Emerging Executive Functions: an investigation of latent structure in toddlerhood and prediction from prenatal stress exposure, sex and early maternal caregiving behaviours.

Thesis submitted in accordance with the requirements of the University of Liverpool for the degree of Doctor of Philosophy by Helen Chadwick.

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List of Abbreviations.

ABN:	Arched Back Nursing
BSID:	Bayley Scales of Infant Development
CFA:	Confirmatory Factor Analysis
EF:	Executive Function
IC:	Inhibitory Control (factor score)
LG:	Licking and Grooming
LHC:	Life History Calendar
MDI:	Mental Development Index (of the BSID)
PCA:	Principal component analysis
PDI:	Psychomotor Development Index (of the BSID)
PFC:	Prefrontal Cortex
PNS:	Prenatal Stress
SLE:	Stressful Life Event
STAI:	Spielberger State Trait-Anxiety Inventory (state anxiety subscale)
TS:	Tactile Stimulation
VC:	Verbal Comprehension
WCHADS:	Wirral Child Health and Development Study
WM:	Working Memory (factor score)

Abstract.

The study of Executive function (EF) in early child development is not fully established, with much still to be confirmed regarding its latent structure and early precursors. Proposed mechanisms underlying reported inverse associations between prenatal stress (PNS) and general cognitive development suggest that brain regions associated with early EF may be specifically vulnerable, yet this is a largely unstudied area. Postnatal caregiving may modify PNS effects on EF providing a possible early intervention target to improve developmental outcomes. This thesis comprises three linked studies. Study 1 examined latent EF structure in toddlers. Study 2 investigated associations between different forms of PNS in each trimester of pregnancy and toddler EF, and tested whether they were sex-dependent. Study 3 tested the moderating influence of early maternal caregiving on associations observed in study 2. **Methods:** This research was embedded within the prospective longitudinal Wirral Child Health and Development Study. **Study 1:** 254 toddlers (mean age 31.4m) completed a battery of EF tasks. **Study 2:** Mothers reported anxiety symptoms (STAI) at 20 weeks' and 32 weeks' gestation, and number of stressful life events (SLEs) via an investigator-led interview for each pregnancy trimester and at multiple postnatal timepoints. Toddler latent EF abilities derived in study 1 were the cognitive outcome. **Study 3:** Mothers reported frequency of stroking their baby at 4 and 9-12 weeks old, yielding a tactile stimulation index of early caregiving. At 6 months, maternal sensitivity was observed and rated during free play. **Results: Study 1:** CFA applied to EF data yielded 2 distinguishable yet moderately correlated (r = .43) factors:- working memory (WM) and inhibitory control (IC). **Study 2:** Multiple linear regression models revealed that 32-week STAI scores in interaction with sex predicted toddler WM (β =0 .55; $p < .05$), accounting for 2% of the variance in scores after accounting for pre and postnatal confounders; and 1st trimester SLEs in interaction with sex predicted IC (β = 0.45; p = .05), accounting for 2% in outcome. Both interactions arose from a similar pattern of opposite associations between PNS and EF in males and females. **Study 3:** PNS effects were not moderated by maternal tactile stimulation. The prediction of toddler IC by the 1st trimester SLE by sex interaction was moderated by maternal sensitivity. This 3-way interaction accounted for 3% of variance after accounting for confounders. **Conclusions:** EF exhibits an integrative latent structure in toddlerhood; PNS was associated with poorer EF in males and relatively enhanced EF in females; exposure to higher maternal sensitivity in the first months of life eliminated the observed sex-dependent PNS effects on toddler IC but not WM. Findings are discussed in the context of Developmental Origins of Health and Disease model and evolutionary perspectives on sex-dependent development.

Declaration

No portion of the work referred to in the thesis has been submitted in support of an application for another degree or qualification of this or any other university or other institute of learning.

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The current thesis employs much data from multiple measures, the amalgamation of which simply would not have been possible without the help of the conscientious and industrious WCHADS research team, past and present. Thanks go to the 'posh coffee' crew; Louise Fisher, Miriam Refberg, Stuart Kehl, and Matthew Bluett-Duncan for their frenzied coding and entry of Life History Calendar data and to Nicky Wright for her co-ordination of this. Not to mention the light relief that was brought with 'posh coffee Fridays' – thanks Matt for ensuring the survival of these! Particular thanks also go to Andrea Clark and Nikhil Darshane for their tireless coding of children's responses at age 2 ½. I would also like to thank Kate Abbott for her advice and understanding in the frustrations of the writing up process, not least those surrounding formatting, the practicalities of Word and APA formatting regulations; and to Nicky Wright for always being on-hand for 'another quick stats question' and her guidance in grasping the use of Mplus. Further thanks to the whole

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Preface

This work was conducted by the author whilst working as a full-time Research Assistant on the Wirral Child Health and Development Study (WCHADS) since the infants in the study were 6 months of age. The author (Helen Chadwick née Jones) took the lead role in the selection and oversight of administration of study measures used in this thesis to assess executive functioning in toddlerhood and broader cognitive functioning, and in the collection of questionnaire data regarding maternal tactile stimulation. She conducted 54 complete assessments at age at 12 months, 86 at age 2 ½ years and 44 at 3 ½ years.

1. Brief Introduction to the Thesis.

Executive function (EF) is the higher order cognitive processing that underlies our ability to engage in complex goal directed behaviour under changing environmental demands. The study of EF very early in development is far less established than in adult populations. Determining the latent structure of early EF may inform our understanding of the time course of the development of mature EF, may facilitate the study of specific links between EF and emerging academic abilities and the development of certain child mental health problems. Previous literature has reported inverse associations between maternal prenatal stress (PNS) and general cognitive development in infancy and early childhood. Proposed mechanisms underlying this association suggest that executive functions, more specifically, may be vulnerable to PNS, though few have examined this issue in early development when there is less opportunity for postnatal environmental influences to confound observed associations. The animal literature has documented sex differences in this association with a relative vulnerability being reported in male offspring. Few previous studies in human populations have examined sex differences in the association between PNS and infant cognitive development and to our knowledge none have examined this in relation to EF abilities in toddlerhood. Animal literature has further reported that the observed negative impact of PNS on offspring cognitive development can be reversed or prevented by specific maternal caregiving behaviours after birth. In human populations, similar moderating effects of postnatal caregiving on prenatal stress effects have been reported, though the caregiving behaviours that have been studied are understandably very different to those examined in the animal literature and none, to our knowledge, have examined EF as

opposed to more general measures of cognitive development.

The current thesis comprises three linked experimental chapters studying same sample drawn from the study population of an ongoing prospective longitudinal investigation into the earliest origins of conduct disorders in children, the Wirral Child Health and Development Study (WCHADS). The thesis examines (a) the structure of executive function (EF) at its earliest emergence, in toddlerhood (b) the prediction of toddler EF by indices of maternal prenatal stress (PNS) including an examination of sex-dependent effects in this regard and (c) the moderation of any observed prenatal stress effects on EF by early maternal caregiving behaviours. Each study is reported with its own background, methods, results and discussion section. Information regarding recruitment and sampling for the over-arching WCHADS study design is given in full within study 1 (chapter 2) in order to provide a broader context for the sampling and recruitment of the study sample used in the current thesis.

2. Study 1. The Latent Structure of Emerging Executive Function in Toddlerhood.

2.1. Background

2.1.1. Executive Function. Executive Function can be defined as the processing that allows us to engage in flexible goal-directed behaviour in novel situations under changing environmental demands (e.g. Anderson, 2004; Hughes, 2002). It involves the dynamic integration of multiple higher-order neural processes such as inhibition, working memory, planning and cognitive flexibility depending upon the demands of the current task. Such processing is thought to be achieved via the recruitment and coordination of more basic cognitive processes that are relevant to the current goal. Both the neuropsychological and functional imaging literature have highlighted the frontal lobes, particularly the pre-frontal cortex, as important neural substrates of successful executive function performance in adults (Luria, 1973, Stuss and Benson 1986, Jurardo and Roselli, 2007).

The study of executive function in adults is well established. Indeed, Goldstein (1944) reported that First World War veterans with frontal lobe lesions were able to perform routine tasks but were impaired in completing new or complex tasks that required planning or abstract thinking. The study of executive function in childhood, however, has lagged behind the adult literature.

One reason for this is associated with the fact that executive functions are closely associated with pre-frontal cortex function. As the pre-frontal cortex does not reach full functional maturity until late adolescence or early adulthood (e.g. Sowell et al., 1999), this region has long been considered functionally silent during childhood and infancy. Robust evidence of the late maturation of this region has resulted in a longstanding general acceptance that executive functions do not emerge until a

similar age. As reported by Hughes and Graham (2002), this widely held view was initially further supported by studies in primates and early research with head injured children that suggested that paediatric neural injury to the pre-frontal cortex did not manifest until adulthood.

Advances in the developmental literature, however, have begun to contradict the assumption of such a late emergence of executive function. Firstly, the pre-frontal cortex can indeed be considered to reach functional maturity in adolescence or early adulthood, however as with other neural regions, indices of development are observable much earlier. Whole brain weight is known to increase throughout childhood from 400g at birth to around 1500g upon reaching maturity in early adulthood, with much of this growth occurring during early childhood (Casaer, 1993). Indeed whole brain volumes have been reported to reach 80% of adult levels as early as 2 years of age (e.g. Knickmeyer , Gouttard, Kang, Evans, Wilber, Smith, Hamer, Lin, Gerig, & Gilmore, 2008).

Structural and functional maturity of neural regions, however, is achieved via the fine-tuning of neurons and neural systems, through processes such as dendritic arborisation, myelination, and synaptogenesis. Physiological development of the brain culminates in apoptosis and synaptic pruning. The pre-frontal cortex is among the last of the neural regions to complete this stage of development, and therefore does not achieve structural maturity until late adolescence/early adulthood (Giedd et al., 1999; Gogtay et al., 2004; Toga et al., 2006). However, structural and functional maturational processes of the pre-frontal cortex have been shown to display peaks and troughs of activity at a number of points during childhood and adolescence; with different processes displaying different developmental patterns (see Fuster 2002 for a review). For example, there is a progressive increase in grey matter from 4 to 12

years of age, with a simultaneous decrease in synaptic density; and a gradual decrease in grey matter thereafter. This decrease in synaptic density and proceeding reduction in grey matter reflects processes of synaptic pruning, the method by which efficient neural networks are developed. White matter volume however, continues to increase through childhood and adolescence and into adulthood. White matter volume reflects the myelination of cortico-cortical axons, which enhances the speed of axonal conduction (and therefore neural processing). Thus the pre-frontal cortex, though not structurally and functionally mature until late adolescence or early adulthood is functionally active from infancy. This protracted development of the pre-frontal cortex may reasonably be accompanied by protracted development of the functions it sub-serves during the same period. As such, though fully mature executive function may not be achieved until adolescence or even early adulthood; the structural and functional architecture sub-serving executive abilities, though immature, begins to develop much earlier (Fuster, 2002).

Secondly, although functional imaging and lesion studies show the pre-frontal cortex to play a vital role in successful executive function task performance, the structural and functional integrity of this region alone is by no means sufficient for successful executive control. There is robust evidence for the involvement of both subcortical and posterior cortical regions during executive function tasks (see Jurardo and Roselli, 2007 for a review), likely due to the recruitment and integration of more basic cognitive processes during their execution. Such regions reach structural and functional maturity much earlier in development than the pre-frontal cortex. This, together with evidence of neural activity in frontal regions prior to adolescence, suggests that rudimentary forms of executive abilities may begin to emerge in childhood.

Thirdly, more recent studies of the neurocognitive outcomes of paediatric brain injury, have now contradicted previously held views of the late emergence of associated deficits in executive function performance with deficits revealed in both the acute and chronic phase post injury in children and adolescents (see Babikan and Asarnow, 2009, for a review). Such findings, in contradicting previous assumptions in the literature, led to a growing research interest into the normative development of executive function through infancy and childhood through to adolescence.

Given the longstanding belief that executive functions emerge in adolescence or even later, the available tasks designed to assess such functions were initially predominantly tailored for adult populations. These measures were necessarily complex, drawing on the coordination of multiple executive processes simultaneously, and typically relied heavily on verbal abilities, either in their required responses (e.g. Stroop, Verbal Fluency task) or in the comprehension of complex verbal instructions (e.g. Tower of London). As such, one of the first challenges for researchers investigating early developing rudimentary executive processes was the development of suitable tasks to test these functions in children.

A growing research interest in the early development of executive functions, related to the conflicting evidence for its developmentally late emergence, has naturally led to the increased availability of developmentally appropriate executive function tasks for young children. With the ability to reliably assess executive functions from childhood through to adulthood, research interest has turned to attempts to characterise its structure across development.

2.1.2. The Latent Structure of Executive Function: A Unitary Construct.

There is debate in the literature as to whether executive function can be considered a unitary construct or a set of dissociable component processes. Proponents of a unitary conceptualisation of executive function assert that executive abilities are mediated by a single unifying factor. Advocates of this unitary conceptualisation of executive function have independently implicated (i) the "central executive" of Baddeley's influential model of working memory (ii) the "supervisory attentional system" of Norman and Shallice and (iii) general intelligence; as central factors mediating the cognitive-control role of executive function (De Frias et al 2006; Duncan et al. 1996; Kimberg et al 1997; Parkin and Java, 1999). Support for this view can be drawn from studies reporting significant inter-correlations amongst performance on different measures of executive function, suggesting a common underlying processing mechanism might mediate their successful completion (e.g. Friedman, Miyake, Young, DeFries, Corley, and Hewitt, 2008).

However, in light of the rather broad role of executive function, in coordinating multiple cognitive processes in order to achieve successful goal-directed behaviour, tasks assessing this ability in adults are typically complex and multi-faceted. Thus, different tasks may measure both distinct and overlapping executive processes. This potential lack of discriminant validity raises issues for the conclusions that can be drawn regarding component executive process abilities. Specifically, reported inter-correlations between performance on different executive tasks may reflect overlapping processing requirements rather than a common overarching executive process mediating performance across tests. For example, tests of inhibitory control often require the participant to hold a rule in mind regarding the specific response to inhibit and therefore also rely on working memory capacities.

Similarly, in tests of cognitive flexibility participants must not only flexibly switch their attention or response set, but must also inhibit the propensity to respond in a previously effective response set, and remember under what conditions or in response to what stimulus they must switch sets. Thus, such tasks may also require inhibitory control and working memory capacities as well as targeting cognitive flexibility for their successful completion.

Consequently, inter-correlations amongst performance on tasks of inhibition, cognitive flexibility and working memory may result from tapping the same component process (e.g. working memory) rather than a single overarching 'executive' function. Furthermore, given that executive function performance involves the recruitment and coordination of more basic cognitive processes; assessments of executive function necessarily also involve lower level cognitive processes (e.g. categorization, naming) for their successful completion. Inter-correlations between task performances may therefore represent similarities in non-executive requirements of the tasks. This difficulty in the conclusions that can be drawn from associations between performance on different executive function tasks, regarding the unitary versus componential structure of executive function, is frequently referred to as the 'task impurity problem' in the literature (e.g. Phillips, 1997).

2.1.3. The Structure of Executive Function: A Componential Perspective.

A componential (sometimes described as 'fractionated') perspective of the structure of executive function posits that a set of dissociable processes are implemented in the completion of successful goal-directed behaviour. Such abilities include planning and organisation, problem solving, self-monitoring, cognitive and attentional

flexibility, inhibitory control, and working memory. In support of this view, Godefroy et al., (1999) highlight that patients with frontal lobe lesions often display within-subject dissociations in their executive function impairments, i.e. performing well on some executive tasks and poorly on others. Additionally, findings from the functional imaging literature have revealed that specific executive processes are associated with activation in differing regions/ networks of regions in the pre-frontal cortex (e.g. Curtis et al., 2000; Koechlin et al., 2000).

