC and CA23/TIC subfields we found that Pearson's correlation, Spearman rho and partial correlation coefficients were not significant. Neither were they significant for CA4DG and CA23 subfields. Thus, PNMS did not predict volumes of CA4DG and CA23 subfields.

Exploratory research question *(2b) To what extent does PNMS affects the right versus left HC and AG?*

Results for exploratory research question 2-b are presented in Table 6.
In boys, when comparing the correlation between stress and normalized right and left HC (RHC/TIC, LHC/TIC) and AG (RAG/TIC, LAG/TIC), controlling for the correlation between right and left HC and AG, we found that subjective stress significantly affected RHC/TIC more than left ($z = 3.031$ $p = 0.002$). Subjective stress was not significantly associated with LHC/TIC ($p = 0.841$) and was significantly associated with larger RHC/TIC ($p = 0.023$). Objective stress and cognitive appraisal in boys did not affect right and left AG/TIC differently.

In girls, perusal of the partial correlations in Table 5 does not reveal any strong pattern of associations between PNMS and HC or AG that would suggest that one side is more sensitive to PNMS than the other. In fact in girls, there was no significant difference of the effect of PNMS on right and left HC/TIC and AG/TIC. Thus, it seems that in boys only, the RHC/TIC is more sensitive to subjective stress then the LHC/TIC.

Hypothesis 3: The effects of PNMS on HC and AG volumes will be stronger when stress occurs during early gestation.

All results for hypothesis 3 are presented in Table 7.

To answer hypothesis 3 we have not tested for moderation by timing on non-normalized HC and AG volumes and TIC unless timing significantly moderated the effect of PNMS on normalized HC and AG volumes. In fact, as shown in Table 2, an effect is not specific to changes in HCV and AGV unless the analysis is significant for normalized HC and AG volumes.

In boys, for the HCV, Pearson correlation coefficients between timing of exposure to the storm and RHC/TIC and LHC/TIC were not significant (Table 4). When looking for a moderating effect of timing, we found no significant interaction of objective stress x timing of exposure to the storm, subjective stress x timing of exposure to the storm nor of cognitive appraisal x timing of exposure to the storm on RHC/TIC and LHC/TIC.

For AG in boys, Pearson correlation coefficients between timing of exposure to the storm and RAG/TIC and LAG/TIC were not significant (Table 4). When looking for a moderating effect of timing we found no significant interaction of objective stress x timing of exposure to the storm nor of cognitive appraisal x timing of exposure to the storm on RAG/TIC and LAG/TIC. However, the subjective stress x timing interaction was significantly related to RAG/TIC ($p = 0.019$) (Figure 1). When subjective stress scores were equal to or greater than a log value of 2.60 (original IES-R scale 12.46), there was a significant ($0.006 < p < 0.05$) effect of timing on RAG/TIC; for these boys, the later they were exposed to the storm in gestation, the larger the normalized RAG. When mothers were exposed to the storm from day 151 (week 22) onwards, there was a significant positive effect of subjective stress on RAG/TIC: for boys exposed to the ice storm after 21 weeks, the greater their mothers' subjective stress, the larger the RAG. When mothers were exposed to the storm before day 151, which includes the mothers exposed to the storm during preconception, there was no significant effect of subjective stress on RAG/TIC. Overall, the model explained 40.0% of the variance in the children's RAG/TIC, including 15% of variance explained by the interaction. To draw more thorough conclusion regarding effect size and specificity we also tested the moderation by timing on RAG and TIC. We found no significant moderation effect on TIC but found that timing significantly moderated the effect of subjective stress on RAG. We can conclude that the moderating effect of timing is associated with the RAG (Table 2).

In girls, Pearson correlation coefficient between timing of exposure to the storm and normalized right and left HCV was not significant (Table 4). When looking for a moderating effect of timing we found no significant interaction of objective stress x timing of exposure to the storm nor of subjective stress x timing of exposure to the storm cognitive appraisal x timing of exposure to the storm on normalized right and left HCV.

When studying the association between timing and AG in girls we found that timing was significantly associated with normalized right AGV (Table 4). However, when looking for a moderating effect of timing we found no significant interaction of objective stress x timing of exposure to the storm nor of subjective stress x timing of exposure to the storm cognitive appraisal x timing of exposure to the storm on normalized right and left AGV.

<u>Hypothesis 4</u>a: It is hypothesized that smaller HC mediates negative effects of PNMS on IQ and memory in the children at 11½ years of age.

The mediation was only tested when both paths of the indirect effect (PNMS to brain, and brain to cognition) were significant or trending ($p < 0.1$).

In boys, we found significant effects of PNMS on most cognitive outcomes (Table 8). When testing hypothesis 4-a, we found that the only significant association between PNMS and HC/TIC was more subjective stress predicting larger RHC/TIC when controlling for timing, handedness and smoking (Table 4). When testing the second path of the indirect effect (Table 9) we found that RHC/TIC was not associated with any cognitive outcomes. Thus, it is not likely to find any indirect effects so none were tested.

In girls, Pearson's correlation coefficient showed that more subjective stress but not objective stress and cognitive appraisal, was associated with lower IQ (Table 8). When testing for potential covariates we found that higher SES predicted better IQ (Table 8). To answer hypothesis 4-a, we found that more objective stress predicted larger RHC/TIC and LHC/TIC when controlling for timing and handedness (Table 4). Thus, we looked for a significant or trending association between RHC/TIC and LHC/TIC and behavioural outcomes to then, finally test for a mediation effect. When testing the second path of the indirect effect we found that both RHC/TIC and LHC/TIC showed a non-significant trend of being associated with IQ when controlling for timing, handedness and SES (Table 9). Thus, we tested for a mediation effect and found that more objective stress predicted larger

RHC/TIC, which in turn predicted better IQ (indirect effect: 0.297 and confidence interval [0.0059; 0.9845]). To further understand the effect size and specificity we looked for a mediation effect with TIC or RHC as mediators. However, PNMS in girls was not associated with TIC or RHC thus we did not test for a mediation effect using those mediators. We can conclude that the mediation effect is very much due to changes specifically in the RHC (Table 2).

In girls, we did not find significant effects of PNMS on memory outcomes (Table 8). To answer hypothesis 4-a we proceeded in the same manner as with the IQ outcomes, and tested the second path of the indirect effect (brain to cognitive outcome). We found that RHC/TIC and LHC/TIC were not associated with any of the memory outcomes in girls (Table 9). Thus, it is not likely to find any indirect effects so none were tested.

Hypothesis 4b: It is hypothesized that a change in AGV (smaller or larger) mediates the negative effects of PNMS on internalizing and externalizing problems in the children at 11½ years of age.

The mediation was only tested when both paths of the indirect effect (PNMS to brain, and brain to behaviour) were significant or trending ($p < 0.1$).

In boys, we saw from the results hypothesis 1 section that PNMS was not associated with RAG/TIC and LAG/TIC controlling for timing, handedness and SES (Table 4). Thus, we will not test for mediation effects but will however present the results of the Pearson correlation coefficients between PNMS and behavioural outcome as well as between potential covariates and behavioural outcome and AGV/TIC and behavioural outcome.