In their influential paper, Miyake, Friedman, Emerson, Witzki and Howerter (2000) investigated the unitary versus componential structure of executive function in adults, employing tasks commonly accepted to draw on the abilities of set shifting, working memory, and inhibition of a pre-potent response; as well as tasks considered to rely on more complex aspects of executive control requiring simultaneous monitoring and integration of multiple processes for successful performance. The authors employed confirmatory factor analysis (CFA) in an attempt to overcome the task impurity problem and determine whether variability in performance on tasks of working memory, inhibition and cognitive flexibility in healthy adult participants could be attributed to separable processing functions or whether performances loaded onto a single common latent factor. Miyake et al.'s findings did not fully support either a unitary or a componential perspective, and the authors instead suggest an 'integrative' framework of executive function, in which both the unity and diversity of executive function is upheld. Specifically, the CFA identified three distinct latent variables of working memory, inhibition and set shifting. However, the correlations among the three latent variables were moderately high (ranging from 0.42 to 0.63) and a model in which the three factors were distinguishable yet moderately related provided the best fit for the data. The authors further applied structural equation

modelling to assess how these three factors related to performance on more complex executive function tasks commonly used in the neuropsychological literature (specifically the Wisconsin Card Sorting Task and the Tower of London task). Their findings suggested that the separable processes identified from CFA (i.e. working memory, inhibition and set-shifting) were differentially required for successful completion on such tasks. Specifically, shifting abilities were important for performance on the Wisconsin Card Sorting Task, whereas inhibitory control was important for performance on the Tower of London task.

Miyake et al.'s (2000) seminal findings of an integrative structure of EF have been replicated during adulthood and mid to late childhood. For example, Lehto et al. (2003) assessed the appropriateness of an integrative model of executive function performance in children aged 8 - 13 years. In accordance with Miyake et al. (2000), they reported a three-factor model comprising the latent variables of working memory, inhibition and set-shifting, in which the three factors were partially dissociable yet moderately inter-correlated. Similarly, Huizinga, Dolan and Van der Molan (2006) assessed the structure of participant performance on multiple measures of executive function across four different age groups (specifically, 7, 11, 15 and 21 year olds). Results yielded two common factors across all age groups; working memory and set shifting. Performance on the individual inhibition measures, however, did not load onto a common inhibition factor. Huizinga et al. (2006) suggest that this finding may have been due to subtle differences in task requirements amongst the inhibition measures, in concert with the wide age range of their sample. However, including the three individual inhibition measures in their analysis, alongside the latent factors of working memory and shifting, they still found an adequate fit for a model of partially dissociable yet moderately correlated executive

function components. More recently, applying both performance measures and parental report of executive function abilities (BRIEF, Gioia, Isquith, Guy and Kenworthy, 2000) and employing CFA, Cassidy's (2016) results revealed three distinct yet moderately correlated cognitive constructs (working memory, set-shifting and verbal fluency) in children and adolescents aged 7 - 18 years. Therefore, evidence of an integrative structure of executive function performance, characterised by both unity and diversity, in which component processes are partially separable yet moderately correlated, has been replicated from middle childhood through to adulthood.

Thus, these findings suggest that, from middle childhood through to adulthood, the term executive function refers to a set of partially dissociable yet moderately related higher order component cognitive processes that rely on input from more basic processing for successful goal-directed behaviour. Such processes are called upon to differing degrees for successful executive function performance, depending on the specific goals and constraints of the relevant task. The coordination of these component processes allows for flexible goal-directed behaviour in novel situations under changing environmental demands.

Miyake et al.'s (2000) evidence that performance on more complex executive function tasks assessing problem solving and planning (i.e. Wisconsin Card Sorting Task, Tower of London task) can be fractionated into component processes, (e.g. working memory, inhibition and set-shifting) offers the opportunity to develop simpler less ambiguous and therefore more developmentally appropriate tasks for younger children. This, together with evidence supporting the position that executive functions may begin to emerge earlier in development than previously thought has resulted in the development of a number of developmentally appropriate assessment

tools for assessing executive functions as they develop in paediatric samples.

2.1.4. The Value of Researching Executive Function Abilities in Paediatric Samples

Research findings examining the early development of executive function may have both theoretical and clinical implications, as well as the potential for informing educational practices. From a theoretical standpoint, research examining early executive function has the potential to inform researchers regarding the structure of mature executive functions. Tasks assessing executive function in adults are typically complex and multi-componential in order to retain the 'executive' requirement of task performance. Thus, difficulty arises in drawing confident conclusions regarding the specific component processes associated with variability in performance on such measures. Tests designed to assess executive functions in young children must be simpler and less ambiguous, and are frequently aimed at relying on single component executive processes for successful completion. Thus, research examining early emerging executive functions may well help researchers to tease apart the developing structure of complex mature executive functions.

From a clinical perspective, well-defined developmental trajectories of normative executive function could help provide a comparison against which dysfunction may be identified early in childhood. In turn, early identification of executive dysfunction in the context of developmental psychopathologies may expand the window of opportunity for targeting early interventions aimed at modifying the maladaptive behavioural and cognitive manifestations of the EF deficits observed. Executive dysfunction has been reported in attention deficit hyperactivity disorder (ADHD), autistic spectrum disorders (ASD), oppositional

defiant disorder (ODD), conduct disorder (CD) and Tourette syndrome (TS); with distinct and overlapping component processing deficits implicated across disorders (e.g. Sergeant et al; 2002, Rajendran and Mitchell; 2007, Guerts et al; 2004).

From an educational perspective, research has shown EF abilities to be associated with broader academic abilities in typically developing children. Specifically, cross-sectional associations have been reported in pre-school samples between EF performance and academic abilities across a number of domains, including literacy skills (Allan and Lonigan, 2011), language (Weiland, Barata and Yoshikawa, 2014) and mathematics (Clark, Pritchard and Woodward, 2010). However, due to the correlational nature of these studies, it is not clear whether EF abilities are predictive of variability in academic skills or vice versa.

Prospective longitudinal research in this area does report that early EF is related to later academic abilities over time offering some evidence to suggest that early EF abilities support the development of later academic skills. Blair and Razza (2007), for example, found that aspects of executive function assessed in the pre-kindergarten year accounted for unique variance in emerging mathematic and literacy ability in kindergarten. Similarly, Welsh, Nix, Blair, Bierman, and Nelson, (2010) reported that growth in working memory and attentional control skills over the pre-kindergarten year made unique contributions to the prediction of math and reading achievement assessed at the end of the kindergarten year. The predictive power of EF in developing academic abilities may span even longer periods than those reported in the aforementioned studies. Specifically, Alloway and Alloway (2010) found that working memory at age five predicted literacy and numeracy abilities at age eleven. However, these longitudinal studies still do not exclude the possibility of a third variable, such as verbal comprehension, accounting for the

shared variance in EF and academic skills over time.

Stronger support implicating EF abilities to underlie developing academic skills comes from studies examining interventions aimed at improving EF. For example, Raver et al. (2011) found that children in preschool classes whose teachers had been trained in strategies aimed at improving children's EF abilities improved significantly more than children in comparison classes. Furthermore, children in the intervention classes improved in academic abilities (specifically vocabulary, letter-naming and maths) significantly more than control children, and this improvement in academic abilities was mediated largely by improvements in EF.

In summary, investigation of the development of EF is important for at least three reasons (i) to advance understanding of the normal development of mature EF (ii) to elucidate the cognitive underpinnings of a number of neurodevelopmental or child mental health difficulties (atypical development) and (iii) to aid identification of early targets for intervention that might support children's future educational achievement. Together these motivations have fuelled a surge of research interest in the early development of EF which, by necessity has led to research teams devising new developmentally appropriate assessment tools for assessing EF earlier and earlier in childhood.

2.1.5. Examining the Structure of Executive Function Performance in Early Childhood. The pre-school age range has attracted much recent research attention regarding the development of executive function. Some of the most frequently studied executive processes observed at this age are working memory, inhibitory control and set shifting/ cognitive flexibility (see Garon, Bryson and Smith 2008 for a review), with some authors also reporting rudimentary planning abilities

(e.g. Fabricius, 1988; Welsh et al, 1992). Longitudinal investigation of executive function performance suggests that children exhibit rapid growth of such abilities across this age range, with significant gains in performance on measures of working memory, inhibitory control, set shifting and planning (e.g. Hughes, 1998; Hughes, Ensor, Wilson and Graham, 2010; Hughes and Ensor, 2007; Carlson, 2005; Espy, Kaufman, McDiarmud and Glisky, 1999; Fuhs and Day, 2011).

These research efforts have demonstrated early emerging EF abilities in children aged 2 – 6 years employing a set of commonly used developmentally appropriate assessment tools. However, the cognitive constructs underlying performance on such tasks are still not well understood. Considering this, research efforts have turned to the examination of the underlying structure of EF performance in 'pre-school' samples. Though there is empirical support for Miyake's integrative model of the structure of executive function in late childhood through to adulthood (Cassidy et al., 2016; Huizinga et al. 2006; Lehto et al. 2003; Miyake et al. 2000,) as reviewed in section 2.1.3 above, it does not necessarily follow that that executive function is organised in the same way earlier in development. Research findings examining the structure of EF in reported 'preschool' samples are far from reaching a consensus, with findings reported to support both a componential structure (a set of dissociable processes that are implemented in the completion of EF tasks) and a unitary structure (in which a common over-arching EF ability is considered to underlie performance across EF tasks). Furthermore, closer examination of such reports together with more recent findings in this area suggest that an integrative structure of EF (in which component EF abilities are partially separable but moderately correlated) may better characterise the structure of EF early in development. One difficulty in evaluating findings across studies arises from the lack

of a consistent demarcation of the age range that constitutes the 'pre-school' age range. Mean ages of samples in this area of the literature have ranged from 2 years and 4 months to 6 years. This issue will be discussed further in section 2.1.6 below following a review of findings examining the structure of EF in this early period of development.

Applying principle components analysis (PCA) to pre-schoolers performance on multiple measures of executive function, Hughes et al. (1998) and Espy et al. (1999) report that variation in EF performance can be explained by a number of dissociable factors, specifically working memory, inhibitory control, and set shifting. Evidence that these component executive processes display separable developmental trajectories (e.g. Carlson 2005; Klenberg, Korkman, and Lahti-Nuuttila, 2001) also supports a componential perspective of the structure of EF in the pre-school period.

In comparison to PCA, the use of confirmatory factor analysis (CFA) has been suggested a more robust statistical method to examine the structure of EF (e.g. Miyake et al., 2000) since it allows researchers to overcome the task impurity problem inherent in tasks available to assess this cognitive function. CFA allows researchers to separate out shared variance unrelated to the latent structure (such as lower order cognitive functions that may be involved in multiple tasks within a task battery), examining only that resulting specifically from the latent ability or abilities under study.

A number of authors that have employed CFA to young children's EF performance have reported findings to suggest that executive function has a unitary structure early in development. For example, Wiebe, Espy and Charak (2008), employed CFA to examine the structure of EF in 243 pre-schoolers (aged 2 to 6 years). Weibe et al. (2008) administered multiple tests of EF, considered a priori to

place demands on working memory and inhibitory control. They then applied CFA to the data to assess the relative fit of a single factor model, a two-factor model and three factor model of performance. The authors reported that although the two and three factor models exhibited acceptable fit to the observed data, they did not result in a significant improvement in fit over the single factor model. Therefore, the unitary model of the structure of EF performance was retained on the basis of parsimony. Though Wiebe et al.'s (2008) study exhibits a number of strengths such as the relatively large sample size, as well as the number of tasks employed to assess child EF (six), the age range of the children was rather large, with no information provided regarding the numbers of younger versus older children within the sample.

In a later study, Wiebe, Sheffield, Nelson, Clark, Chevalier and Espy (2011) also reported findings supporting a unitary structure of EF in the preschool period, employing a sample exhibiting a smaller age range. Specifically, Wiebe et al. (2011) employed CFA to assess the structure of executive function performance in 228 3 year olds using developmentally appropriate measures of inhibitory control and working memory. In testing the relative fit of a number of different models, they reported that their results supported a single latent EF construct in agreement with Wiebe et al.'s (2008) findings. However, like Wiebe et al. (2008), the analysis also revealed that a two-factor model, separating inhibitory control and working memory fit the data equally well, but the two-factor model was rejected as it did not result in a significant improvement in fit over the unitary model and the correlation between the two factors was high (r = .8).

In a sample of 191 children assessed longitudinally at age 4 and 6 on tests of planning, inhibitory control and working memory, Hughes et al. (2010) examined the developmental stability of the structure of executive function between these ages.

The authors reported that a single latent construct fit the data well at both ages, supporting the stability of a unitary structure of EF across this age range. However, as the authors included only one indicator representing each of the three possible sub-components assessed (working memory, inhibition and planning), it was not possible to assess the relative fit of a componential model to their data. As such, though providing additional support for a unitary model of EF early in early childhood, and as outlined by Hughes et al. (2010), their "results do not challenge the fractionated model of EF" (p.31).

In the largest such study to date, Willoughby, Blair, Werth and Greenberg (2010) applied CFA to the performance of 975 3-year olds on a battery of tasks assessing EF. They reported that children's performance across the multiple tasks administered was 'adequately' summarised by a single factor. Like Wiebe et al. (2008) and Wiebe et al. (2011), while a two-factor model of performance also fitted the data well; this model was rejected on the basis of parsimony.

More recent findings examining this issue suggest that an integrative structure (in which component executive processes are distinguishable yet correlated) may better characterise executive function in young children. Lonigan, Lerner, Goodrich, Farrington and Allan. (2016) assessed EF in both English speaking and Spanish speaking children aged three years to five years nine months (mean age 4 ½ years). A formal test of relative model fit revealed that a two-factor solution provided a better fit to the data than a unitary model of performance. Specifically, the CFA revealed two distinct factors (working memory and inhibitory control), with the two factors significantly correlated (at r =.7 in both English speaking and Spanish speaking children). However, like Wiebe et al.'s (2008) study, the age range of the children assessed was rather wide and no information was given regarding the

proportion of the sample falling within smaller age brackets.

Lerner and Lonigan (2014) also provide findings supporting an integrative structure of EF in early childhood, within a smaller age range. Specifically, they applied CFA to the performance of 289 children (aged 3 years, 9 months to 5 years, 3 months) on a battery of EF tasks. Testing the relative fit of a unitary versus a two-factor model of performance, they found that a two-factor model, separating working memory and inhibitory control, provided a significant improvement in fit to the data over the unitary model. The two factors exhibited a significant positive correlation of r =.8.

Usai, Viterbori, Traverso and De Franchis (2014) provide further support for an integrative structure of EF in early childhood, albeit in a slightly older sample. Specifically, they used CFA to examine the structure of executive function performance on a battery of tasks considered a priori to assess working memory, set shifting and inhibitory control in children aged 5 and 6. Formally testing the relative fit of a unitary, a two factor and a three factor model, they reported that a two factor structure (inhibition and working memory/set shifting) provided the best fit for the data at both ages, with a significant positive correlation (r = .6) between factors at both ages.

Similarly, Miller, Giesbrecht, MÜller, McInerney and Kerns (2012) conducted CFA on children's performance on a battery of executive function tasks assessing working memory, inhibitory control and set-shifting at age 3 to 5 years (mean age 4.2 years). Formal model fit comparisons revealed a two factor model, separating working memory and inhibitory control provided the best fit for the data, with a significant positive correlation between the two factors of r = .6.

To our knowledge, only one previously published study has examined the

unitary versus componential latent structure of EF in children as young as the current study sample. Specifically, Mulder, Hoofs, Verhagen, Van der Veen and Leseman (2014) examined the structure of EF in a large sample of toddlers (N = 2,437) at a mean age of 2 years, 4 months. They found that a 2-factor model of performance provided the best fit for their data, with the modelled factors exhibiting a significant moderate positive correlation (r = .4). Though the modelled factors comprised a distinction between 'Hot' EF (processing of affectively laden stimuli) versus 'Cool' EF (affectively neutral stimuli), rather than the characterisation of the purely cognitive latent structure of EF, the findings lend support to the position that toddler EF may be characterised by an integrative rather than a unitary latent structure.

In summary, there are findings supporting both a unitary and an integrative structure of EF in early childhood. The discrepancies across studies may be borne out of a number of limitations within and across them. These will be discussed in the following section.

2.1.6. Limitations of previous studies examining the structure of EF in preschool samples. Firstly, the findings from a number of previous CFA studies supporting a unitary structure of EF do not provide conclusive evidence in opposition to an integrative model. For example, though Hughes et al. (2010), Willoughby et al. (2010), Wiebe et al. (2008) and Wiebe et al. (2011) each report that a unitary structure of performance provided the 'best' fit to their data, a componential structure also provided adequate fit or could not be tested. The decision to accept the unitary structure was typically based on the fact that a componential model of performance did not result in a significant improvement in fit over the unitary model (or could not be tested) and thus the unitary model was retained as 'best' on the basis of

parsimony, as is frequently the case in CFA studies.

Second, other studies investigating this issue, one reporting a unitary structure of EF (Wiebe et al., 2008) and one reporting an integrative structure of EF (Lonigan et al., 2016) have employed samples exhibiting relatively wide age ranges (Table 1). Since the pre-school period is a time of rapid growth in EF and this growth is characterised by differing trajectories of significant gains across different executive processes, (Hughes 2011), it is also possible that the overall structure of these abilities alters across this period of development. For example, Cassidy (2016) suggests that early EF may emerge as a unitary function and becomes gradually more differentiated and integrated across development. So, research findings in this area of the literature based on samples exhibiting wide age ranges (such as Espy et al., 1999; Lerner & Lonigan, 2014; Lonigan et al.2016; Lerner et al., 2016; Miller et al., 2012 and Weibe et al., 2008) may actually be predominantly driven by the performance of a subsample within a particular age bracket rather than across the entire sample. Indeed, Wiebe et al.'s (2008) findings reveal that the unitary model of performance on the task battery administered explained more variance in performance for younger than for older children. Thus, the reported results of a unitary structure of EF may have been driven predominantly by task performance of the younger children in their sample, as opposed to the entire age range. This is speculative since no information was given regarding the proportion of the sample that fell within smaller age brackets, nevertheless, it may be prudent to employ samples exhibiting relatively narrow age ranges when examining the structure of EF as it is developing.

Third, though not all authors (e.g. Hughes et al., 2010; Usai et al., 2014; Willoughby et al., 2010; Wiebe et al., 2011) refer to their samples as ‘pre-school samples’ the age range of those referred to as ‘pre-school samples’ often clearly

straddles the early school age (seeTable **1**). This may result from the different locations in which the studies were administered and related differences in educational systems. Nevertheless, it is an important consideration in attempting to characterise the early development of EF within specific development periods.

Table 1

M*ean age and Age range of samples from cited studies examining the structure of EF in 'pre-schoolers'.*

Authors [Location of study]	Child Age	
	Mean	Range Minimum to Maximum
Mulder et al. (2014) [Netherlands]	28 months	20 months to 37 months
Wiebe et al. (2011) [Midwestern US]	36 months	36 months to 36 months[1] (SD=12.8 days)
Espy et al. (1999) [Arizona, US]	40 months	23 months to 66 months
Hughes et al. (1998) [London, UK]	47 months	39 months to 55 months
Wiebe et al. (2008) [US, more precise location not provided]	47 months	28 months 72 months

[1] Minimum and maximum age are equal as all children attended their assessment within two weeks of their third birthday.

Table 1 (continued)

Mean age and Age range of samples from cited studies examining the structure of EF in 'pre-schoolers'.

Authors	Child Age	
[Location of study]	Mean	Range Minimum to Maximum
Miller et al. (2012) [Southwestern Canada]	50 months	36 months to 60 months
Hughes et al. (2010) [Cambridge, UK]	Time 1: 51 months Time 2: 72 months	43 months to 59 months 65 months 83 months
Lonigan et al. (2016) [Central Florida and New Mexico, US]	54 months	38 months to 69 months
Lerner and Lonigan (2014) [North Florida, US]	56 months	45 months to 63 months
Usai et al. (2014) [Northern Italy,]	Time 1: 60 months Tine 2: 72 months	63 months to 76 months 63months to 90 months

Finally, a further limitation of the aforementioned lies in the lack of reporting on the socio-demographic characteristics of the samples studied (see **Table 2**). A limited range of socio demographic backgrounds within samples and particularly where there is an under representation of those from more deprived circumstances

may result in a restricted range of children's abilities being studied, with an under representation of children at the lower end of ability. Though a number of studies have specifically selected samples that include families at socio-demographic risk, such as those with lower family income (Hughes et al., 2010; Lonigan et al., 2016; Willoughby et al., (2010); Wiebe et al., 2011), the rest simply did not assess (or at least did not report) the level of such socio- demographic risks in their samples (Espy et al., 1999; Hughes et al., 1998; Lerner & Lonigan, 2016; Usai et al., 2014; Wiebe et al., 2008). Socio-demographic characteristics of interest commonly not reported or assessed across studies include maternal age, education level and psychosocial risk. Thus, it is not clear whether children from more deprived backgrounds, with less well-educated parents or at higher psychosocial risk have been adequately represented in previous research examining the structure of EF in the pre-school period, and therefore whether the full range of children's abilities has been sampled.

The next section outlines the aims of the current study which was designed to improve on some of the limitations discussed above.

Table 2

Demographic characteristics reported in previous studies examining the structure of EF in early development.

Authors	Maternal age	Maternal education	Deprivation	Psycho-social risk
Hughes et al. (1998)	Not reported	Not reported	Not reported	Not reported/ assessed
Espy et al. (1999)	Not reported	Years in education: Mean = 17years 1 month	Not reported	Not reported/ assessed
Wiebe et al. (2008)	Not reported	Years in education: Mean 14 years 1 month SD=2years 3months range = 8y to 20y	Not reported	Not reported/ assessed
Hughes et al. (2010)	Not reported	Educational qualification: 10% none 31% elementary (age 16) 27% higher (age 18) 32% degree	40%: family income below the national median 16%: living in poverty	Not reported / assessed

Table 2 (continued)

Demographic characteristics reported in previous studies examining the structure of EF in early development.

Authors	Maternal age (years)	Maternal education	Deprivation	Psycho-social risk
Willoughby et al. (2010)	Mean = 29.4	Years in education: Mean 13	77 % 'in low income stratum'. Further details not provided.	Not reported/ assessed
Wiebe et al. (2011)	Not reported	Years in education: Mean 14 years 8 months	Roughly equal numbers of low socio-demographic risk and high socio-demographic risk (defined by eligibility for public medical assistance).	Not reported/ assessed
Miller et al. (2012)	Not reported	Median level of Education received: some college or university (no further details provided).	Not reported	Not reported/ assessed

Table 2 **(**continued)

Demographic characteristics reported in previous studies examining the structure of EF in early development.

Authors	Maternal Age	Maternal Education	Deprivation	Psycho-social risk
Lerner and Lonigan (2014)	Not reported	Not reported	Not reported	Not reported/ assessed
Usai et al. (2014)	Not reported	Not reported	Not reported	Not reported/ assessed
Mulder et al. (2014)	Not reported	Not reported	41.5%: low/ middle income 58.5 %: high SES Classification based on parental education level.	Not reported/ assessed
Lonigan et al. (2016)	Not reported	Median level of Education received: - 62% high school, - 21% some college - 17% college completion or higher.	Median household income: - $20,000 or less - Family income > $40,000: 7% (No further details provided).	Not reported/ assessed

2.1.7. Aims of the Current Study. To help clarify conflicting findings regarding the structure of EF in the pre-school period and to add to previous research in the area, the current study aimed to (a) employ CFA to characterise the structure of executive function very early in its development, at an age that has thus far been largely unstudied by research in the field (b) study a sample of toddlers exhibiting a relatively narrow age range clearly defined within the pre-school period and (c) include participants from a range of socio-demographic backgrounds in order to study the structure of EF across the full range of toddler abilities.

The current research was embedded within The Wirral Child Health and Development Study (WCHADS); a prospective longitudinal study funded by the Medical Research Council. The design, sample recruitment and procedures of the WCHADS will first be outlined (see section 2.2.3 to 2.2.5) in order to provide a context for the current study. The method for the current research investigation will then be described in full in section 2.4.

2.2. WCHADS Method

2.2.1. WCHADS Ethics Statement. Ethical approval for phases 1 to 8 of data collection on the WCHADS was granted by the Cheshire North and West Research Ethics committee on the 27th June 2006 (reference number 05/Q1506/107) Ethical approval for phase 9 of data collection, at which point toddler EF was assessed was granted by the Cheshire North and West Research Ethics committee on the 7th June 2010 (reference number 10/H1010/4). The letters confirming ethical agreement for these phases of study are in Appendix 1. Participants gave written informed consent for data collection at multiple phases within the WCHADS. Information sheets that are relevant to the current thesis are given in Appendix 2. The author joined the WCHADS team during phase 2 of data collection and was named as a researcher on the information sheets for postnatal phases of the study.

2.2.2. WCHADS aims. The WCHADS is an ongoing prospective longitudinal study, starting in pregnancy with follow-up of mothers and their infants through development up to age 9 years to date. The WCHADS was designed with an over-arching aim to investigate early risk and resilience influences on the development of later behavioural and conduct problems in childhood.

2.2.3. WCHADS Design. The WCHADS adopted a two-phase stratified design with a consecutive sample of first time pregnant mothers being recruited as the 'extensive sample' for long term follow up. A sub-sample of these families were then asked to also take part in an 'intensive' arm of the study with more detailed developmental assessments and maternal assessments over time. Details regarding the basis for this stratification procedure are given in section 2.2.4.3 below. The current thesis focuses on data gathered from the stratified 'intensive' sub-sample of WCHADS participants with detailed developmental assessments administered over

time. All data analyses take account of the stratification variable. Figure 3 outlines the progression of the intensive sample through WCHADS study phases spanning the period of data collection for the current thesis. Recruitment and sampling of the extensive sample will first be described as background information (section 2.2.4.1), followed by the approach to recruitment of the intensive sample that were used in the current thesis (section 2.2.4.3)

2.2.4. WCHADS Sampling Procedure. The WCHADS used a two-stage epidemiological recruitment strategy. All participants were recruited from a single maternity unit serving the Wirral, Merseyside. The population of the Wirral is a socioeconomically diverse one which is overall relatively deprived and has a majority of white British families with low rates of ethnic diversity.

2.2.4.1. Recruitment to the extensive sample. In the first stage of recruitment, at their 20 week antenatal scan, a consecutive sample of 1881 expectant mothers that had previously expressed interest in hearing more about the study (at their booking visit for antenatal care) were approached by research midwives and written informed consent to participate was requested. Women were invited to participate in the WCHADS based on the following inclusion criteria: (i) primiparous, (ii) English speaking and (iii) 18 years of age or above at the time of recruitment. Consent was gained from 1286/1881 (68.4%) of those eligible. This is illustrated in *Figure* 1. This sample was termed the "extensive" community sample. Immediately after written informed consent was gained, participants completed phase 1 of data collection, comprising a brief interview and a questionnaire pack.

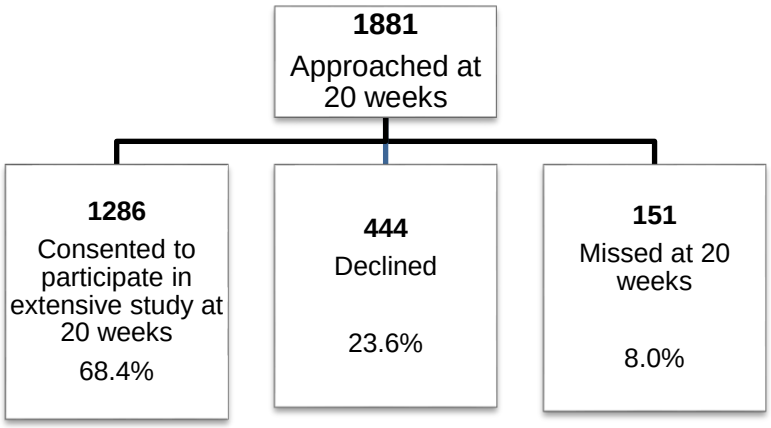


Figure 1

Antenatal Recruitment to the WCHADS extensive sample.

2.2.4.2. Comparison of consenters and non-consenters to the extensive sample. At initial approach about the study, at their booking visit for antenatal care (8-12 weeks gestation), basic demographic data was collected from all women that expressed a wish to hear more about the study at their following (20 week) scan appointment. This included age and postcode. A comparison between women who subsequently consented to the extensive study and those who declined was conducted. Those who declined were significantly younger (mean age 25.2 years, SD 5.9) than those who consented (mean age 26.7 years SD 5.9) $t\ (1927) = -5.32$, $p < .01$; *d =0.25).* More of the women that declined participation (47.5%) were in the most deprived quintile compared to those that consented (41.3%) to participate ($\chi^2\ (1) = 6.61$, $p< .01$; $\varphi = 0.005$).

2.2.4.3. Recruitment to the intensive sample. The current thesis sample was drawn from the WCHADS intensive sample that was recruited to the WCHADS in the following manner. On the basis of their responses to Phase 1 (20 weeks) of data collection, 554 'extensive sample' participants were selected to be approached for inclusion in a sub-sample stratified for psychosocial risk, termed the 'intensive' sample. Selection for the intensive sample was based on responses to the Dunedin Relationship scale (Moffitt, Caspi, Krueger, Magdol, Margolin, Silva, and Ros, 1997), which aimed to screen women for the presence of psychological abuse in their intimate partner relationship over the past 12 months. All participants who scored above a threshold for a higher risk of psychological abuse from mother to partner or vice versa (N= 283, 51.1%), plus a random sample of those scoring below this threshold, (N = 271, 48.9%) were invited to join the intensive sample. The threshold (a score of four or higher for psychological abuse from either the woman toward her partner, vice versa, or both) was set on the basis of data from the Dunedin Multidisciplinary study (Moffitt et al., 1997). All WCHADS researchers were blind to the risk status of participants and remained so throughout subsequent phases of study.

Informed consent to participate in the intensive study was gained from 341 (61.6%) of those women approached for inclusion in the 'intensive' sample. 213 women refused inclusion in the 'intensive' sample and opted to remain in the extensive study. Subsequently, at WCHADS phase 3 of data collection (birth and delivery records) 316 participants from the intensive sample remained eligible for postnatal follow up. This is illustrated in Figure 2. This comprised 163 participants (52%) scoring above threshold on the stratifying variable and 153 (48%) scoring below threshold.

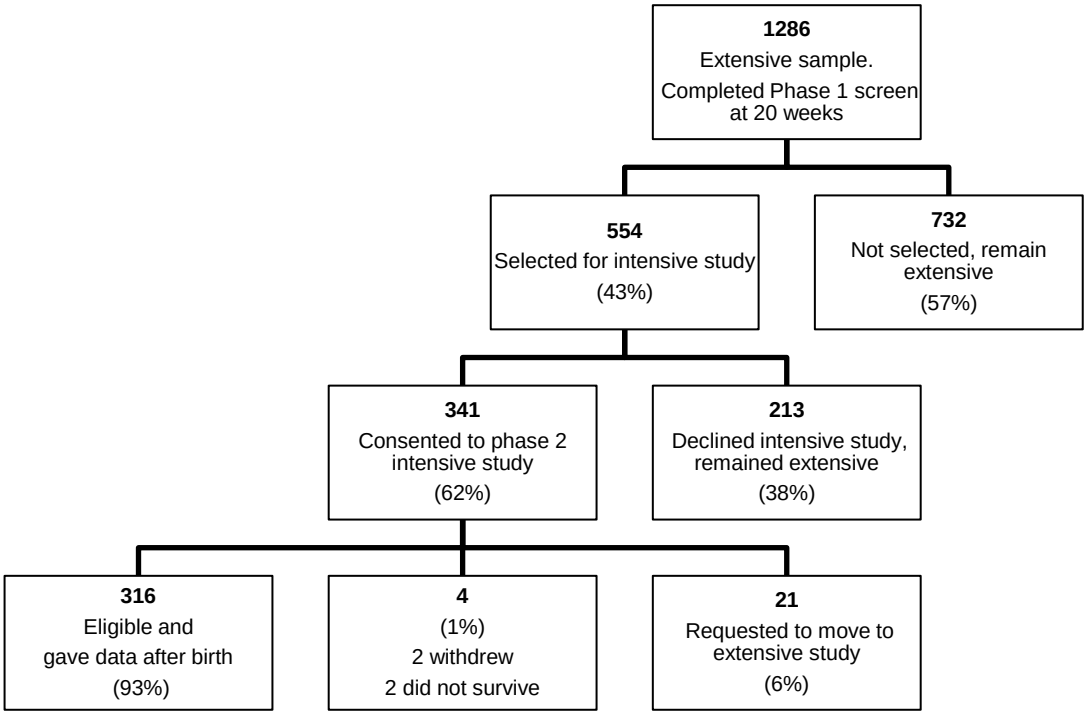


Figure 2

Antenatal Recruitment to the WCHADS intensive sub sample.

2.2.4.4. Comparison of consenters and non-consenters to the intensive sample. Statistical comparison of those who consented to take part in the intensive study and those who declined was conducted based on age, deprivation (revised English Index of Multiple Deprivation, Noble et al., 2004) and psychological abuse scores. Women who consented to the intensive study were significantly older (mean age 27.4 (SD 6.2) than those who declined (mean 24.4 SD (5.1); t (397.6) = 5.40, $p < .01$; $d = 0.53$) and fewer of those who consented were in the most deprived quintile of the IMD (40.1% compared with 50.3%; Chi-squared (1) = 6.61, P<0.01; φ =

0.003). Participating women did not report different levels of psychological abuse perpetrated by partners (mean consenters = 1.75 (SD 2.7), mean non-consenters =1.91 (SD 2.6); t= 0.63, p>0.05, *d* = 0.06) or by the women toward their partners (mean consenters = 2.66 (SD 2.6), mean non-consenters = 2.85 (SD 2.7); t= -0.77, p>0.5, *d* = 0.07).