In boys, Pearson correlation coefficients showed that PNMS was not associated with internalizing problems (Table 8). However, Spearman's rho coefficient showed that more maternal drinking during pregnancy and more maternal psychological functioning were associated with more internalizing problems in the child (Table 8). When testing the second path of the indirect effect we found that RAG/TIC and LAG/TIC were not associated with internalizing problems when controlling for timing, handedness, SES, maternal psychological functioning and maternal drinking during pregnancy (Table 10).

Pearson's correlation coefficients showed that PNMS was not associated with externalizing problems (Table 8). However, Spearman's rho coefficient showed that more

maternal drinking during pregnancy was associated with more internalizing problems in the child (Table 8). When testing the second path of the indirect effect we found that RAG/TIC and LAG/TIC were significantly associated with externalizing problems when controlling for timing, handedness, SES, maternal psychological functioning and maternal drinking during pregnancy (Table 10). However, because right and left AG/TIC was not significantly associated with PNMS, we did not test for a mediation effect.

In conclusion, there were no significant mediation models in boys; however, timing moderated the effect of subjective stress on RAG/TIC. Thus, we were led to test a moderated mediation effect in this model. We found that in boys, timing significantly moderated the indirect effect of subjective stress on externalizing problems through RAG/TIC (confidence interval for the Index of moderated mediation: [0.0012; 0.0355]). RAG/TIC significantly mediated the effect of subjective stress on externalizing problems when mothers were exposed to the storm during late gestation.
When exposed to the storm during late gestation, more subjective stress predicted larger RAG/TIC, which in turn predicted more externalizing problems in boys.

In girls, we saw from the results hypothesis 1 section that PNMS was not associated with right and left AG/TIC controlling for timing and handedness (Table 4). However, there was a trend suggesting that more objective stress was associated with larger right and left AG/TIC when controlling for timing and handedness. Thus, we looked for a significant or trending associations between RAGV and behavioural outcomes to then, finally test for a mediation effect.

In girls, Pearson's correlation and Spearman's rho coefficients showed that no PNMS predictor variables were associated with internalizing problems (Table 8). When testing the second paths of the indirect effect we found that right and left AG/TIC were not associated with internalizing problems when controlling for timing and handedness (Table 10), thus, we did not further test for a mediation effect.

Pearson's correlation coefficients showed that more subjective stress, but neither objective stress nor cognitive appraisal, predicted more externalizing problems (Table 8). Pearson's correlation and Spearman's rho coefficient showed that potential covariates were not associated with externalizing problems (Table 8). When testing the second path of the indirect effect we found that larger right and left AG/TIC predicted more externalizing

problems when controlling for timing and handedness (Table 10). Because both pathways of the indirect effect are significant, we tested for a mediation effect. We found that in girls, more objective stress predicted larger LAG/TIC which in turn was associated with more externalizing problems (effect: .286 and confidence interval [0.0182; 0.9922]). These findings were similar, and significant, with the RAG/TIC (effect: .2998 and confidence interval [0.0114; 1.0111]).

The overall conclusion for hypothesis 4 is that in girls more objective stress predicted larger RHC/TIC, which in turn predicted better IQ. Moreover in girls, more objective stress predicted larger right and left AG/TIC, which in turn predicted more externalizing problems. Lastly in boys, when exposed to the storm during late gestation, more subjective stress predicted larger RAG/TIC, which in turn predicted more externalizing problems.

VII. Discussion

Firstly, the aim of the present study was to determine the association between disaster-related PNMS and child HC and AG volumes. Secondly, we aimed to investigate the extent to which PNMS affects HC subfield volumes and to compare the effect of PNMS on right versus left HC and AG. Thirdly we aimed to determine the extent to which timing of the ice storm in utero moderates the effects of PNMS on HC and AG volumes, and lastly we aimed to investigate the extent to which changes in HC and AG volumes mediated the effects of disaster-related PNMS on child behavioural and cognitive outcomes. By using a natural disaster as the source of stress, our method included the ability to distinguish between the degree of objective exposure of the mother to the hardship from the storm, her degree of subjective distress as well as cognitive appraisal, and to test the relative contribution of these three aspects of the PNMS experience. Given the sudden onset of the storm compared to other forms of prenatal "stress" such as anxiety, we were also able to test, and control for, the effects of timing of the stressor in utero. Because boys and girls respond to PNMS differently, we tested our hypotheses in boys and girls separately.

Hippocampus:

Firstly, we hypothesized that higher levels of objective and/or subjective stress and/or negative cognitive appraisal in the mother would predict smaller HCV in the child.

When investigating the effect of PNMS on HCV, we found that in boys, more subjective stress in the mother predicted larger normalized RHCV. In girls, more objective stress predicted larger normalized right and left HCV. Cognitive appraisal did not predict HCV in either boys or girls. When comparing the correlation coefficients of PNMS on left versus right HC, we found that in boys, subjective stress significantly affected right HC more than left. These findings are supported and strengthened by our subsequent analyses looking at the effect of PNMS on HC subfields, where we found that in boys subjective stress predicted larger normalized RCA1 volume, and in girls objective stress predicted larger RCA1 volume. Interestingly, other studies have reported greater sensitivity of the right hemisphere to early androgens exposure (De Lacoste et al., 1991) supporting the idea that testosterone in utero leads to a more rapid growth of the right hemisphere and/or retards the growth of the left hemisphere. This is the first study in humans to report an effect of PNMS on HC subfields, more specifically, CA1. This region has been referred to as "vulnerable sector" or "Sommer sector" (Duvernoy, 2005) as it is most sensitive to insults such as anoxia, and the main site of neuronal loss in aging and Alzheimer's disease (Duvernoy, 2005). With this study, we support CA1 as the "vulnerable sector" and extend its sensitivity window to prenatal insults.

Although our results show consistency with each other, they are contrary to our hypothesis, which was that more stress would cause a decrease in HCV. The existing literature suggests possible underlying mechanisms to explain our findings. During normal HC development, the pre-pubertal phase is characterized by a marked overproduction of axons, dendrites, synapses and receptors associated with an increase in HCV (Rakic, 1991). This is followed by a period of rapid pruning and elimination between puberty and adulthood associated with a decrease in HCV (Andersen and Teicher, 2004). Thus, theoretically the 11-year-old participants in our study are right around that age where the HC switches from intense proliferation (HCV growth) to pruning (HCV decrease). However, the traumatic interference hypothesis (Arnsten and Goldman-Rakic, 1998) suggests that early life stress may affect the pruning phase, thus leading to a larger HCV at this age due to a lack of pruning rather than "growth" per se. The traumatic interference hypothesis proposes that the prefrontal cortex sends inputs to other cortical areas to enhance pruning during normal development. However, it seems that early life stress would inhibit prefrontal cortex inputs to other cortical areas

resulting in a decrease in pruning in those areas, which could at least partially explain the larger structures associated with early life stress. Given that the prefrontal cortex sends many inputs to the HC (Duvernoy, 2005), there might be a similar prefrontal cortex involvement where prenatal stress would inhibit normal pruning in the HC leading to an increase in HCV. In addition to altering pruning mechanisms, early life stress might also affect the initial stage of neurogenesis. A study showed that early maternal separation led to temporally specific changes in HC neurogenesis, such that very young adult rats displayed enhanced HC neurogenesis whereas middle-aged animals displayed reduced HC neurogenesis. This study, in addition to showing a deregulation in neurogenesis, also suggests that early life stress may have a delayed negative effect on the HC. In agreement with this finding, many studies support the idea that early stress induced alteration in human HC size are not apparent until at least early adulthood (Bremner et al., 1997, Driessen et al., 2000, De Bellis et al., 2001, Andersen and Teicher, 2004, Koehl et al., 2009).