2.2.5. WCHADS study phases. Following phase 1 consent and data collection (20 weeks gestation), and spanning the time period relevant to the current thesis, extensive participants completed additional phases of data collection at birth (phase 3: labour, delivery and birth records); at 9-12 weeks post-partum (phase 5: postal questionnaire pack); and at 12 months post-partum (phase 7: postal questionnaire pack and infant health records). New consents were completed at a home visit at 3½ years post-partum (phase 10: research health visitor home visit) and subsequent assessments not reported here.

During this period, in addition to phases 1, 3, 5, 7 and 10 of data collection described above, all intensive participants (from which the current thesis sample is drawn) were invited to complete additional phases of data collection at 32 weeks gestation (phase 2: questionnaire pack and detailed investigator led interview); at 4-8 weeks post-partum (Phase 4: infant observational assessment); at 6 months post-partum (Phase 6: mother and child observational assessment and investigator led maternal interview); at 12 months post-partum (phase 8: mother and child observational assessment and detailed investigator led maternal interview); and at 2 ½ years post-partum (phase 9: mother and child observational assessment and detailed investigator led maternal interview). The current chapter of this thesis reports data collected from the WCHADS intensive sample at phases 9 and 10.

Chapter 2, examines the prediction of toddler EF performance from maternal prenatal stress and reports data collected from the WCHADS intensive sample at phases 1, 2, 3, 6, 8 and 9. Chapter 3 of this thesis, examining the moderation of the prenatal stress prediction of toddler EF performance by maternal caregiving behaviours reports data from the WCHADS intensive sample at phases 1, 2, 3, 4, 5 6. 8 and 9.

Figure 3 provides an overview of the progression of the intensive sample through study phases spanning the period of data collection for this thesis. Phases relevant to the current thesis are highlighted, with phases employed for the analyses in each chapter indicated. Details of the assessments carried out relevant to each analysis are also presented within the figure.

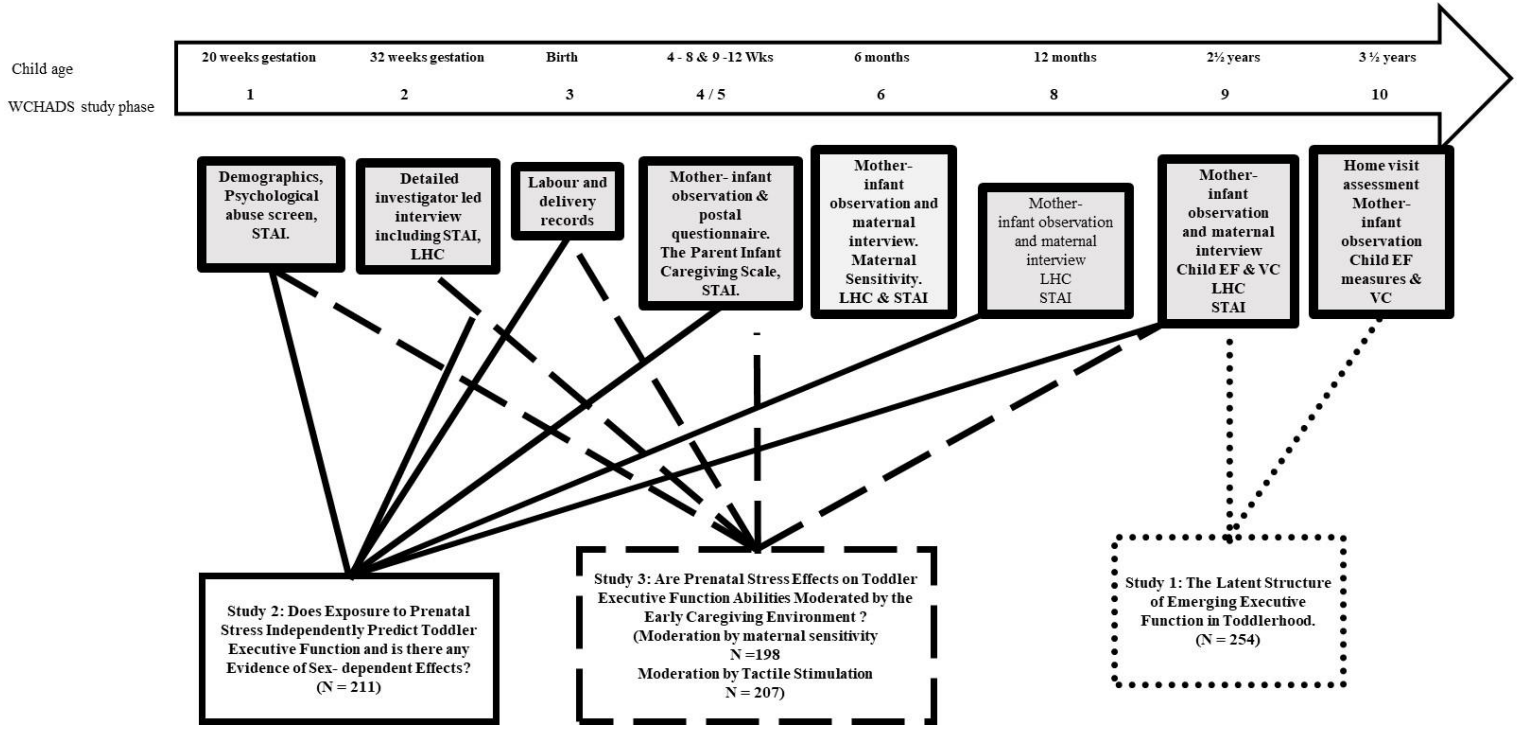


Figure 3

Intensive subsample progression through WCHADS study phases with measures employed in the current thesis during the period spanning the current thesis.

LHC= Life history calendar; STAI = Spielberger State-Trait Anxiety scale (state anxiety subscale), EF = Executive Function, VC = Verbal Comprehension.

2.3. Study Aims: Testing the Latent Structure of Executive function in Toddlerhood

Though outlined in a previous section, the aims of the current study are first restated to aid the reader.

To help clarify conflicting findings regarding the structure of EF in the pre-school period and to add to previous research in the area, the current study aimed to (a) employ CFA to characterise the structure of executive function very early in its development, at an age that has been largely unstudied by research in the field to date (b) study a sample of toddlers clearly defined within the pre-school period (c) include participants from a range of socio-demographic backgrounds in order to study the structure of EF across the full range of toddler abilities.

Confirmatory Factor Analysis was applied to performance data from 254 toddlers (at age 2 ½) on a battery of tasks that had differing executive function demands, drawn from previous literature, in order to assess the fit of a unitary and a componential model of performance to the observed data. The validity of the factor scores derived was also assessed by examining relationships with concurrent age and verbal ability and with later EF performance (assessed at age 3 ½). Due to the limited amount of previous literature examining the structure of executive function in toddlerhood and the fact that available studies in the pre-school period have yielded conflicting findings, the analysis of the structure of executive function in the current thesis was exploratory. Therefore no a priori hypotheses were made regarding factor structure. At the time of the assessment EF abilities there were no available validated tests of EF for children aged 2 ½, against which construct validity could be assessed. Therefore construct validity was assessed by examining bi-variate correlations between EF performance and other aspects of development that have previously been

found to be associated with EF in early development, specifically age (e.g. Carlson, 2005) and verbal ability (e.g. Hughes and Ensor 2007). It was hypothesised that EF at age 2 ½ would correlate positively with age at testing and with indices of verbal ability (vocabulary and verbal comprehension). In addition, EF performance at age 2½ was expected to correlate positively and significantly with EF performance at age 3 ½.

2.4. Method:

2.4.1. Design

The current study includes both cross sectional and prospective comparisons. The structure of EF in toddlers was examined cross-sectionally with the use of confirmatory factor analysis. The validity of the derived factor scores was examined cross-sectionally at 2 ½ (phase 9), via bi-variate correlations with concurrent toddler age and verbal ability, and prospectively via bi-variate correlations with the EF performance of the same sample at age 3 ½ (phase 10).

2.4.2. Participants. The sample for the current analysis consisted of the 254 toddlers that attended their WCHADS phase 9 mother-child observational assessments and completed executive function measures. 256 toddlers (81% of those determined at birth as eligible for postnatal follow-up) attended their phase 9 assessment with their mother; however two of these toddlers refused to engage in any of the executive function measures, one due to apparent shyness and the other due to an apparent lack of understanding of researcher requests (this child had recently received a diagnosis of Autistic Spectrum Disorder). Data from these two toddlers were therefore excluded from the current analysis.

At the time of toddler executive function performance measurement, the current sample ranged in age from 27.3m to 41.2m, with a mean age 31.4 m (SD 2.4). This will further be reported as 2 ½ for brevity. The sample consisted of 125 (49%) boys (mean age 31.6m, SD 2.5) and 129 (51%) girls (mean age 31.2m, SD 2.2). 38.9% of the sample were in the most deprived quintile, 19% in the second most deprived quintile, 26.9 % in the mid quintile, 6.3 % in the second least deprived quintile and 8.9% in the least deprived quintile of the IMD (Noble, Wright, Dibben, Smith, McLennan, Anttila, Barnes, Mokhtar, Noble, Avanell, Gardner, and Lloyd, 2004). 51 % of mothers left full-time education at age 16 or below and 95.3% were of white British ethnic origin.

246 of these 254 toddlers (97%) completed an additional administration of measures of verbal ability, and a subset of EF measures, at the WCHADS phase10 research home visit assessment. At this follow up appointment, children ranged in age from 36m to 50m (mean age = 42.2m, SD =1.9m) and the sample consisted of 120 (49%) boys (mean age = 42.5m SD = 2.7m and 126 (51%) girls (mean age = 42.1m, SD = 1.9 m).

2.4.3. Procedure

2.4.3.1. WCHADS phase 9 (2 ½ year assessment) procedure. When toddlers were approaching 2 ½ years of age, their mothers were contacted and invited to attend the WCHADS study base to complete the phase 9 (2 ½ year) mother-toddler observational assessment. Upon arrival at the study base, two researchers met with mother and toddler. One researcher presented toys to occupy the toddler while the other provided an information sheet for the mother, regarding the procedures of the phase 9 mother-toddler observational assessment. Mothers were given time to read

the information sheet and had any subsequent queries answered. If the mother was happy to continue, consent forms were completed. A copy of the WCHADS phase 9 information sheet can be found in appendix 2. After obtaining informed consent, mother-infant dyads completed up to 2 hours of observational assessment with breaks built in as part of the over-arching study design. There was some variation in the duration of assessments according to individual participant needs. The assessments of toddler executive function for the current thesis lasted overall around forty-five minutes.

Measures of executive function performance were presented in a standard order for all participants; the order of presentation follows the order in which the measures are described in section 2.4.4.1. Cameras were positioned in the assessment room to allow recording of the assessment from four angles. The cameras fed to a TV screen and DVD recorder behind a partition wall so that the assessment could be observed concurrently by a second researcher. Two researchers were present to complete each assessment; one researcher administered the experimental measures while the other researcher observed and recorded toddler responses from behind the partition wall. DVD recordings were reviewed following the assessment to ensure accurate coding.

2.4.3.2. WCHADS phase 10 (3 ½ year assessment) procedure. When children were approaching age 3 ½, their mothers were contacted again and invited to take part in a WCHADS phase 10 research home visit. Those participants that expressed an interest in taking part were visited by a member of the WCHADS research team. Upon arrival, the researcher provided an information sheet to the mother regarding the procedures of the phase 10 research home visit. Whilst mothers

were given time to read the information sheet, the researcher introduced themselves to the child and engaged with them so that the child became familiar with them. After obtaining written informed consent, mother-infant dyads completed around 2 hours of assessment, with breaks built in as necessary, as part of the over-arching study design. During this time children completed measures of verbal ability and executive function performance for the current thesis which lasted in total around 20 minutes.

2.4.4. Measures

2.4.4.1. 2½ year (WCHADS Phase 9) Executive Function performance. The toddler measures of executive function are described below in the order in which they were administered. This was the same for all participants. It should be noted that due to the paucity of research examining executive function in children as young as the current study sample, there were (at the time of study design) no standardised assessments of executive function for children of this age. Therefore, the tasks administered were drawn from previous published research examining the development of executive function in young children, and from personal communication with researchers in the field. They have previously been used with children around the same age.

Whisper task (adapted from Kochanska, Murray, Jacques, Koenig and Vandergeest, 1996; Kochanska, Murray and Harlan, 2000). This task was adapted (on the basis of children's performance during pilot testing) from procedures used in previous literature examining the development of effortful control (Kochanska et al., 1996, 2000) in 2 year olds. The task was presented in line with procedures employed in the Cardiff Child Development Study (Hay, D personal communication). The task requires children to inhibit the pre-potent tendency to shout out responses, and

instead whisper, in an exciting identification game. The researcher presented a closed box containing 10 plastic animal figures. The animal figures were not visible to the child. The researcher then told the child that they were going to play a whispering game together and asked the child if they could whisper. The researcher spoke in a whisper throughout the remainder of the task. When it had been established that the child was able to whisper, the researcher asked the child if they would like to see some animals. The researcher explained that the animals were asleep but that s/he would carefully get them out of the box one-by-one. The child was asked to name each of the animals, but informed that they must whisper so as not to wake the animals.

Scoring and outcome measure: The child's response on each of the ten trials (i.e. animal presentations) was scored as follows:

0 = shout;

1 = normal/ mixed voice;

2 = whisper.

The outcome variable derived for use in the confirmatory factor analysis, Whisper, was the average score (0-2) for trials on which the child provided the correct animal name response.

This scoring scheme, following Sabbagh, Xu, Carlson, Moses and Lee (2006) was adopted as children showed variability in their knowledge of the correct animal names and therefore the number of trials for which they received a score.

Spin the pots (E.g. Hughes and Ensor, 2005). This multi-location search task was developed by Hughes and Ensor (e.g. Hughes and Ensor, 2005) to assess working memory early in development. A lazy susan tray with eight visually distinct

small pots was presented to the child. The researcher removed all the lids from the pots and placed an attractive sticker in six of them, whilst ensuring the child was attending to where the stickers were being placed. When all six stickers had been placed, the researcher explained that s/he had run out of stickers, therefore two of the pots (these were pointed out to the child in turn) were empty. The same two pots were left empty for all children. The researcher then covered the lazy susan tray with a piece of fabric, spun it, removed the fabric and asked the child to choose a pot to find a sticker. This was repeated until the child had retrieved all six stickers, up to a maximum of sixteen trials.

Scoring and outcome measures: Each trial was considered correct if the child found a sticker. The following three summary scores were then computed for use in the confirmatory factor analysis, based on previous literature employing this or similarly structured tasks with pre-schoolers.

- Pots Score: Reversed error score, following Hughes and Ensor (2005). This was calculated as 16 (the maximum number of trials permitted) minus the number of incorrect searches.
- Pots Span: Span score, (following Wiebe, et al., 2011). This was the number of trials in the toddlers' longest run of consecutively correct responses.
- Pots Perseverative errors: Reversed perseverative error score (following Brito, Grenell and Barr, 2014). This was calculated as 16 (maximum number of trials permitted) minus the number of trials on which a pot was chosen that had also been chosen on the immediately preceding trial.

Baby stroop task (adapted from Hughes and Ensor, 2005, on the basis of personal communication with Hay, D). This task, analogous to that developed by Hughes et al. (e.g. Hughes and Ensor 2005), and adapted for use on the Cardiff Child Development study (Personal communication) required toddlers to inhibit the pre-potent tendency to match items by size. Toddlers were presented with two laminated pictures; one large bear and one small bear. The toddler was then asked point to the "big" and "little" bears in turn, in order to ensure they understood the concept of big and little. The researcher then explained that:

> *"These bears are funny bears because the big bear likes to eat with the little spoon (the researcher places a little spoon on the picture of the big bear) and the little bear likes to eat with the big spoon (the researcher places a big spoon on the picture of the little bear); and the big bear likes to drink from the little cup (the researcher places a little cup on the picture of the big bear) and the little bear likes to drink from the big cup (the researcher places a big cup on the picture of the little bear)."*

The big and little spoons were identical in all but size, as were the big and little cups. The experimenter then presented the toddler with a series of big and little cups and spoons, one by one, in a standard order and asked the toddler to give each one to the 'bear that liked to use it'. The task was initially administered consisting of 4 trials. Part way through the study, the number of trials administered was increased to 12 in order to increase variability in performance across the sample.

Scoring and outcome measures: Each trial was scored as correct or incorrect according to whether or not the child placed the cup/spoon on the appropriate bear.

The following three summary scores were then computed for use in the confirmatory factor analysis:

- Stroop 1 to 4 = number of correct responses across trials 1 - 4
- Stroop 5 to 8 = number of correct responses across trials 5 - 8
- Stroop 9 to 12 = number of correct responses across trials 9 – 12

Snack delay (adapted from Kochanska et al., 2000). This task required toddlers to delay the gratification of retrieving a small edible treat placed in front of them. It was administered in the same way as Kochanska et al.'s (2000) original task with additional trials. The researcher placed an A4 laminated card displaying two hand prints, a transparent plastic beaker and a small hand bell on the table in front of the toddler. The researcher asked the toddler to place their hands on the hands card. They then placed a small edible treat (jelly tot/raisin) on the table and placed the inverted plastic beaker over the treat. The toddler was then told that they must wait until the researcher rings the bell before they could reach for and take the treat. The researcher administered one practice trial (in which feedback regarding performance was given) followed by 6 test trials, each with an increasing delay before ringing the bell (5s,10s,15s,20s,25s,30s). The experimenter looked away from the toddler, pretending to be otherwise engaged, during the delay periods and carefully lifted the bell (but did not ring it) half way through each delay period. The rule was restated for each trial.

Scoring and Outcome measures: Performance on each trial was scored according to the following scale:

1= ate treat before experimenter lifted bell

2= ate treat after experimenter lifted bell

3=touched but did not eat treat before experimenter lifted bell

4=touched but did not eat treat after experimenter lifted bell

5=touched cup or bell before experimenter lifted bell

6=touched cup or bell after experimenter lifted bell

7=waited for duration of the delay period without touching cup, bell or sweet.

The child also received 2 bonus points per trial if they kept their hands on the mat for the duration of the delay period. The following three summary scores were computed for use in the confirmatory factor analysis:

- Snack delay average 1 = average score across trials 1 and 2 (5s and 10s)
- Snack delay average 2 = the average score across trials 3 and 4 (15s and 20s)
- Snack delay average 3 = the average score across trials 5 and 6 (25s and 30s)

Detour reaching task (Hughes and Ensor, 2005). The first part of the detour reaching task (referred to here as the 'knob-route') required the child to inhibit a direct reach towards a desired object, and instead complete a novel means-end action to retrieve the item. The second part of the task (referred to here as the 'switch route') required the child to inhibit both a direct reach and the previously successful means end action in order to complete a new two-step means end action in order to retrieve the item.

The following description of the apparatus for the detour reaching task was taken directly from Hughes and Ensor's original description (Hughes and Ensor, 2005 pp. 503).

The box was made of aluminium and measured 30 x 30 X 30 cm. In its front face was a centrally located, circular opening, 15 cm in diameter, cut in a Perspex square. Mounted inside the box was a pyramid-shaped platform with a narrow, flat top on which a large marble rested. On either side of the opening in the front face of the box were photoelectric cells by means of which an infrared beam was directed toward the top of the platform. When the beam was broken, a trapdoor at the top of the platform was automatically activated. Immediately behind the marble's resting position was a small metal paddle that could be flipped forward by turning a round knob in the right, external side of the box. By this means, the marble could be projected forward and down a small chute running between the platform and a catch tray at the front of the box. A yellow light mounted at the right-hand top of the front face of the box remained lit when the knob route was available. The knob route could be blocked by throwing a small bolt located in front of the paddle, thus obstructing its forward movement. Throwing this bolt triggered a green light on the left of the front face of the box and caused the yellow light to be extinguished. The green light signalled to the subject that the switch route was now required rather than the knob route. To use the switch route, the subject had to operate a small, toggle switch on the left side of the box. This switch was inoperative when the knob route was available and the yellow light was on. The left side of the box also contained a small door (15 x 20 cm), by means of which the experimenter could manipulate the bolt and reposition the marble at the end of each trial.

The researcher placed a large marble on the platform inside the box and invited the toddler to retrieve it. Reaching directly into the box caused a trapdoor in the platform beneath the marble to open and the marble to drop out of sight. After the

toddler had experienced this three times, the researcher drew their attention to the yellow light on the front of the box and explained that the yellow light indicated that they must turn the knob on the right hand side of the box to retrieve the marble. The researcher pointed out the knob to the toddler, ensuring they were attending to its location. The researcher then demonstrated how to retrieve the marble using the knob twice, ensuring the toddler was attending each time. The marble was then placed back on the platform inside the box and the toddler was asked to retrieve it. Praise was given if the toddler retrieved the marble successfully; reminders regarding the use of the knob were given if the toddler reached directly into the box. This was repeated for five trials. If the toddler retrieved the marble successfully on three consecutive trials, the experimenter threw the bolt so that the knob route was blocked. This caused the yellow light to go out and a green light to come on.

The experimenter then drew the toddler's attention to the green light and explained that the green light indicated that the knob no longer worked and invited the toddler to try turning the knob so that they would experience this themselves. The experimenter then explained that the green light indicated that the toddler must now flick a small switch on the left hand side of the box (drawing their attention to the switch as they explained) and this would allow them reach inside and get the marble, without it falling through the trapdoor. The experimenter demonstrated this twice ensuring the toddler was attending each time, and then invited the toddler to retrieve the marble themselves. Praise was given if they retrieved the marble successfully; reminders regarding the use of the switch route were given if the toddler reached directly into the box or attempted to retrieve the marble using the knob route. This was repeated until the toddler achieved three consecutively correct responses up to a maximum of 12 trials. For the switch route trials, a more exciting flashing ball was

used to maintain interest.

Conceptually, the two parts of the detour reaching task (i.e. knob route trials and switch route trials) differentially draw on working memory and inhibitory control for their successful performance. Specifically, in part one ('knob route trials') the child must inhibit the propensity to reach directly into the box, and instead turn the knob on the side to retrieve the marble. In part two ('switch route trials') the child must, not only inhibit this action and a direct reach, but also remember to flick the switch on the opposite side of the box and then reach inside to retrieve the marble. In order to reflect this (and guided by personal communication with Hughes), the following two summary scores were constructed for use in the confirmatory factor analysis:

- **Detour Knob score** detour reach knob route trials: Each trial was considered correct if the child retrieved the marble on their first attempt and the summary score was determined by the following categorical scale:

  0= did not achieve criterion of 3 consecutively correct responses within 5 trials on part 1 ('knob route')

  1= reached criterion of 3 consecutively correct responses within 4 or 5 trials on part 1 ('knob route')

  2= reached criterion of 3 consecutively correct responses within the first 3 trials on part 1 ('knob route')

- **Detour Switch score** detour reach switch route: Each trial was considered correct if the child retrieved the marble on their first attempt and the summary score was determined by the following categorical scale:

0= did not achieve criterion of three consecutively correct responses on part one (‘knob route’) therefore part two (‘switch route’) not administered.

1= achieved criterion of three consecutively correct responses on part one (‘knob route’), but did not achieve criterion of 3 consecutively correct responses on part two (switch route) within the maximum 12 trials permitted.2= achieved criterion of 3 consecutively correct responses on part two (switch route) within 4 to 12 trials.

3= achieved criterion of 3 consecutively correct responses on part two (switch route) within the first three trials.

Table 3 summarises the tasks administered and the derived indicator labels used in the confirmatory factor analysis.

Table 3

Executive Function tasks administered and corresponding indicator scores.

Task	Indicator	Description
Spin the pots	Pots Score	16 – number of incorrect searches
	Pots Span	Longest run of consecutively correct searches
	Pots Perseverative errors	Number of trials a pot that had been chosen on the preceding trial was chosen
Baby Stroop	Stroop 1 to 4	Total correct responses over trials 1 to 4
	Stroop 5 to 8	Total correct responses over trials 5 to 8
	Stroop 9 to 12	Total correct responses over trials 9 to 12
Snack delay	Snack delay average 1	Average score over trial 1 (5s delay) and trial 2 (10s delay)
	Snack delay average 2	Average score over trial 3 (15s delay) and trial 4 (20s delay)
	Snack delay average 3	Average score over trial 5 (25s delay) and trial 6 (30s delay)
Detour reaching task	Detour Knob	Score for detour reaching task part 1 ('knob route trials')
	Detour Switch	Score for detour reaching task part 1 ('switch route trials')
Whisper	Whisper	Average score for trials on which child gave correct animal name response.

2.4.4.2. 2 ½ year (WCHADS Phase 9) Verbal Ability Measure. British Ability Scales II (BAS-II) Elliot, 1996.

The BAS-II (Elliot, 1996) is a standardised battery of individually administered subtests assessing different aspects of cognitive ability. Two subtests from the BAS-II (Elliot, 1996) were administered to assess toddler verbal ability, specifically the verbal comprehension subtest and the naming vocabulary subtest.

The verbal comprehension subtest requires respondents to point to pictures or manipulate objects in response to verbal instructions from the administrator. Items are administered in standard order with trials getting progressively more difficult as the subtest progresses, For example, an early trial asks "show me the bears eyes" (while presenting a picture of a teddy bear) whilst a later item asks "Before you give me the van, give me the little house" with a set of small wooden props presented (consisting of a big and little house, a big and little tree, a van a car, a bridge and two children).

The naming vocabulary subtest, a series of pictures are presented in a flip book format and the respondent is required to name each picture. Again, trials become progressively more difficult as the subtest proceeds. For example, an early item presents a picture of a shoe, a later item presents a picture of a thermometer. The raw scores for each of these subtests were used in analyses.

2.4.4.3. 3 ½ year (WCHADS phase 10) Executive Function performance.

Spin the pots (E.g. Hughes and Ensor, 2005). This was administered in the same way as at the 2 ½ year assessment, with a different set of props. The outcome measure was the reversed error score, following Hughes and Ensor (2005). This was calculated as 16 (the maximum number of trials permitted) minus the number of incorrect searches.

Whisper task (adapted from Kochanska et al., 1996). This was administered in the same way as at the 2 ½ year assessment, using a different set of plastic animal figures. The child's response on each of the ten trials (i.e. animal presentations) was scored as follows:

0 = shout;

1 = normal/ mixed voice;

2 = whisper.

The outcome score was the average score (0-2) for trials on which the child provided the correct animal name response, following Sabbagh, et al. (2006)

2.5. Statistical Methods.

The distributions of scores on each measure (indicator) were first examined for normality. Visual inspection of histograms and values of skewness and kurtosis indicated that data were non-normally distributed for all but two indicators (Detour Knob score and Whisper average score, see appendix 4). Descriptive statistics for toddler performance on each of the indicators and bivariate correlations amongst indicator scores were then calculated using SPSS Version 22 (see

Table 4 and Table **5**). Confirmatory factor analysis (CFA) was then applied, using Mplus 7 (Muthen and Muthen 1998 - 2012) to examine whether a single latent

executive function factor could explain variation in toddler EF performance on the tasks administered, or whether performance was better explained by a number of separable factors underlying separate executive functions. Guided by Brown (2006), weighted least squares (WLSMV) estimation was used for CFA since all but two indicators had non-normal distributions (see appendix 4) and a number of indicators were scored on an ordinal scale. The WLSMV estimator does not assume normally distributed variables and is considered the best option for modelling categorical data (Brown, 2006).

To evaluate the goodness of fit of each of the models tested, the Chi-square (χ^2) test of model fit, the root mean square error of approximation (RMSEA), the comparative fit index (CFI), and the Tucker-Lewis index (TLI) were examined. The Chi-square test of model fit assesses the difference between the observed (from the sample data) and expected (according to the model specified) covariance matrices, with a good fitting model resulting in a non-significant *p*-value, indicating that the expected and observed covariance matrices are not significantly different. Chi-square values closer to zero, reflecting a smaller difference between the hypothesised and observed covariance matrices indicate better model fit. The Chi-square test of model fit is sensitive to sample size and deviations from normality in the distribution of the outcome data, such that one may fail to reject an inappropriate model in small sample sizes; and may reject an appropriate model in large sample sizes or with highly skewed or kurtotic data (Curran et al., 1996). Thus, additional measures of model fit are also routinely taken into account.

The root mean square error of approximation (RMSEA) evaluates how well the hypothesised model reproduces the observed data whilst avoiding the sample size sensitivity of the chi-square test. Values range from zero to one, with smaller values

indicating better model fit. A value of 0.06 or less is indicative of acceptable model fit with a value of zero indicating perfect fit (Hu and Bentler, 1999).

The comparative fit index compares the 'fit' of the hypothesised model to the fit of the independence or null model (a model in which the variables are assumed to be uncorrelated). 'Fit' refers to the difference between the observed and hypothesised covariance matrices (as indicated by the chi-square index). Therefore, the CFI represents the ratio between the discrepancy of the specified model to the observed data and the discrepancy of the null model to the observed data, and thus indicates the extent to which the model specified fits the data better than the null model. Values range from zero to one, with larger values indicating better fit; Hu and Bentler, (1999) suggest a cut-off value of 0.95 or greater to indicate acceptable model fit.

The Tucker Lewis Index (TLI) also assesses the proportion of improvement in fit of the hypothesised model over a null model. Again, values range from zero to one, with larger values indicating better fit and a cut-off of 0.95 or greater indicating acceptable model fit (Hu and Bentler, 1999). Factor loadings of each of the indicators in the specified models were also considered, with an a priori threshold for factor loadings > 0.35 considered acceptable (following Comrey and Lee, 1992).The validity of the factor scores derived was then assessed by examining bi-variate correlations between factor scores and concurrent measures of development expected to be associated with EF ability (namely age and measures of verbal ability). The predictive validity of the factor scores for later EF ability was also assessed by examining bi-variate correlations between the factor scores derived at age 2.5 years and later Inhibitory Control (IC) and Working Memory (WM) performance approximately one year later at age 3 ½ years.

2.6. Results

Descriptive statistics of toddler performance for each of the indicator scores, and bi-variate correlations between these scores are presented in

Table 4 and Table **5** respectively.

Table 4

Descriptive statistics for toddler scores on EF performance indicators

Indicator	N	Mean	*SD*
Pots Score	254	10.69	3.56
Pots Span	254	3.30	1.21
Pots Perseverative Errors	254	15.19	1.40
Stroop 1 to 4	237	1.27	1.47
Stroop 5 to 8	144[a]	1.48	1.19
Stroop 9 to 12	141[b]	1.22	1.09
Snack Delay Average 1	236	7.42	2.00
Snack Delay Average 2	237	6.63	2.37
Snack Delay Average 3	222	5.97	2.42
Whisper Average score	247	0.96	0.79

	N	Median	Mode	Range
Detour Knob score	240	1	2	2
Detour Switch score	237	1	0	3

[a b] The smaller number of toddlers for these indicator scores is due to a change in the administration procedure part way through the study. Eight additional trials were administered to the last 144 toddlers.

Table 5

Bivariate correlations (Spearman's Rho) amongst EF performance indicators.

	Pots score	Pots span	Pots perseverative errors	Stroop 1 to 4	Stroop 5 to 8	Stroop 9 to 12	Snack delay average 1	Snack delay average 2	Snack delay average 3	Detour knob score	Detour switch score	Whisper average score
Pots score	-											
Pots span	**.44****	-										
Pots perseverative errors	**.51****	**.32****	-									
Stroop 1 to 4	-.13	-.03	-.07	-								
Stroop 5 to 8	-.11	-.13	-.12	**.52****	-							
Stroop 9 to 12	**-.18***	-.08	-.11	**.51****	**.55****	-						
Snack delay average 1	.13	**.18****	**.14***	-.00	.01	-.11	-					
Snack delay average 2	.05	**.14***	.07	-.05	-.07	-.14	**.62****	-				
Snack delay average 3	**.14***	.20**	**.16***	-.12	-.14	-.16	**.59****	**.74****	-			
Detour knob score	.09	**.13***	**.15***	-.02	-.14	-.03	**.24****	**.18****	**.19****	-		
Detour switch score	.10	.11	**.18****	-.08	-.13	-.05	**.25****	**.17***	**.15***	**.88****	-	
Whisper average score	**.14***	.07	.10	-.04	**-.17***	-.04	**.21****	**.17****	**.22****	**.15***	.10	-

** $p < 0.05$; ** $p < 0.01$*

2.6.1. Missing Data and Floor and Ceiling Effects. Varying levels of missing data were evident across indicators of EF performance and there were a range of reasons for missingness Table 6 displays a summary of these. Examination of the distributions of data (see appendix 4) also revealed possible floor and ceiling effects. Missing data and possible floor and ceiling effects will be described in the following section, providing a rationale for the final set of EF indicators included in the CFA.