Thus, the PNMS induced delay in alteration in HCV, combined with the influence of PNMS on normal neurogenesis and pruning processes, could explain the increase in HCV seen in 11 ½ children exposed to the ice storm in utero.

Next, we investigated the moderating effect of the timing of the stressor in utero on the association between PNMS and brain volume. The lack of an effect of gestational timing was somewhat surprising, given the important differences in neuronal growth in the HC between early and late gestation. The HC is almost fully developed by the end of the second trimester, so we would expect to see stronger effect of PNMS on HCV when exposure to the storm was in early gestation compared to late gestation. Thus, it would suggest that the effect seen in childhood was due to a sustained shift in the developmental trajectory after birth, rather than just mediated by a single insult in utero.

Following from this, we investigated the extent to which the effects of disaster-related PNMS on cognitive outcomes were mediated by changes in HCV. We found that in girls, exposure to more objective stress during pregnancy predicted higher IQ in the child, and this association was mediated, in part, by larger RHC/TIC volume in the child. We found no association between HCV and memory. While it is surprising that more stress would predict better IQ, some studies have suggested that moderate levels of early life stress,

rather than being overall unfavourable, may program the HC such that it is optimally adapted to a stressful context later in life (Avital and Richter-Levin, 2005, Tang et al., 2006, Champagne et al., 2008, Dipietro, 2012, Suri et al., 2013). For example, Suri et al 2013 reported that exposure to early life stress in rats led to improved cognitive performance selectively on stress-associated, HC-dependent learning task at younger ages, whereas in middle-aged animals, early life stress reduced performance on cognitive tasks. Thus, in our study we might be witnessing an initial positive effect of PNMS on IQ scores (cognitive tasks) in a stress-related situation (i.e., IQ testing). Unfortunately, we cannot ascertain the existence of a delayed decline phase in cognitive abilities and HCV as our brain imaging study is not yet longitudinal. This might explain why we are not seeing an association between PNMS, HC and memory. Nonetheless, we have brain and cognitive data in those same children at age 16½ that have yet to be analysed. Thus, future analyses could test for the occurrence of delayed negative effect of PNMS on HC and HC-cognitive related tasks.

Interestingly, more objective and subjective PNMS predicted smaller TIC in boys but not in girls. The existing literature concurs with those findings in that stress has been strongly linked with total brain volume loss (Lupien et al., 2009). Interestingly, a few studies showed that males with PTSD had smaller intracranial and cerebral volumes compared to females with PTSD (De Bellis et al., 1999, De Bellis and Keshavan, 2003). These finding may suggest that males are more vulnerable to the effects of severe stress in global brain structure than females. However, the neurobiological changes explaining why total brain is more affected in males whereas females show more changes in subcortical areas are unknown.

Amygdala:

Our first hypothesis was that higher levels of objective or subjective stress and negative cognitive appraisal would predict a change (either larger or smaller) AGV. We found no significant association between objective stress, subjective stress or cognitive appraisal and AGV in either boys or girls. Nonetheless, in girls there was a trend where more objective stress predicted larger normalized right and left AGV. It is during foetal development that the AG shows the highest level of cell proliferation and differentiation as well as the highest rate of gene expression sustaining gliogenesis compared to any other

period in life (Kang et al 2011 Nature). Thus, exposure to stress during the prenatal period may have stable and permanent consequences by decreasing cell proliferation and neuronal differentiation and increasing gliogenesis, which may account for the larger AGV observed (Salm et al., 2004, Buss et al., 2012).

We found no significant difference in the effect of PNMS on right versus left AG.

Following from this, we aimed to assess the extent to which timing of exposure to the storm in utero moderated the relationship between PNMS and AGV. We found that in boys only, higher subjective stress scores predicted significantly greater normalized RAGV in response to stress when mothers were exposed to the storm from pregnancy week 22 onwards. These findings were surprising given that the AG develops during the first trimester. However, higher subjective stress may have affected the AG at any point during prenatal or postnatal development. Factors such as decrease pruning may be the cause of the larger AGV.

The last step in our analysis was to investigate the extent to which the effects of disaster- related PNMS on behavioural outcomes were mediated by changes in AG volume. The present findings represent, to the best of our knowledge, the first report linking PNMS with subsequent AGV and externalizing problems in childhood.

In boys, because normalized RAGV was strongly associated with externalizing problems, we looked for a moderated mediation effect and found that when exposed to the storm during late gestation, more subjective stress predicted larger normalized RAGV, which in turn predicted more externalizing problems. In girls, exposure to higher objective stress during pregnancy predicted more externalizing problems in the child, and this association was mediated, in part by larger normalized right and left AGV. Although the type of stressor differs between boys and girls, the general effect is the same: More stress causes more externalizing problems, and this is in part explained by greater AGV. Although the existing literature suggests that more externalizing problems are associated with smaller AG in adults (Matthies et al., 2012, Bobes et al., 2013, Gopal et al., 2013), the age at testing may influence the effect seen of PNMS on AGV. In fact, Buss et al's study (2012), reported that higher maternal cortisol levels during pregnancy predicted more internalizing problems in girls at 11, and that this association was mediated, in part, by larger AGV. Nevertheless, Buss et al. did not investigate this relationship using externalizing problems.

Interestingly, as we have seen previously, there is a sizable effect of PNMS on TIC in

boys but not in girls, thus, the effects of PNMS on HCV and AGV reported in this study seem to be stronger in girls. This could be explained by sex-specific programming effect such as sex- specific placental adaptation to stress exposure (Clifton, 2010) or the notion of increased susceptibility of the female brain to its milieu given the more rapid neurodevelopmental trajectory in females compared with males (Buss et al., 2009).