Spin the pots task. Distributions of data revealed no floor or ceiling effects on this task. There were no sources of missing data for any of the indicators derived from this task.

Baby stroop task. Three issues arose that led to a decision not to include data from this task in the CFA. Each will be described in turn. First, it was noted during administration of the assessments that it was not always clear that toddlers had understood the instructions of this task and therefore whether they were attempting to follow task demands, as opposed to just playing with the props of the task. Consequently, it is not clear whether low scores on the task for some toddlers represent lower executive function ability or simply lack of understanding of the demands of the task.

Second**,** examination of the distributions of the available data for indicators from the baby stroop task supported the notion that toddlers either could not understand the instructions of the task or there were floor effects in performance. Specifically, 48.9% of toddlers achieved the minimum score of zero for stroop 1 to 4 compared with only 13.9% achieving the maximum score of four. Similarly, for stroop 5 to 8 and stroop 9 to 12 respectively, 24.5% and 34.5% of toddlers received the minimum score of zero with only 6.8% and 2.8% receiving the maximum score of four.

Third, by design there was a notable amount of missing data for indicators derived from the baby stroop task, particularly stroop 5 to 8 (42 %) and stroop 9 to 12 (44 %), This arose because the number of trials was initially set at four and subsequently increased to 12 in order to increase variability in scores across the sample. As such, 87 toddlers (34 %) received only four trials on the task, and therefore had missing data for later trials summarised by the stroop 5 to 8 and stroop 9 to 12 indicators. Of the 167 toddlers that received 12 trials on the task, there was a further source of missing data, in that some toddlers appeared to become bored of the repetitive nature of the task and either refused to continue or simply stopped engaging with the researcher (n= 12; 7 %, for stroop 5 to 8, n = 12 and n =15, 9 %, for stroop 9 to 12).

These three issues raised significant doubt as to the validity of the data collected on the task. A decision was made to exclude the task from the CFA analysis.

Snack Delay Task. There was a marginal amount of missing data for the three indicators derived from the Snack delay task. However, the distribution of data for two indicators, Snack Delay Average 1 (i.e. the average score across the five second and ten second delay trials) and Snack Delay Average 2 (the average score across the 15 second and 20 delay second trials) suggested possible ceiling effects in performance. Specifically, 41.5% and 26.6% of toddlers achieved maximum scores, compared with only 1.7% and 5.5% obtaining the minimum possible scores, respectively. Therefore, only the Snack Delay Average 3 indicator (average score across the 25s and 30 second delay trials) from the snack delay task was included in the confirmatory factor analysis. Distributions of scores on this indicator did not appear to suggest ceiling or floor effects.

Detour reaching task. Examination of distributions of scores also revealed possible floor effects on the Detour Switch score indicator from the detour reaching task. Specifically, 41.4 % of toddlers received the lowest possible score of zero for this indicator, with only 9.7% achieving the maximum score of three. In addition to this, there was a strong correlation (r_s = 0.88) between the two indicators (Detour Knob score and Detour Switch score) derived from the task. This, together with the nature of the coding schemes applied to derive these indicators (which rely on the child passing criterion on the Detour Knob route before going on to the Detour Switch route), introduced the possibility of duplication of data by including both indicators in the confirmatory factor analysis. Therefore, the Detour Switch score indicator, the harder level of the task, was not included in the confirmatory factor analysis.

Whisper task. There was a marginal amount of missing data for toddler scores on this task (2.8%). Distributions of data did not suggest floor or ceiling effects.

Table 7 details the final set of indicators included in the confirmatory factor analysis, with the tasks from which they were derived.

Table 6

Missing data and reasons for missingness.

Task	Indicator	% missing	Reasons for missingness (%)
Spin the pots	Pots Score	0	
	Pots Span	0	
	Perseverat-ive errors	0	
Baby Stroop	Stroop 1 to 4	7.1	Refused to engage (4.7) Off task behaviour (2.4)
	Stroop 5 to 8	42.1	Refused to engage (3.9) Off task behaviour (3.5) No clear response (0.4) Invalid administration (0.4) Only 4 trials administered (33.9)
	Stroop 9 to 12	44.1	Refused to engage (3.9) Off task behaviour (4.7) No clear response (0.4) Invalid administration (1.2) Only 4 trials administered (33.9)
Snack Delay	Snack Delay Average 1	7.1	Refused to engage (3.5) Invalid administration (1.2) Task discontinued due to infant distress (0.4) Technical problems (2)
	Snack Delay Average 2	6.7	Refused to engage (3.5) Invalid administration (1.2) Task discontinued due to infant distress (0.4) Task discontinued due to off task behaviour (0.4) Technical problems (1.2)

Table 6 (Continued)

Missing data and reasons for missingness

Task	Indicator	% missing	Reasons for missingness (%)
Snack delay (Continued)	Snack Delay Average 3	12.6	Refused to engage (4.3) Invalid administration (6.7) Task discontinued due to infant distress (0.4) Task discontinued due to off task behaviour (0.4) Technical problems (0.8)
Detour reaching task	Detour Knob score	5.5	Invalid administration (2.4) Task not administered due to family time constraints (1.2) Task discontinued due to infant distress (0.4) Technical problems (1.6)
	Detour Switch score	6.7	Refused to engage (2.4) Invalid administration (1.2) Task not administered due to family time constraints (1.2) Task discontinued due to infant distress (0.4) Technical problems (1.6)
Whisper	Whisper Average	2.8	No response to all trials (2) Not administered (0.8)

Table 7

Executive Function tasks administered and corresponding indicator scores derived and used in confirmatory factor analysis.

Task	Indicator	Derivation of score
Spin the pots	Pots Score	16 - number incorrect searches
	Pots Span	Longest run of consecutively correct searches
	Pots Perseverative errors	Number of trials a pot that had been chosen on the preceding trial was chosen.
Snack delay	Snack Delay Average 3	Average score over trial 5 (25s delay) and trial 6 (30s delay)
Detour reaching task	Detour Knob score	Score for detour reaching task part 1 ('knob route trials')
Whisper task	Whisper Average	Average score for trials on which the child gave the correct animal name response.

2.6.2. Confirmatory factor analysis. Two competing models were tested to examine whether toddler performance on the tasks administered was accounted for by a unitary latent executive function factor (Model 1) or whether variation in performance was better explained by two separable factors, namely working memory and inhibitory control (Model 2). Since a number of indicators were derived from the same task, models were specified to indicate that these indicators were correlated with each other. Specifically, since Pots Score, Pots Span and Pots Perseverative errors were different measures from the same task (spin the pots), models were specified to allow these indicators to be correlated.

The overall model fit statistics for the competing models can be seen in Table 8, factor loadings of indicators in each model can be seen in **Error! Reference source not found.** and model diagrams can be seen in **Error! Reference source not found.**

Table 8

Model fit statistics for models tested.

Model	$\chi 2$	df	p	RMSEA	CFI	TLI
1. Unitary Model (EF)	4.15	6	.66	0.00	1.0	1.03
2. Two-Factor model (WM & IC)	8.61	8	.38	0.02	1.0	0.99

First, a unitary model of performance, in which all indicators loaded on a common EF factor, was tested. Model 1 fit the data well, with a non- significant χ^2 value and adequate fit statistics. Each indicator loaded significantly on the EF factor, however, factor loadings from four of the six indicators (Pots Score, Pots Span, Pots Perseverative errors and whisper) were observed to be somewhat low (following Comrey and Lee ,1992) ranging from .25 to .34 and below the a priori criterion set. The higher factor loadings of Snack delay average 3 and Detour Knob score suggested that the model was driven primarily by indicators from tasks considered a piori to assess toddler inhibitory control. As such, the resultant unitary factor model better represented inhibitory control, rather than executive function more broadly.

Next a two-factor model (Model 2) comprising a working memory factor and an inhibitory control factor was tested to determine whether a two-factor solution might better summarise toddler executive function performance at this age. Again, the overall model fit statistics indicated that this model fit the data well, exhibiting a non-

significant χ^2 value and adequate fit statistics (see Table 8); all indicators loaded significantly on to their respective factors. Furthermore, indicator loadings were now observed to be good to excellent, (ranging from .53 to .79) for three of the previously mentioned indicators (Pots Score, Pots Span, Pots Perseverative errors) and acceptable, at .35 for the fourth (Whisper), following Comrey and Lee, (1992) see **Error! Reference source not found.**.

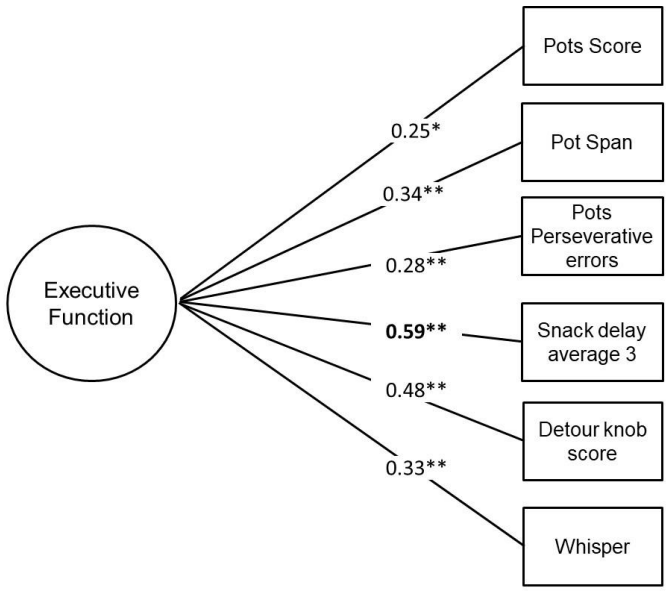
Overall, model 2 was considered the best fitting model for the observed data based on the model fit statistics, the factor loadings of the indicators included, together with the fact that conceptually model 1 appeared to represent a model of inhibitory control specifically rather than EF more broadly. The modelled factors of working memory and inhibitory control in model 2 exhibited a moderate positive correlation of *r* =.43. A formal test of relative model fit between model 1 and model 2 was not possible due the application of the WLSMV estimation in MPlus. This was necessary due to the presence of categorical data and the non-normal distributions of data for the indicators employed in the CFA.

Table 9

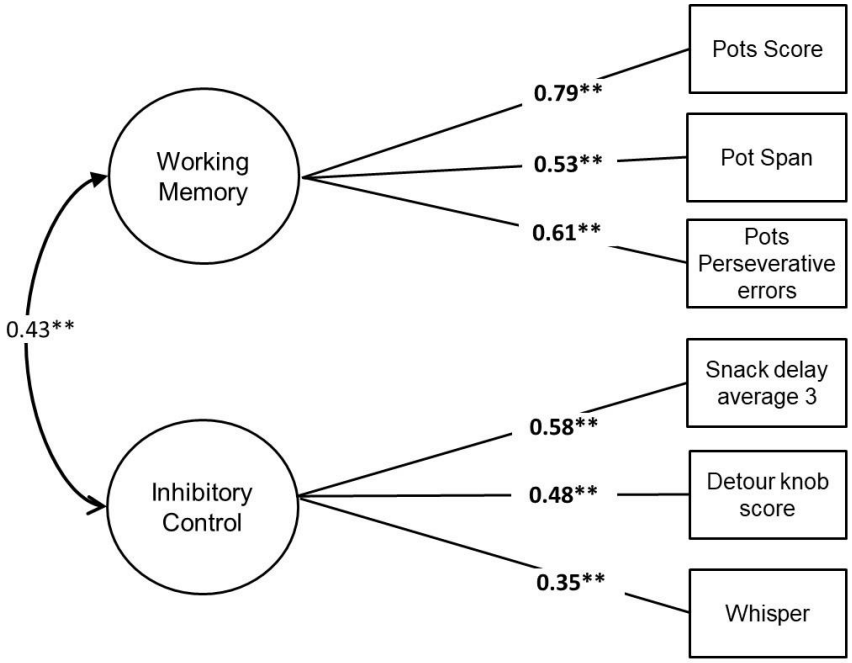
Standardised Factor loadings and proportion of variance in indicator scores accounted for by modelled factors.

	Model 1		Model 2		
	Standardised factor loadings	R^2	Standardised factor loadings		R^2
Indicator	EF		WM	IC	
Pots Score	0.25*	.06	**0.79****		0.62
Pots Span	0.34**	.11	**0.53****		0.28
Pots Perseverative errors	0.28**	.08	**0.61****		0.38
Snack delay average 3	**0.59****	.35		**0.58****	0.34
Detour Knob score	**0.48****	.23		**0.48****	0.22
Whisper	**0.33****	.11		**0.35****	0.12

EF = executive function, *IC*=inhibitory control, WM = working memory; R^2=proportion of variance in indicator accounted for by factor, * $p < 0.05$; ** $p < 0.01$, factor loadings exceeding the a priori criterion of 0.35 are highlighted in bold type



Model 1 : the Unitary model (Executive Function)



Model 2: the 2 Factor model (Working Memory and Inhibitory Control)

Figure 4

Path diagrams displaying two alternative CFA models designed to test the structure of toddler EF tested at age 2 ½.

Standardised factor loadings and coefficients are shown. * $p < 0.05$; ** $p < 0.01$. Factor loadings exceeding the a priori criterion of 0.35 are highlighted in bold type-face.

2.6.3. Power Analysis. Post hoc power analysis was conducted using the software package G-Power (Faul, Erdfelder, Lang and Buchner, 2007). The power to detect large effects (w = 0.5 following Cohen, 1988) was 0.99, the power to detect medium effects (w = 0.3 following Cohen, 1988) was 0.95 and the power to detect small effects (w = 0.1 following Cohen 1988) was 0.16.

2.6.4. Validity of derived factor scores. The construct validity of the WM and IC factor scores was assessed by examining bi-variate correlations with concurrent age and measures of verbal ability (Table 10). Associations were positive and significant in the expected direction showing that higher IC and WM scores were associated with older age and verbal naming and comprehension scores at the time of testing. This finding supports the notion that future work examining children's performance on EF tasks should take account of children's emerging verbal ability at that time as these factors may influence task performance.

The predictive validity of the WM factor score was assessed by examining its bi-variate correlation with children's performance on a repeat administration of the spin the pots task (drawing on working memory ability) at 3 ½. A significant positive association was found between WM indices at the two time points. The predictive validity of the IC factor score was assessed by examining its bi-variate correlation with a repeat administration of the Whisper task (drawing on inhibitory control ability) at 3 ½ (Table 11)

Table 10

Bi-variate correlation (Spearman's Rho) between EF factor scores (WM and IC) and concurrent age and verbal ability.

Developmental indices	WM factor score	IC factor score
Age at time of 2 ½ year assessment	.20**	.26**
N	254	254
BAS Verbal comprehension score	.29**	.32**
n [1]	228	228
BAS Verbal Naming score	.19**	.33**
n [2]	229	229

** $P < 0.01$; [1] the smaller n for these comparisons is due to missing data for verbal comprehension subtest scores as a result of child refusal/ lack of engagement (n =18) or invalid test administration (n =8); [2] the smaller n for these comparisons is due to missing data for verbal naming subtest scores as a result of child refusal/ lack of engagement (n =12) or invalid test administration (n= 13).

Table 11

Bivariate correlation (Spearman's rho) between age 2 ½ EF factor scores (WM and IC) and age 3 ½ working memory and inhibitory control task scores.

	WM factor score age 2 ½	IC factor score age 2 ½
Age 3 ½ Spin the pots (WM task) score (16- N incorrect responses)	.22**	.17**
n[1]	245	245
Age 3 ½ Whisper (IC task) score (average inhibition score for correct animal name responses)	.03	.24**
n[2]	243	243

*** P < 0.01;*

[1] the smaller n for these comparisons is due to missing data for Spin the pots task scores as a result of child refusal/ lack of engagement (n = 1)

[2] the smaller n for these comparisons is due to missing data for Whisper task scores as result of child refusal/ lack of engagement (n =2) or child inability to speak (n= 1).

2.7. Discussion

The goal of current research was to characterise the structure of EF in a sample of toddlers within a relatively narrow age range compared with previous studies in this area of the literature, and at an age that has previously been largely unstudied. This was achieved by administering a battery of EF tasks that were drawn from the developmental literature and considered a priori to place demands on working memory or inhibitory control to a sample of 254 toddlers with a mean age of 31 months, ranging between 27 months and 41 months. Using confirmatory factor analysis (CFA), the fit of a unitary and a two-factor model of performance was examined.

CFA revealed that toddler EF performance was best summarised by a two-factor model in which task indicators loaded onto two separable factors that were moderately correlated. Thus, the results support an integrative structure of EF during toddlerhood, in which the two latent factors of working memory and inhibitory control are clearly distinguishable yet related constructs. Assessment of the validity of the derived factor scores indicated that they were a valid representation of toddler WM and IC abilities (see Table 10 and Table 11). Specifically, significant positive associations were revealed between WM factor scores and toddler age at time of assessment, toddler verbal ability (comprehension and vocabulary) and performance on the working memory task at 3 ½. Similarly, significant positive association were found between IC factor scores and toddler age at time of assessment, toddler verbal ability (comprehension and vocabulary) and performance on the WM and IC task at 3 ½.

2.7.1. Study Findings in the Context of Previous Research. The current study provides evidence that the latent structure of EF very early in its development, at age 2½, exhibits an integrative structure characterised by both unity and diversity. Specifically, EF performance loaded on to two clearly distinguishable latent factors of working memory and inhibitory control, and these two latent factors were moderately correlated. The current findings are in line with those reported in adults, adolescents and older children, in which an integrative model of EF was also found to provide the best fit for the observed data (e.g. Miyake et al., 2000; Lehto et al., 2003; Cassidy et al., 2016; Huizinga et al., 2006).

The current findings differ from some previous studies reporting on the structure of EF in early childhood with slightly older samples, such as those of Hughes et al. (1998) and Espy et al. (1999) who applied principal components analysis (PCA) to pre-schoolers performance on a battery of EF tasks. Both Hughes et al. (1998) and Espy et al. (1999) reported that variation in executive function performance was explained by a number of separate component processes. However, the application of PCA in the investigation of the latent structure of EF may be limited by the task impurity problem inherent in tasks assessing this cognitive domain. Such tasks commonly rely on distinct and overlapping executive, as well as non-executive processing requirements. Thus, the factor structures reported by Espy et al. (1999) and Hughes et al. (1998) may reflect differences in the non-executive demands of the tests administered, rather than a set of separable latent sub- components of EF per se. Indeed, Hughes et al.'s (2010) findings revealed that when the effects of non-verbal general cognitive ability, verbal ability and age were regressed out, a slightly different factor solution was revealed. Specifically, one of the EF tasks administered loaded equally onto two of the extracted factors, rather than onto to a single factor as had been the case when these effects were not regressed

out. It could be argued that the factor structure revealed when the effects of these non-executive processes were regressed out could be considered support for an integrative model of EF. That is, performance on some of the tasks loaded onto distinct factors and was therefore separable, whereas performance on another task loaded equally onto two of the extracted factors, suggesting some but not entirely common processing requirements.

The statistical approach adopted in the current study, CFA, is considered a more robust statistical method for examining the structure of EF (e.g. Miyake et al., 2000), since it allows for separating out shared variance unrelated to the latent abilities under study (such as non-executive processes). A number of groups have applied CFA to examine the structure of EF in preschool and early childhood samples previously. The current results are in contention with some such findings and in agreement with others. For example,Weibe et al. (2011), examining the structure of EF performance in three year olds and Hughes et al. (2010) in four and six year olds longitudinally; reported that a unitary model of executive function provided the best fit for the observed data. However, as conceded by Weibe et al. (2011) a componential model of executive function in which performance loaded on to two separable factors also provided adequate fit to the data, but was rejected on the basis of parsimony. Thus, both a unitary model and a two-factor model separating working memory and inhibitory control adequately fit the observed data. With no significant improvement in fit of one model over the other, the simpler model (unitary) was retained, as appears to be the convention in CFA studies of the structure of EF.

The main difference between the findings reported in the current study and those of Weibe et al. (2011) lies in the strength of the correlation observed between the factors derived from the two-factor models. Specifically, Weibe et al.'s (2011) two-factor

model exhibited a high correlation (*r* = .8*)* between the WM and IC factors derived suggesting that the two factors had little unique explanatory power. In contrast, the WM and IC factors retained in the current analyses displayed a moderate significant correlation of *r* = .4, suggesting that though related constructs, WM and IC were clearly distinguishable latent abilities in the current sample. Furthermore, though the unitary model tested in the current study provided adequate fit to the data and it could be suggested that this model should be retained on the basis of parsimony, the factor loading of individual indicators onto the unitary factor derived was only adequate for a subset of tasks. This subset were all considered a priori, based on their application in previous studies of EF in children, to place demands on inhibitory control. In contrast, those indicators considered a priori to draw more heavily on working memory demands exhibited inadequate factor loadings based on the a priori criterion set. Thus, conceptually, the unitary model represented a model of inhibitory control rather than EF more broadly.

Although, Hughes et al.'s (2010) findings supported a unitary structure of EF early in development (mean age 4 years 3 months) they were unable to examine the fit of a componential model to their data as the number of indicators of EF performance was too few. Therefore, as reported by Hughes et al. (2010) in their discussion, they could not dismiss the possibility that a componential or integrative model of performance may provide a better fit to the observed data.

The current findings are also in agreement with more recent reports examining the structure of executive function in early childhood, but with slightly older samples. Lonigan et al., (2016), Lerner & Lonigan (2014), Usai et al., (2014) and Miller et al., (2012) all reported that an integrative structure of executive function, consisting of two separable but moderately correlated factors (working memory and inhibitory control)

provided the best fit for their data. Discrepancies amongst previous studies and the differences between the findings the current study and those of Wiebe et al. (2011) and Hughes et al. (2010) may be explained in a number of ways. Firstly, the age of children at EF assessment varies across studies and given the rapid development of EF over the childhood years, there may be variations in its structure across development. Similarly, differences across studies in the tasks employed to assess EF may account for the discrepant findings. Indeed, Miller et al. (2012) report that "the structure of EF that emerges from CFA is influenced by task and performance indicator selection". Specifically, in testing the fit of two separate model series to the same data, they found that selecting performance indicators similar to those employed by Wiebe et al. (2011) replicated the favoured unitary structure of Wiebe et al. (2011); however, when different indicators were selected, a two-factor model of performance provided the best fit for the data.

To our knowledge, only one previous study (Mulder et al., 2014) has examined the latent structure of EF as early in development as the current study. Though Mulder et al.'s (2014) study was concerned with different aspects of latent structure than the current study (specifically examining performance on 'Hot' and 'Cool' EF tasks) the findings converge. Both studies report an integrative structure of EF, in which latent subcomponents are distinguishable yet moderately correlated, to best characterise EF performance at this very early stage in development.

2.7.2. Implications of the Current Findings. The current study found that, in a population with a diverse socioeconomic background, EF at age 2 ½ exhibits an integrative latent structure. This is an important addition to the existing developmental literature with only one previous study examining the structure of EF at this early age (Mulder et al., 2014).These findings pave the way for further research into the development of component EFs over time and how these may relate to developmental disorders and the development of psychopathology over time. Greater understanding of the development of component EFs and their correlates in typically developing children provides a comparison against which disorder may be identified. With a number of developmental disorders characterised by impairments in EF this could help to identify such disorders early, thus expanding the window of opportunity for intervention aimed at improving outcomes. The potential for earlier identification of such disorders would also help to provide a greater understanding of them through increasing the opportunity for research investigating their development. The current findings may also allow a more fine-grained analysis of the association between EF abilities and academic achievement by allowing investigation of the specificity of the association between different EF components and achievement outcomes.

2.7.3. Methodological strengths.

2.7.3.1. Age range of toddlers. The current sample of toddlers was recruited over a relatively narrow age range (13 months) in comparison to the majority of previous CFA studies of EF performance in pre-schoolers. With the exception of Wiebe et al.'s (2011) sample (who were all assessed within weeks of their third birthday), samples from previous CFA studies examining the structure of EF in early childhood have varied in age ranges from 16m to over 3 years (Table 1 in section 2.1.6). Such wide age ranges are an important consideration since EF is thought to develop rapidly over the preschool and school age period. In samples with larger age ranges, the structure of EF may vary within the sample and depending upon the distribution of ages across the sample, the structure of EF in younger children may be masked by that of older children or vice versa.

2.7.3.2. Sample characteristics and recruitment procedure. The sample for the current study comprised mother-infant dyads from the WCHADS intensive study sample. The WCHADS intensive study sample was selected using stratification based on psychosocial risk resulting in an over-representation of risk compared with a community population. The intensive sample exhibited higher levels of deprivation than the broader UK population, with 39% living in conditions equivalent to those observed in the most deprived quintile of the IMD for the UK at the time of recruitment in 2007 and 2008 (Noble, et al., 2004), and more participant mothers left full-time education at age 16 or below (51%) than the broader UK population. Thus, the current study sample exhibited higher levels of psycho-social risk, socio- demographic risk and lower levels of maternal education than typically observed in the broader UK population. As a result of this, a broader range of toddler abilities is likely to have modelled in the current data

set compared with one from a more socio-demographically restricted sample. A systematic comparison of the demographic characteristics of this sample with the research conducted previously is hampered by the sporadic reporting of these descriptive data in the literature (see Table 2 in section 2.1.6). Six out of the eleven studies reviewed, who examined the structure of executive function early in development, failed to report the demographic characteristics of their samples. Therefore, it is not clear whether their samples exhibited adequate variability in socio-demographic risk. The remaining five studies that reported these characteristics used proxy measures of relative deprivation such as parental education level, family income or eligibility for public medical assistance. The current study improved on this by using a well validated measure of relative deprivation (IMD, Noble et al., 2004) that considers multiple aspects of the environment to derive an index of deprivation. Furthermore, these studies did not report complete information regarding the distributions of participants across the range of socio-demographic risk, thus it still not always clear that the full range has been adequately sampled.

2.7.3.3. Sample size. Whilst there was some missing data for some indicators of EF performance the size of the current sample (N=254) is one of the larger samples amongst studies designed to examine the structure of EF in young children.

2.7.3.4. Low levels of missing data. Data from just two toddlers (1%) was excluded from the CFA, as they would not engage in the EF tasks (nor in tasks associated with the wider WCHADS study assessment), one due to apparent shyness and the other due to a lack of understanding of researcher requests.

For the remaining 254 toddlers included in the analysis, missing data ranged from 0% to 13% across performance indicators. In comparison to previous research in this area of the literature and considering the younger age of the current sample (mean age 31 months), this level of missing data is relatively low for the field (see Table 12) and may be expected due to the demands being placed on EF abilities just as these functions are coming on line in toddlers.

Table 12

Mean age, sample sizes and proportion of missing data in previous research examining the structure of EF in early childhood.

Study	N	Mean age	Missing data (%)
Hughes et al. (1998)	45	3y11m	10% participant's data excluded from due to failure to complete at least one task
Espy et al. (1999)	117	3y 4m	1% to 16% across tasks
Weibe et al. (2008)	243	3y 11m	23% to 44% across tasks
Hughes et al. (2010)	191	T1: 4y 3m T2: 6y	0%
Weibe et al. (2011)	228	3y	0% to 14% across tasks.
Miller et al. (2012)	129	4y 2m	0% to 15% across tasks
Lerner and Lonigan (2014)	289	4y 8m	14% to19% across tasks.
Mulder et al. (2014)	2437	2y 4m	"64% completed all 5 tasks 23% completed 4/5 tasks 8% completed 3/5 tasks 4% completed 2/5 tasks" Further details not reported
Usai et al. (2014)	175	T1: 5y T2: 6y	17% excluded due to scoring < 10th percentile on a measure of intelligence.
Lonigan et al (2016)	241	4y 6m	0%

2.7.4. Methodological Limitations

2.7.4.1. Shared method variance. In conducting CFA, it has been proposed that it is preferable to employ a single outcome variable (as opposed to multiple variables) from each task administered, to represent performance indicators. This is because different indicators derived from a common task are typically more highly correlated with each other than with indicators derived from other tasks, due to shared method variance (Gorsuch 1983). As Lee et al. (2013) assert, employing multiple indicators from the same task to model a latent factor runs the risk of focusing the factor solution on incidental task specific similarities across indicators from common tasks rather than true variance attributable to the latent construct/constructs purportedly underlying task performance. Since the three indicators loading onto the modelled working memory factor were derived from a common task, it could be argued that the shared variance contributing to the modelled working memory factor was solely due to shared method variance rather than a latent working memory factor per se. Administration of additional tasks considered a priori to place demands on toddler working memory ability may have resolved this issue. However, the EF tasks were embedded within a longer mother-toddler developmental assessment as part of the wider WCHADS study. Therefore, time constraints determined by participant availability and, importantly, toddler fatigue precluded this. We attempted to account for shared method variance amongst the three indicators derived from the Spin the Pots task, by specifying a CFA model in which the error variances of these indicators were allowed to correlate (following Brown, 2006).

2.7.4.2. Inadmissible task data. As outlined in section 2.6.1, data from the baby stroop task was excluded from the CFA analysis. Exclusion of this data may be considered a limitation of the current study by resulting in a smaller number of indicators of EF ability for confirmatory factor analysis. However, it was considered that inclusion of the data would have compromised the validity of the results for a number of reasons (a) as it was not clear that task scores reflected toddler EF performance rather than their comprehension of task instructions more generally and (b) examination of the distributions of scores suggested there may have been floor effects in toddler performance on the task.

2.7.4.3. Lack of formal comparison of competing models. The current findings are also limited through the lack of formal comparison of the unitary and two factor models tested. The reasons for this are two-fold (a) the models tested were not nested models, this is a requisite for formal model comparisons in Mplus and (b) the ordered categorical nature of some indicators of EF in the models tested required use of the WLSMV estimator in tests of model fit. Use of this estimator precludes formal model comparison tests.

2.7.5. Conclusions, Implications and Directions for Future Research. The current study examined a population with diverse socioeconomic backgrounds, likely sampling across the range of toddler abilities. The findings strongly support an integrative structure of EF in toddlerhood. This is an important addition to the existing developmental literature as only one previous study (Mulder et al., 2014) has examined the structure of EF at this early age, with similar findings, but a slightly different focus. Together, these findings pave the way for further research into the development of

component EFs and how these may relate to neurodevelopmental disorders and the development of psychopathology over time. Greater understanding of the development of component EFs and their correlates in typically developing children provides a comparison against which disorder may be identified. With a number of developmental disorders characterised by impairments in EF, such research could help to identify difficulties early, thus expanding the window of opportunity for early intervention aimed at improving outcomes. The current findings may also allow a more fine-grained analysis of the specificity of associations between early EF abilities and different domains of subsequent academic performance. Finally, empirical support for the reliable measurement of executive functions at this early stage, in toddlerhood, will enable studies to be conducted that examine the earliest prenatal and postnatal predictors of emerging executive functions with the potential to inform early interventions to support their development over time.

3. Study 2: Emerging Executive functions in toddlerhood: Does exposure to prenatal stress predict development in a sex dependent manner?

3.1. Background

This chapter of the current thesis examines the association between maternal prenatal stress (PNS) and toddler executive function ability (EF) and on the basis of previous animal and human research makes the case for an investigation of possible sex-dependent effects. The conceptualisation of the term stress will first be outlined. This is followed by a review of findings in the human and animal literature investigating the association between PNS and measures of cognitive development, with findings investigating sex dependent effects in this association also reviewed. It will become apparent from the previous literature that findings in animal models focus mainly on spatial learning and memory, and in the human literature on measures of general cognitive development. A proposed mechanism for the reported association is then highlighted, providing the rationale for examining the influence of PNS on executive function abilities specifically, as opposed to the more general measures of cognitive development employed in the majority of previous literature examining this association in early development. Limitations in this field of the literature are then highlighted in providing the rationale for the current study.

3.1.1. Definitions of prenatal stress. Stress can be considered a psycho-physiological construct in that it is accepted to have both psychological and physiological connotations. Lazarus and Folkman, (1984) offers an influential account of the psychological aspects of this dual definition; describing the experience of stress to be the result of an individual's perception of an imbalance between the demands of a

situation and their perceived capacity to cope.

In terms of the physiological aspects of this dual definition; one of the major physiological stress response systems and one that has received considerable research attention in the investigation of the effects of PNS on offspring development is the hypothalamic-pituitary-adrenal (HPA) axis. The HPA axis involves the initiation of a cascade of hormones released to mobilise an organism's resources, in order to prepare them for action. In response to a stressor (an event or situation perceived by the individual to disrupt homeostasis) corticotropin releasing hormone (CRH) is released from the hypothalamus. CRH then stimulates the release of adreno-corticotropic hormone (ACTH) from the pituitary gland, which in turn stimulates the release of glucocorticoids (cortisol in humans and other primates, corticosterone in rats) from the adrenal cortex. The glucocorticoids bind with glucocorticoid receptors in the brain, stimulating a negative feedback response, thus shutting off the stress response at the hypothalamus.