In terms of the influence played by the various elements of stress i,e; objective stress, subjective stress and cognitive appraisal on the brain, we report that objective stress seems to be the strongest predictor of brain, while subjective stress has some mild influence on the brain, and cognitive appraisal was not associated with any brain outcomes. Our findings with objective stress are surprising because they don't fit the conventional model of subjective stress and cortisol where more subjective stress during pregnancy causes an increase in maternal cortisol, which affects foetus development. Unfortunately, no human studies have investigated the effect of objective stress on the brain so it is difficult to discuss our findings on the brain in the light of previous research. Nonetheless, both subjective and objective stress have been associated with cognitive and behavioural outcome in the child (Watson et al., 1999, Wadhwa et al., 2001, Gutteling et al., 2005, Rice et al., 2007, Chuang et al., 2011, Liu, 2011). Moreover, in previous Project Ice Storm studies, objective stress was most strongly correlated with physical, physiological and cognitive measures (Laplante et al., 2004, King et al., 2005, King and Laplante, 2005, Laplante et al., 2007, Laplante et al., 2008, King et al., 2009, King et al., 2012). In particular, it was reported that objective stress was strongly associated with DNA methylation (Cao- Lei et al., 2014), insulin secretion (Dancause et al., 2013) and cytokine production (Veru et al., 2015), suggesting that the mechanisms through which objective stress affects offspring development may bypass the maternal HPA axis and may use other pathways to influence fetal DNA methylation, metabolism and immune function.

Lastly, our present findings linking objective stress with IQ and objective/ subjective stress with externalizing problems are supported by, and similar to, previous Project Ice Storm findings with assessments done at 2, 4 ½, 5 ½, 6 ½, 8 ½ and 9 years of age (Laplante et al., 2004, Laplante et al., 2008, King et al., 2012).

One of the limitations in this study was that data on pubertal stage was not collected during the assessment at 11½ years of age. However, age of menarche was collected in 23

female participants at the 13 ½ year assessment. The mean age of menarche was 12. Results showed that earlier menarche was associated with larger HCV at 11 ½ (Table 4). This may suggest that earlier menarche is associated with a higher rate of neurogenesis during the pre pubertal phase, leading to larger HC size at 11 ½. Having pubertal stage information for the full sample would have greatly improved our understanding of the effect seen, and may have helped remove the confounding effect of pubertal hormone surge on the brain and behaviour. Another limitation in this study was that many factors were looked at relative to our small sample size. Moreover, delivery complications were not explored and we did not have the resources and statistical power to consider the mechanisms by which PNMS might influence brain development. Lastly, many other areas of child development, including attention problems, may also have been affected by the ice storm but they were outside the scope of this master's thesis.

Our study's main strength was the use of a random occurring natural disaster as a stressor rather than psychological state and non-independent life events. This allowed us to draw causal conclusions about the effect of PNMS on child brain outcomes removing the influence of genetic factors. Moreover, we were able to assess the various elements of stress soon after the event occurred and with thoroughly adapted questionnaires thus providing us with a highly reliable measure of the various stress levels. Additional strengths of our present study include the prospective design and the longitudinal behavioural and cognitive assessments done in the children. Moreover, follow up assessments including MRI scans done at 16 are currently underway. Last but not least, segmentation of all child brain MRI was done manually by a highly reliable rater. This may be especially important for the assessment of AG and HC volumes because it has been suggested that automatic segmentation protocols are less reliable for smaller subcortical structures (Luft et al., 1996). Lastly, although outside of the scope of this study, further analyses will investigate the extent to which PNMS affects the prefrontal cortex.

Conclusion: Findings from the present study provide support for the hypothesis that cognitive abilities and susceptibility to behavioural problems may, in part, be programmed in utero, and that this effect may be mediated through changes in the anatomy of the HC and AG. Furthermore, the study shows that exposure to a stressors during gestations exerts a lasting influence on child development. The results of the present study add to the growing

awareness of the importance of the intrauterine environment and reveal a new pathway through which the objective exposure to a stressor may affect the offspring.

VIII. References

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APPENDIX

Table 1 Questions used to assess the four dimensions (Threat, Loss, Scope, and Change) of our Objective Stress Questionnaire that the mothers completed shortly after the ice storm.

Threat		Loss		Scope		Change	
1	Were you injured? No = 0 Yes = 1	1	Did your residence suffer damage as a result of the ice storm? No = 0 Yes = 2	1	How many days were you without electricity? 0 = 0 – 5 days 1 = 6 – 13 days 2 = 14 – 19 days 3 = 20 – 21 days 4 = >22 days	1	Did your family stay together for the duration of the ice storm? Yes = 0 No = 1
2	Was anyone close to you injured? No = 0 Yes = 1	2	Did you experience a loss of personal income? No = 0 Yes = 2	2	How many days were you without the use of your telephone? 0 = 0 days 1 = .01 – 1 day 2 = 2 – 4.5 days 3 = 5 – 7 days 4 = 8+ days	2	Did you spend any time in a temporary shelter? No = 0 Yes = 1
3	Were you ever in danger due to:	3	How much was the total financial loss including income, food, damage to home? 0 = < $100 1 = $100 - $1000 2 = $1000 - $10000 3 = $10000 – 100000 4 = > $100000			3	How often were you required to change residence during the ice storm? 0 = 0 1 = 1 time 2 = 2+ times
3. 1	…the cold No = 0 Yes = 1					4	Did you take in guests during the ice storm? No = 0 Yes = 1
3. 2	…exposure to downed electrical power lines No = 0					5	Did you experience an increase in physical work during the ice

	Yes = 1		storm? 0 = less or same 1 = little or lot more
3. 3	...exposure to carbon monoxide No = 0 Yes = 1		6 Number of nights away from home: 0 = none 1 = 1 – 7.5 nights 2 = 8+ nights
3. 4	...lack of potable water No = 0 Yes = 1		
3. 5	...lack of food No = 0 Yes = 1		
3. 6	…falling branches and ice No = 0 Yes = 1		
8 points	8 points	8 points	8 points

Table 2 Defining effect size and specificity using 3 outcome markers: local volume, global volume and ratio.

Effects	HC AG	HC/TIC AG/TIC	TIC
No effect	–	–	–
Effect present in TIC not in HC or AG	–	–	*
Effect present but diluted by TIC	*	–	–
Effect present, not specific to HC or AG	*	–	*
Small effect	*	*	*
Effect present, Specific to HC or AG	*	*	–
	–	*	*
	–	*	–

Table 3 Descriptive analysis of variables.

Sex Child	Variables	N	Min	Max	Mean	SD
Boys	Objective stress Storm32	31	5.00	24.00	11.839	4.810
	Subjective stress IES-R_log	31	0.00	4.03	1.968	1.189
	Number of days of pregnancy when ice storm happened	31	-62	274	78.45	102.849
	Gestational age at birth (weeks)	31	38.43	43.86	40.226	.976
	Maternal psychological well-being GHQ-28	30	0.00	.64	.106	.155
	Socioeconomic Status (SES) Hollingshead scale	31	11.00	61.00	29.839	12.718
	Number of cigarettes/day	31	0.00	21.50	2.419	5.622
	Number of glasses of alcohol /Week	31	0.00	1.00	.047	.184
	IQ WISC-IV	30	88.00	143.00	111.700	12.089
	Visual immediate memory CMS	30	13	30	22.47	3.471
	Verbal immediate memory CMS	30	12	31	22.87	4.776
	Visual delayed memory CMS	30	14	27	21.73	2.947
	Verbal delayed memory CMS	30	12	29	20.70	4.187
	General memory CMS	30	57	104	87.77	11.261
	Internalizing problems CBCL	30	34	72	54.73	10.106
	Externalizing problems CBCL	30	33	66	48.03	8.732
	Right HC (RHC)	31	2017	3406	2916.29	317.169
	Left HC (LHC)	31	2036	3649	2983.29	337.015
	Right CA1 (RCA1)	31	523.0	962.0	759.774	97.651
	Left CA1 (LCA1)	31	510.0	873.0	720.774	81.791
	Right CA4 and DG (RCA4DG)	31	436.0	739.0	640.355	70.627
	Left CA4 and DG (LCA4DG)	31	448.0	779.0	644.097	78.325
	Right CA2 and CA3 (RCA23)	31	76.0	203.0	158.419	28.985
	Left CA2 and CA3 (LCA23)	31	55.0	183.0	131.581	30.188
	Right AG (RAG)	31	823	1283	993.71	113.135
	Left AG (LAG)	31	820	1256	1021.39	110.287
	RHC/TIC	31	.16	.23	.205	.021
	LHC/TIC	31	.16	.25	.210	.021
	RCA1/TIC	31	.04	.07	.053	.006
	LCA1/TIC	31	.04	.06	.051	.005
	RCA4DG/TIC	31	.03	.05	.045	.004
	LCA4DG/TIC	31	.03	.05	.045	.005
	RCA23/TIC	31	.01	.02	.011	.002
	LCA23/TIC	31	.00	.01	.009	.002
	RAG/TIC	31	.06	.09	.070	.007
	LAG/TIC	31	.06	.09	.072	.007
	Total intracranial volume (TIC)	31	1243630	1603640	1421954.52	97770
Girls	Objective stress STORM32	32	4.00	24.00	11.031	4.816
	Subjective stress IES-R_log	32	0.00	3.43	1.758	1.110
	Number of days of pregnancy when ice storm happened	32	-73	274	94.59	103.788
	Gestational age at birth (weeks)	32	37.57	41.43	39.754	.927

Maternal psychological well-being GHQ-28	31	0.00	.43	.060	.115
Socioeconomic Status (SES) Hollingshead scale	32	11.00	65.00	27.094	12.105
Number of cigarettes/day	32	0.00	25.00	1.750	4.930
Number of glasses of alcohol /Week	32	0.00	2.00	.127	.421
Age of Menarche	23	8.00	14.20	12.011	1.215
IQ WISC-IV	32	90.00	132.00	111.625	12.312
Visual immediate memory CMS	32	15	29	22.13	3.696
Verbal immediate memory CMS	32	10	32	23.41	5.073
Visual delayed memory CMS	32	11	28	21.94	4.150
Verbal delayed memory CMS	32	13	32	22.81	4.540
General memory CMS	32	61	116	90.28	12.855
Internalizing problems CBCL	32	33	80	49.47	11.376
Externalizing problems CBCL	32	34	64	45.81	8.716
Right HC (RHC)	32	2337	3201	2822.03	262.574
Left HC (LHC)	32	2411	3322	2905.28	260.402
Right CA1 (RCA1)	32	563.0	895.0	724.625	74.107
Left CA1 (LCA1)	32	545.0	821.0	687.813	81.400
Right CA4 and DG (RCA4DG)	32	452.0	716.0	631.969	67.021
Left CA4 and DG (LCA4DG)	32	510.0	769.0	645.844	67.184
Right CA2 and CA3 (RCA23)	32	116.0	208.0	160.750	23.027
Left CA2 and CA3 (LCA23)	32	59.0	169.0	130.750	25.088
Right AG (RAG)	32	716	1159	969.97	112.871
Left AG (LAG)	32	769	1136	966.66	95.344
RHC/TIC	32	.18	.25	.210	.021
LHC/TIC	32	.18	.27	.216	.020
RCA1/TIC	32	.04	.07	.054	.005
LCA1/TIC	32	.04	.06	.051	.005
RCA4DG/TIC	32	.04	.06	.047	.005
LCA4DG/TIC	32	.04	.06	.048	.005
RCA23/TIC	32	.01	.02	.012	.002
LCA23/TIC	32	.00	.01	.010	.002
RAG/TIC	32	.05	.08	.072	.008
LAG/TIC	32	.06	.08	.072	.007
TIC	32	1177870	1579900	1348204.38	81096

Note. IES-R: Impact of Event Scale Revised questionnaire; GHQ: General Health Questionnaire; WISC: Wechsler Intelligence Scale for Children; CMS: Child Memory Scale; CBCL: Child Behaviour Checklist; HC: Hippocampus; CA: Cornu Ammonis; DG: Dentate Gyrus; AG: Amygdala; TIC: Total Intracranial Volume

Table 4 Pearson's, Spearman's rho and partial correlation coefficients between predictor and TIC, HC and AG variables (N). Partial correlations coefficients controlling for handedness, timing of exposure to the storm and additional covariates (see footnotes).

Sex Child	Variables	Correlation Coefficient	TIC	RHC/TIC	LHC/TIC	RHC	LHC	RAG/TIC	LAG/TIC	RAG	LAG
Boys	Objective stress	Pearson's (31)	-0.392*	0.052	0.083	-0.199	-0.163	0.055	0.089	-0.175	-0.161
		Partial (30)	-0.486****	0.097	0.081	-0.174	-0.179	-0.084	-0.175[1,2]	-0.256	-0.210
	Subjective stress	Pearson's (31)	-0.320[†]	0.290	-0.032	0.078	-0.206	0.045	0.135	-0.148	-0.076
		Partial (30)	-0.467****	0.435****[3]	0.041	0.122	-0.208	0.175	0.138[1,2]	-0.180	-0.071
	Cognitive appraisal	Pearson's (30)	0.128	-0.002	-0.145	0.093	-0.028	0.144	0.077	0.192	0.153
		Partial (30)	0.182	-0.035	-0.149	0.042	-0.043	0.159	0.029[1,2]	0.232	0.166
	Number of days of pregnancy when ice storm happened	Pearson's (31)	0.050	0.102	0.073	0.112	0.08	0.256	0.203	0.227	0.195
	Gestational age at birth (weeks)	Pearson's (31)	0.001	-0.045	0.112	-0.038	0.094	-0.354[†]	-0.322[†]	-0.312[†]	-0.306[†]
	Handedness	Pearson's (31)	0.074	-0.130	-0.010	-0.088	0.026	0.101	0.038	0.163	0.109
	Number of cigarettes /day	Spearman's rho (31)	0.047	-0.407[*]	-0.384[*]	-0.284	-0.282	-0.204	-0.288	-0.077	-0.258
	Glasses of alcohol / week	Spearman's rho (31)	0.105	-0.267	-0.234	-0.169	-0.163	0.072	0.182	0.168	0.235
	Socioeconomic status	Spearman's rho (31)	0.189	-0.314[†]	-0.328[†]	-0.154	-0.122	-0.408[*]	-0.355[*]	-0.285	-0.269
	Maternal psychological functioning	Spearman's rho (30)	-0.123	-0.175	-0.133	-0.181	-0.210	0.243	0.434[*]	0.147	0.360[†]
Girls	Objective stress	Pearson's (32)	-0.087	0.321[†]	0.239	0.269	0.178	0.087	0.146	0.028	0.083
		Partial (32)	-0.182	0.451****	0.384****	0.345[†]	0.261	0.320[†]	0.360[†]	0.169	0.212

Subjective stress	Pearson's (32)	-0.099	0.005	0.077	-0.045	0.030	0.229	0.266	0.163	0.200
	Partial (32)	-0.109	0.010	0.087	-0.045	0.034	0.269	0.300	0.178	0.215
Cognitive appraisal	Pearson's (32)	0.066	0.035	0.144	0.071	0.177	-0.207	-0.216	-0.154	-0.173
	Partial (32)	0.054	0.065	0.179	0.096	0.203	-0.152	-0.189	-0.102	-0.147
Number of days of pregnancy when ice storm happened	Pearson's (32)	-0.105	0.172	0.181	0.123	0.123	0.441*	0.285	0.342†	0.209
Gestational age at birth (weeks)	Pearson's (32)	0.167	0.039	0.018	0.156	0.129	-0.008	-0.155	0.067	-0.063
Handedness	Pearson's (32)	-0.122	0.076	0.128	0.011	0.054	0.133	0.212	0.059	0.121
Age of Menarche	Pearson's (23)	0.078	-0.412†	-0.431*	-0.373†	-0.391†	-0.281	-0.223	-0.173	-0.138
Number of cigarettes /day	Spearman's rho (31)	0.006	-0.079	0.133	-0.090	0.123	0.176	0.150	0.162	0.147
Glasses of alcohol / week	Spearman's rho (31)	-0.233	-0.099	-0.133	-0.135	-0.281	-0.100	-0.185	-0.134	-0.252
Socioeconomic status	Spearman's rho (31)	-0.337†	0.197	0.343†	0.115	0.230	0.310†	0.209	0.199	0.064
Maternal psychological functioning	Spearman's rho (30)	0.089	-0.079	-0.053	-0.016	-0.056	-0.127	-0.063	-0.065	-0.006

Note. Cognitive appraisal 0: negative. 1: Neutral or positive.
Handedness 1: right handed. 2: left handed.
†p <.1. *p < .05 (2-tailed). **p < .01 (2-tailed).
[1] Socioeconomic status
[2] Maternal psychological functioning
[3] Number of cigarettes per day
[4] Glasses of alcohol per week

Table 5 Pearson's, Spearman's rho and partial correlation coefficients between predictor and HC subfields variable (N). Partial correlations coefficients controlling for handedness, timing of exposure to the storm and additional covariates (see footnotes).

Sex Child	Variables	Correlation Coefficient	RCA1/ TIC	LCA1/T IC	RCA1	LCA1	RCA4DG /TIC	LCA4DG/ TIC	RCA4DG	LCA4DG	RCA23/ TIC	LCA23/TI C	RCA23	LCA23
Boys	Objective stress	Pearson's (31)	0.107	0.110	-0.108	-0.138	0.020	0.065	-0.230	-0.166	-0.013	0.024	-0.161	-0.084
		Partial (30)	0.142	-0.010	-0.138	-0.317	0.036	0.066	-0.233	-0.238	-0.008	-0.007	-0.196	-0.139
	Subjective stress	Pearson's (31)	0.283	0.095	0.104	-0.103	0.195	-0.135	-0.029	-0.291	0.197	-0.178	0.096	-0.240
		Partial (30)	0.435*[3]	0.306	0.154	-0.044	0.316	-0.097	-0.004	-0.343[†3]	0.167	-0.211	0.006	-0.312
	Cognitive appraisal	Pearson's (30)	0.025	0.021	0.098	0.104	-0.116	-0.166	-0.005	-0.048	-0.100	-0.254	-0.048	-0.195
		Partial (30)	0.022	0.001	0.137	0.127	-0.107	-0.126	-0.009	0.039	-0.056	-0.230	0.020	-0.151
	Number of days of pregnancy when ice storm happened	Pearson's (31)	0.128	0.009	0.138	0.031	-0.095	-0.089	-0.065	-0.052	-0.333[†]	-0.098	-0.324[†]	-0.091
	Gestational age at birth (weeks)	Pearson's (31)	-0.001	0.089	0.002	0.089	0.100	0.210	0.084	0.179	0.067	0.231	0.069	0.222
	Handedness	Pearson's (31)	-0.099	-0.069	-0.060	-0.020	-0.075	-0.101	-0.028	-0.048	-0.071	0.078	-0.055	0.101
	Number of cigarettes /day	Spearman's rho (31)	-0.488[**]	-0.544[**]	-0.377[*]	-0.454[*]	-0.423[*]	-0.552[**]	-0.327[†]	-0.454[*]	-0.211	-0.168	-0.181	-0.138
	Glasses of alcohol / week	Spearman's rho (31)	-0.340[†]	-0.121	-0.191	-0.055	-0.065	-0.129	0.048	-0.025	-0.118	0.061	-0.016	0.114
	Socioeconomic status	Spearman's rho (31)	-0.352[†]	-0.492[**]	-0.212	-0.367[*]	-0.239	-0.266	-0.044	-0.144	-0.03	-0.201	0.096	-0.202
	Maternal psychological functioning	Spearman's rho (30)	-0.059	0	-0.088	0.036	-0.177	-0.250	-0.224	-0.222	-0.216	-0.203	-0.213	-0.154
Girls	Objective stress	Pearson's (32)	0.382[*]	0.307[†]	0.290	0.217	0.198	0.266	0.142	0.213	0.163	0.075	0.139	0.052
		Partial (32)	0.441*	0.284	0.295	0.153	0.268	0.284	0.166	0.182	0.200	0.121	0.148	0.077
	Subjective stress	Pearson's (32)	0.116	0.066	0.048	0.009	0.083	0.127	0.045	0.086	-0.124	0.059	-0.165	0.060
		Partial (32)	0.116	0.057	0.044	-0.003	0.088	0.126	0.045	0.081	-0.123	0.060	-0.167	0.060
	Cognitive appraisal	Pearson's (32)	0.198	0.311[†]	0.220	0.310[†]	0.211	0.252	0.245	0.290	0.152	0.027	0.196	0.035

	Partial (32)	0.211	0.325†	0.226	0.320†	0.224	0.254	0.252	0.288	0.157	0.044	0.199	0.052
Number of days of pregnancy when ice storm happened	Pearson's (32)	0.047	-0.011	-0.007	-0.056	0.059	-0.019	0.017	-0.069	0.016	0.094	-0.006	0.082
Gestational age at birth (weeks)	Pearson's (32)	0.201	0.233	0.290	0.303†	0.090	0.061	0.194	0.168	-0.043	-0.200	0.024	-0.166
Handedness	Pearson's (32)	-0.004	-0.144	-0.069	-0.184	0.065	-0.019	0.003	-0.088	0.032	0.022	-0.015	-0.014
Age of Menarche	Pearson's (23)	-0.348	-0.284	-0.236	-0.182	-0.144	-0.107	-0.073	-0.038	-0.071	-0.092	-0.013	-0.057
Number of cigarettes /day	Spearman's rho (31)	-0.081	0.029	-0.067	-0.004	-0.178	0.019	-0.161	-0.004	-0.180	0.083	-0.187	0.085
Glasses of alcohol / week	Spearman's rho (31)	-0.133	-0.135	-0.248	-0.202	-0.190	-0.106	-0.278	-0.244	0.017	-0.085	-0.002	-0.161
Socioeconomic status	Spearman's rho (31)	0.322†	0.332†	0.171	0.161	0.036	0.348†	-0.063	0.187	0.011	0.005	-0.138	-0.106
Maternal psychological functioning	Spearman's rho (30)	0.288	0.186	0.318	0.135	-0.016	0.031	0.057	0.010	0.049	-0.194	0.026	-0.189

Note. Cognitive appraisal 0: negative. 1: Neutral or positive.
Handedness 1: right handed. 2: left handed.
†p <.1. *p < .05 (2-tailed). **p < .01 (2-tailed).

[1] Socioeconomic status
[2] Maternal psychological functioning
[3] Number of cigarettes per day
[4] Glasses of alcohol per week

Table 6 Z-scores from the comparisons of right and left HC and AG correlated correlations.

Sex of child	Type of stressor	RHC/TIC versus LHC/TIC	RAG/TIC versus LAG/TIC
Boys	Partial correlation	0.732	0.762
	Objective stress	0.120	0.729
	Subjective stress	3.031**	0.297
	Cognitive Appraisal	0.857	1.037
Girls	Partial correlation	0.869	0.735
	Objective stress	0.795	-0.323
	Subjective stress	-0.825	-0.825
	Cognitive Appraisal	-1.229	-1.229

†p <.1. *p < .05 (2-tailed). **p < .01 (2-tailed).

Table 7: Moderations of PNMS on brain outcome by timing of exposure to the storm for the final interaction model

SEX	Outcome	Predictor	Predictor Effect	Timing effect	Model R2	Interaction R2 change	Interaction Effect	P_Value	Star
Boys	TIC	Subjective stress	-52984.487	-514.7129	0.1776	0.066	235.0298	0.1606	
	RHC/TIC	Objective stress	0.0004	0	0.1427	0.0001	0	0.9677	
	LHC/TIC	Objective stress	-0.0002	-0.0001	0.162	0.0524	0	0.2227	
	RHC/TIC	Subjective stress	0.01	0.0001	0.2974	0.0221	0	0.3841	
	LHC/TIC	Subjective stress	0.0035	0.0001	0.1224	0.0172	0	0.4909	
	RHC/TIC	Cognitive appraisal	-0.0024	0	0.1036	0.0006	0	0.8994	
	LHC/TIC	Cognitive appraisal	-0.005	0.0001	0.1014	0.0034	0	0.7664	
	RAG/TIC	Objective stress	-0.0003	0	0.2904	0.0658	0	0.1403	
	LAG/TIC	Objective stress	-0.0004	0	0.3129	0.032	0	0.3115	
	RAG/TIC	Subjective stress	-0.0018	0	0.4004	0.1509	0.000027	0.019	*
	LAG/TIC	Subjective stress	-0.0005	0	0.3035	0.078	0	0.1067	
	RAG/TIC	Cognitive appraisal	0.0037	0	0.2612	0.0155	0	0.4847	
	LAG/TIC	Cognitive appraisal	0.0035	0.0001	0.2057	0.05	0	0.2308	
	RAG	Subjective stress	-64.4487	-0.7845	0.3011	0.1819	0.4516	0.0151	*
Girls	RHC/TIC	Objective stress	0.0018	0	0.2426	0.0106	0	0.544	
	LHC/TIC	Objective stress	0.001	0	0.235	0.0448	0	0.2194	
	RHC/TIC	Subjective stress	-0.0013	0	0.0424	0.0064	0	0.6748	
	LHC/TIC	Subjective stress	0.0018	0	0.0575	0.0003	0	0.9326	
	RHC/TIC	Cognitive appraisal	-0.0034	0	0.0598	0.0198	0.0001	0.4574	
	LHC/TIC	Cognitive appraisal	0.0064	0	0.0806	0.0003	0	0.9312	
	RAG/TIC	Objective stress	0.0002	0	0.3409	0.046	0	0.1809	
	LAG/TIC	Objective stress	0.0003	0	0.2861	0.0448	0	0.2042	
	RAG/TIC	Subjective stress	0.0009	0	0.2842	0.0129	0	0.4909	
	LAG/TIC	Subjective stress	0.0025	0	0.2273	0.0203	0	0.4066	
	RAG/TIC	Cognitive appraisal	-0.0001	0	0.2477	0.0153	0	0.4648	
	LAG/TIC	Cognitive appraisal	-0.0001	0	0.1879	0.0283	0	0.3406	

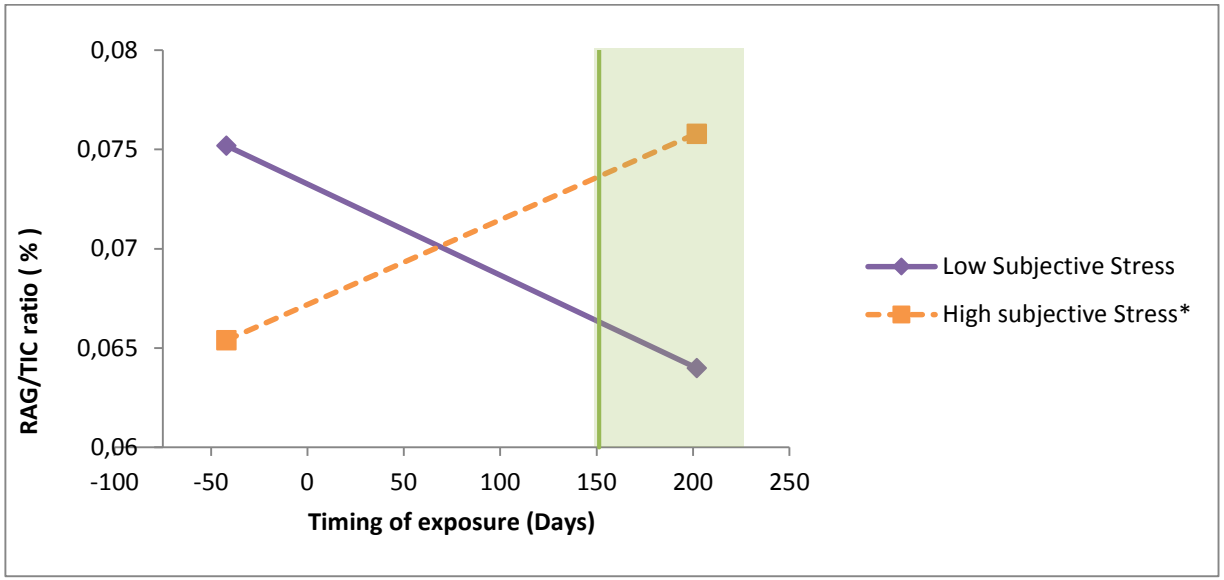

Figure 1: Moderation of subjective stress' effect on RAG/TIC by timing of exposure in boys.
Note. *p < 0.05

Table 8 Pearson correlation and Spearman's rho coefficients between predictor and behaviour and cognitive outcome variables (N)

Sex Child	Variables	Correlation Coefficient	IQ	Visual immediate memory	Visual delayed memory	Verbal immediate memory	Verbal delayed memory	General memory	Internalizing problems	Externalizing problems
Boys	Objective stress	Pearson's (30)	-0.367*	-0.270	-0.307†	-0.008	-0.204	-0.243	0.053	0.133
	Subjective stress	Pearson's (30)	-0.528**	-0.227	-0.387*	-0.366*	-0.392*	-0.472**	0.126	0.124
	Cognitive appraisal	Pearson's (30)	0.218	0.159	0.082	-0.010	0.060	0.092	0.072	0.223
	Number of days of pregnancy when ice storm happened	Pearson's (30)	0.057	0.208	0.222	-0.051	-0.172	0.037	0.079	-0.097
	Gestational age at birth (weeks)	Pearson's (30)	-0.002	-0.216	-0.146	-0.311	-0.307†	-0.351†	0.153	-0.154
	Handedness	Pearson's (30)	-0.173	-0.350†	-0.437*	-0.362*	-0.240	-0.465**	-0.074	-0.063
	Number of cigarettes /day	Spearman's rho (31)	-0.177	-0.324†	-0.319†	-0.425*	-0.236	-0.392*	-0.073	-0.274
	Glasses of alcohol / week	Spearman's rho (31)	0.178	0.274	0.465**	-0.085	-0.213	0.064	0.397*	0.391*
	Socioeconomic status	Spearman's rho (31)	-0.283	-0.503**	-0.337†	-0.339†	-0.012	-0.384*	-0.261	-0.129
	Maternal psychological functioning	Spearman's rho (30)	-0.073	0.204	0.122	-0.124	-0.311†	-0.036	0.529**	0.390*
Girls	Objective stress	Pearson's (32)	-0.047	-0.109	0.108	0.297†	0.318†	0.233	-0.039	0.070
	Subjective stress	Pearson's (32)	-0.448*	0.120	0.250	-0.198	-0.309†	-0.072	0.291	0.396*
	Cognitive appraisal	Pearson's (32)	0.111	-0.024	0.096	0.118	0.093	0.104	-0.051	-0.174
	Number of days of pregnancy when ice storm happened	Pearson's (32)	-0.332†	-0.209	0.058	-0.646**	-0.445*	-0.453**	-0.048	-0.045
	Gestational age at birth (weeks)	Pearson's (32)	-0.138	-0.331†	-0.301†	-0.141	-0.155	-0.302†	-0.260	-0.215

Handedness	Pearson's (32)	0.134	0.018	-0.153	-0.198	-0.322†	-0.236	-0.128	0.145
Age of Menarche	Spearman's rho (23)	0.245	0.283	0.353	-0.138	-0.155	0.100	0.048	-0.254
Number of cigarettes /day	Spearman's rho (32)	-0.25	0.386*	0.136	0.270	-0.027	0.299	-0.036	0.210
Glasses of alcohol / week	Spearman's rho (32)	0.073	0.408*	0.068	0.256	0.228	0.383*	-0.120	-0.009
Socioeconomic status	Spearman's rho (32)	-0.554**	0.057	0.237	-0.001	-0.046	0.044	0.107	0.150
Maternal psychological functioning	Spearman's rho (31)	0.100	0.049	0.307†	0.187	0.172	0.331†	0.189	0.215

Note. Cognitive appraisal 0: negative. 1: Neutral or positive.
Handedness 1: right handed. 2: left handed.
†p <.1. *p < .05 (2-tailed). **p < .01 (2-tailed).

Table 9 Pearson correlation coefficients between dependent variable (HC) and cognitive variables controlling for handedness, timing of exposure to the storm and additional covariates (see footnotes).

Sex Child	Variables	IQ	Visual immediate memory	Verbal immediate memory	Visual delayed memory	Verbal delayed memory	General memory
Boys	RHC	0.208	-0.155[1]	0.096[3]	-0.082[4]	0.248	0.015[1, 3]
	LHC	0.307	-0.136[1]	0.100[3]	0.179[4]	0.237	0.113[1, 3]
	RHC/TIC	-0.073[3]	-0.194[1, 3]	0.096[3]	-0.149[3, 4]	0.072[3]	-0.092[1, 3]
	LHC/TIC	0.048[3]	-0.189[1, 3]	0.096[3]	0.187[3, 4]	0.074[3]	0.017[1, 3]
	TIC	0.414*	0.008 [1]	0.054[3]	-0.038[4]	0.215	0.195[1, 3]
Girls	RHC	0.404*[1]	0.021[3, 4]	0.123	0.162	0.124	0.119[4]
	LHC	0.354†[1]	-0.050[3, 4]	0.111	0.105	0.057	0.072[4]
	RHC/TIC	0.364†[1]	-0.061[3, 4]	0.261	0.202	0.246	0.198[4]
	LHC/TIC	0.324†[1]	-0.137[3, 4]	0.257	0.153	0.188	0.155[4]
	TIC	0.103[1]	0.124[3, 4]	-0.208	-0.087	-0.189	-0.124[4]

Note. †p <.1. *p < .05 (2-tailed). **p < .01 (2-tailed).
[1] Socioeconomic status
[2] Maternal psychological functioning
[3] Number of cigarettes per day
[4] Glasses of alcohol per week

Table 10 Pearson correlation coefficients between dependent variable (AG) and behavioural problems variables controlling for handedness, timing of exposure to the storm and additional covariates (see footnotes).

Sex Child	Variables	Internalizing problems	Externalizing problems
Boys	RAG	0.082[2, 4]	0.243[2, 4]
	LAG	0.089[2, 4]	0.249[2, 4]
	RAG/TIC	0.096[1, 2, 4]	0.450*[1, 2, 4]
	LAG/TIC	0.080[1, 2, 4]	0.407*[1, 2, 4]
	TIC	-0.095	-0.228
Girls	RAG	0.268	0.472**
	LAG	0.258	0.451*
	RAG/TIC	0.177	0.459*
	LAG/TIC	0.144	0.390*
	TIC	0.201	0.141

Note. †p <.1. *p < .05 (2-tailed). **p < .01 (2-tailed).
[1] Socioeconomic status
[2] Maternal psychological functioning
[3] Number of cigarettes per day
[4] Glasses of alcohol per week