In view of this psycho-physiological conceptualisation of the construct of stress, given the range of situations that may challenge an individual's capacity to cope or threaten to disrupt homeostasis, together with the range of situations that may be *perceived* as such by different individuals; research examining the association between PNS and offspring development has approached the measurement of stress heterogeneously across studies. Research in animal models has the advantage of experimental manipulation of the environment in order to systematically introduce external stressors that are expected to challenge the organism's capacity to cope and therefore threaten to disrupt homeostasis. Such studies are therefore able to apply relatively objective measures of stress exposure, including restraint, electric foot shock, subcutaneous injection, disruption of social relationships, predator exposure and

exposure to unexpected loud noise (e.g. Aleksandrov, Polyakova & Batuev, 2001; Chapillon, Patin, Roy, Vincent & Caston, 2002; Lemaire, Koehl, & Abrous, 2000; Nishio, Kasuga, Ushijima & Harada, 2001; Schultz, Pearson, Neeley, Berger, Leonard, Adams, & Stevens, 2011). Other authors have taken a direct physiological approach, mimicking the physiological stress response via the administration of synthetic gluco-corticoids to pregnant animals (Kranendonk, Hopster, Fillerup, Ekkel, Mulder & Taverne, 2006).

In view of the obvious ethical constraints of applying parallel methodology in human participants, researchers in this area have relied on the measurement of naturally occurring stress experienced by pregnant women. However, this too has been approached in a number of different ways. A common approach has been to employ self-report measures of subjective feelings of stress and stress symptoms (e.g. Buitelaar, et al., 2003; Davis and Sandman, 2010). Another approach has been to mimic the methods employed in the animal literature more closely by asking pregnant women to report on the occurrence, during pregnancy, of major life events or lower level 'daily hassles' that are widely deemed as stressful. (e.g. Bergman, Sarkar, O'Connor, Modi & Glover 2007). A number of studies have taken advantage of the occurrence of natural/ man-made disasters (e.g. ice storm) and examined aspects of development of the children of women who were exposed during pregnancy (e.g. LaPlante, Brunet, Schmitz, Ciampi & King, 2008). Other authors have taken a physiological approach, assessing the association between biological markers of the physiological stress response (such as salivary cortisol concentration) during pregnancy and aspects of child development (e.g. Davis and Sandman, 2010).

3.1.2. Prenatal Stress and its Developmental Impact on the Offspring. The notion that a pregnant woman's emotional state may affect the development of her unborn child dates back to the time of Hippocrates (Huizink, Mulder & Buitelaar, 2004). Indeed, over a thousand years ago, appreciation of the importance of prenatal factors led to the institution of the first antenatal clinic in China. Rather than maintaining physical well-being, the clinic aimed to ensure 'tranquillity' in the mother, and therefore in the unborn child (MacFarlane, 1980). Empirical investigation of the importance of prenatal factors is relatively recent in comparison. Findings from research conducted in animal models were the first to confirm adverse effects of prenatal maternal stress on the development of the offspring. In the animal literature, pregnant females are subjected to an experimentally manipulated external stressor. The aim is to induce concomitant changes in the maternal physiology and thus alterations in the foetal environment. Such studies initially examined the effects of PNS on birth outcome; with results reporting spontaneous abortion, smaller litter size, lower birth weight, and poor physical growth in offspring of PNS animals (Paarlberg, Vingerhoets, Passchier, Dekker and Herman,, 1995). Further research in the animal literature revealed that the adverse effects of PNS extend to a range of adult outcomes. Offspring of prenatally stressed rats have been found to exhibit decreased motor abilities, reduced propensity for social interaction, increased anxiety in intimidating or novel situations, a reduction in cerebral asymmetry as well as deficient learning and spatial memory capabilities (Weinstock, 2001; Lemaire et al., 2000; Mychasiuk, Gibb and Kolb, 2011). In non-human primate models, PNS has been shown to affect aspects of cognitive and motor development and fearfulness in the offspring (see Huizink et al., 2004 for a review).

3.1.3. Examining Prenatal Stress and Offspring Cognitive Development in Animal Models.

Of particular relevance to the current study is research examining the effect of prenatal stress on offspring cognitive development. Well- replicated findings in animal models have demonstrated a detrimental impact of PNS on this developmental domain across a number of species. Studies in the animal literature allow causal inference to be drawn due to direct experimental manipulation of stress exposure. In research examining the offspring of pregnant rats that were exposed to an experimentally manipulated stressor, cognitive abilities have been assessed using the Morris water maze, the radial arm maze, the T-maze, the Y-maze and passive avoidance tasks. Despite the differing experimental manipulations of stress exposure in such studies (e.g. restraint, re-housing with an unfamiliar social group, subcutaneous injection, electric foot shock, and crowding), offspring of prenatally stressed dams have been robustly reported to perform more poorly on the aforementioned tasks. Thus, representing relative deficits in spatial learning, cognitive flexibility, spatial working and short-term memory, and (non-spatial) long-term memory, compared to non-PNS offspring (Aleksandrov et al., 2001; Archer and Blackman, 1971; Barlow et al., 1979; Grimm and Frieder, 1987; Hayashi, Nagaoka, Yamada, Ichitani, Miake, & Okado, 1998; Lemaire et al., 2000**;** Szuran, Pilska, Pokomy & Weltzl, 2000; Vallée, Maccari, Francoise, Hervé, Michel & Willy, 1999; Zagron and Weinstock, 2006).

Research in this area has been extended, providing evidence of analogous effects of prenatal stress in other species. For example, Coulon, Nowak,Adanson, Petit, Lévy & Boissy, (2015) found that lambs of ewes that were exposed to a variety of unpredictable stressors during pregnancy (including restraint, transport and rehousing with an unfamiliar social group) displayed deficits in spatial learning compared to control lambs. Prenatal stress has also been shown to induce cognitive deficits in non-human

primate models, though the cognitive capacities implicated differ somewhat to those in other species of animal model. Specifically, in a series of observations by Schneider and Colleagues (2002), pregnant primates were removed from their home cages and subsequently exposed to uncontrollable loud noise bursts. Offspring of the prenatally stressed primates performed more poorly on measures of attention and exhibited delayed object permanence development, compared with offspring of control primates (Schneider, 1992; Schneider, Moore, Kraemer, Roberts & DeJesus, 2002).

Such differences in the findings may reflect tangible differences in the cognitive functions vulnerable to prenatal stress across species; or may simply be due to variations in the functions assessed, or in the developmental stage of the animals at the time of assessment.

3.1.4. Prenatal Stress and Infant Cognitive Development. Direct extrapolation of the findings of an adverse effect of PNS on offspring cognitive development from animal models to human populations is problematic due to interspecies differences in gestational length, gestational age relative to neurological maturation as well as neurological maturation at birth (Beydoun and Saftlas, 2008). Indeed gestational length is known to vary from around 21 days in rats compared with 147 days in sheep, 161 days in Rhesus Macaques and around 264 days in humans (Clancy, 2001). Furthermore, neuronal maturation processes vary in the time course of their trajectories across species, being complete before birth in some species versus continuing postnatally in others (Workman et al. (2013).

Replication of the findings in the animal literature indicating adverse effects of PNS on offspring cognitive development is warranted in human participants and though efforts have indeed been made to this end, direct replication of the methodologies

employed in the animal literature is not feasible due to the obvious ethical constraints of exposing pregnant women to analogous external stressors. Researchers examining the suggested adverse effects of prenatal stress on child cognitive development have therefore relied upon the measurement of naturally occurring stress in pregnant women's everyday lives. This has been approached in a number of different ways. A common approach has been to employ self-report measures of stress symptoms (such as anxiety and depression). Others have utilised pregnant women's reports of their subjective feelings of generalised, or pregnancy specific stress, using instruments such as the Perceived Stress Scale (Cohen, 1983) or the Pregnancy Related Anxieties Questionnaire Revised (Van den Bergh, 1990) respectively.

Another approach may be considered to mimic the methodologies employed in the animal literature more closely. Specifically, by quantifying the presence of external stressors experienced during pregnancy. The stressors assessed have varied across studies from relatively low level 'daily hassles' such as 'could not find important belonging', 'stuck in traffic jam', etc. to more major stressful life events, such as relationship breakdown, bereavement of a close family member or major financial difficulties. Furthermore, one research group has taken advantage of the occurrence of a natural disaster (the 1998 Ice Storm in Quebec) and examined the developmental characteristics of the children of women who were exposed during pregnancy (LaPlante et al., 2004 - 2008).

3.1.5. Research Employing Self-Report Measures of Prenatal Stress. One of the earliest reports of a link between PNS and infant cognitive development in human participants, measured PNS via self-report of stress symptoms. Specifically, Davids, Holden and Gray, (1963) assessed generalised anxiety symptoms using the Taylor Manifest Anxiety scale (MAS) and compared the infants of mothers reporting high versus low PNS, dichotomised as scoring above or below the sample median on this 50-item self-report anxiety questionnaire. Infants of mothers from the high anxiety group (N=24) obtained significantly lower cognitive development scores at 8 months, as measured by the Bayley Scales of Infant Development Mental Development Index (BSID-MDI) compared with infants of their low anxiety counterparts (N=26). However, Davids et al.'s (1963) sample was small and no information was gathered regarding exposure to maternal postnatal anxiety, which may reasonably confound the reported results. Furthermore, the authors reported significant differences between mothers that experienced high versus low anxiety during pregnancy in aspects of personality, child rearing attitudes, satisfaction with the role of being a parent, and interaction with their children during the postnatal assessment. Such factors could conceivably have mediated the reported difference in mental development scores between children of prenatally high versus low anxious mothers but were not taken into account in Davids et al**.'s** (1963) analyses, perhaps due to the small sample size.

More recently, using similar methodology, Brouwers, Van Baar & Pop (2001) provided support for Davids et al.'s (1963) findings with a larger sample (150 mother infant dyads). Prenatal stress was assessed using the Spielberger State-Trait Anxiety Inventory (STAI, Spielberger, 1970) at 32 weeks' gestation. The STAI is a self-report questionnaire consisting of two 20-item subscales; the *state anxiety* subscale measuring anxiety concurrent with the time of scoring, and the *trait anxiety* subscale measuring

dispositional anxiety. State anxiety is characterised by subjective feelings of fear, nervousness, and tension, accompanied by heightened activity of the autonomic nervous system. It is considered a temporary emotional condition referring to an individual's subjective feelings in the present. Trait anxiety on the other hand refers to a more stable dispositional characteristic of an individual; characterised by feelings of stress, worry, discomfort etc. experienced across multiple situations on a daily basis. Brouwers et al., (2001) reported that children of prenatally stressed mothers (those scoring ≥1 standard deviation above the mean on state and/or trait anxiety) scored significantly lower than those of non-anxious mothers on the BSID-MDI at 24 months of age. Furthermore, multiple regression analysis allowed Brouwers et al. (2001) to control statistically for a number of pre- and postnatal variables, specifically birth weight, breast feeding, the 'organisation of the home environment' subscale score of the HOME assessment, prenatal alcohol use, prenatal smoking, maternal depression during the 2 years after birth, and maternal depression symptoms (EPDS score) at 12 months. The analysis revealed high prenatal anxiety (state and/ or trait) at 32 weeks to be a significant independent predictor of BSID-MDI at 24 months. The regression analysis also revealed two additional significant independent predictors of BSID-MDI in this sample, low maternal education and male sex.

At 12 months of age however, there was no statistically significant difference between children of PNS versus non-PNS mothers on the BSID-MDI, though children of PNS mothers exhibited lower scores on a 'task orientation' cluster of the Infant Behaviour Record from the BSID; a measure of attention and reactivity. The authors also reported that even earlier in development, at 3 months of age, children of PNS mothers scored significantly lower on the orientation cluster of the Neonatal Behavioural Assessment Scales (NBAS) an early measure of the ability to attend to

visual and auditory stimuli. These results may reflect the course of normative cognitive development, such that the association with PNS is detected more easily in those functions that are just emerging when assessed. That is, the proposed deficit in cognitive function associated with prenatal stress is highlighted when the cognitive function being assessed is just emerging and so variability amongst infants is high. The BSID-MDI at 12 months of age, being a rather general measure of cognitive ability, tapping multiple aspects of cognition, may lack sensitivity to some aspects of emerging cognitive functions at this age. In addition to this, Brouwers et al.'s (2001) analyses are hampered by a lack of statistical power in detecting more subtle associations due to the small sample size of the prenatal stress group (N=20).

Despite some promising findings, Brouwers et al.'s (2001) study exhibits a number of methodological limitations. First the authors attempted to take into account the contribution of postnatal maternal stress in their analysis by including EPDS score at 12 months as well as the presence of an episode of maternal depression (depression section of the CIDI) at any point during the 2 years after birth. Neither of these factors was found to be a significant predictor of BSID-MDI at 24 months. However, neither the STAI nor the EPDS were administered at the 24 month follow up assessment, thus higher levels of concurrent postnatal stress symptoms cannot be ruled out as a potential confound of the reported results at 2 years. As Brouwers et al. (2001) concede in their discussion "Anxious women may provide different stimulation and interaction experiences to their children than non-anxious women" (p 103) and this may impact upon cognitive development.

Additionally, the women included in the prenatal stress group were heterogeneous in the type of stress they reported (i.e. state versus trait anxiety) and consisted of those reporting high levels of state anxiety or high levels of trait anxiety

and those reporting high levels of both. These subgroups are likely to vary in both the levels and chronicity of prenatal stress experienced across gestation, which may impact heterogeneously on infant cognitive development. However, this was not taken into account by Brouwers et al.'s (2001) analysis; likely due to the small sample size of the prenatal stress group. Related to this, though the overall sample size in this study was three times the size of Davids et al.'s (1963) sample; the number of PNS mothers was actually smaller (N=20), reducing the confidence with which conclusions can be drawn from the reported results. This is an encumbrance of the field. The relatively small numbers of pregnant women experiencing high levels of PNS symptoms such as anxiety can impede the requisite statistical power to draw confident conclusions within studies examining group differences between children of PNS versus non-PNS mothers. An alternative approach would be to examine exposure to PNS using dimensional indicators of anxiety or depression, rather than defining a somewhat arbitrary cut-point to define high versus low exposures.

Huizink, Robles de Medina, Mulder, Visser & Buitelaar (2003) employed a larger sample size (n=170) and reported further evidence in support of the proposed negative association between PNS and cognitive development; albeit examining different aspects of PNS to the previously cited literature. Specifically, Huizink and colleagues assessed Pregnancy-Specific Anxieties employing the Pregnancy Related Anxieties Questionnaire- Revised (PRAQ-R) and amount of daily hassles using the Everyday Problem List. These were assessed at three time points during gestation; early pregnancy (15-17 weeks), mid-pregnancy (27-28 weeks) and late pregnancy (37-38 weeks). High levels of mid-gestational pregnancy-specific anxiety and high levels of reported daily hassles in early pregnancy were independent predictors of BSID-MDI scores at 8 months but not at 3 months of age. These measures of prenatal stress

remained significant independent predictors of BSID-MDI at 8 months after adjusting for gestational age at birth, birth weight and the postnatal stress and depression levels of the mother.

Despite providing further evidence of a negative association between maternal prenatal stress and child cognitive development, Huzink et al.'s (2003) findings are not without limitation. Specifically, though the authors attempted to account for postnatal maternal stress as a possible confounding variable by assessing depression symptoms (EPDS score) and the level of stress that the mother felt she had experienced over the preceding month (Perceived stress scale) at both postnatal follow up appointments; these measures may assess somewhat different aspects of stress compared to those employed in the prenatal period (specifically, the Everyday Problems List and Pregnancy Related Anxieties Questionnaire). The Everyday Problems List may be considered a somewhat more objective measure of stress in comparison to the more subjective EPDS and Perceived stress scale. It is possible that the lack of an association between postnatal stress and infant cognitive development could reflect these methodological differences between the pre and postnatal assessment of stress.

Further investigation of the association between maternal PNS and child cognitive development is reported by Davis and Sandman, (2010). Specifically, Davis and Sandman (2010) followed women (N=125) longitudinally from early pregnancy to 12 months post-partum. Multiple aspects of prenatal stress, specifically state anxiety, perceived stress and pregnancy specific anxiety were assessed at 5 time points across gestation (15 weeks, 19 weeks, 25 weeks, 31 weeks and 37 weeks). Infant cognitive development (BSID-MDI) was assessed at 3 months, 6 months and 12 months post-partum. Increased levels of pregnancy specific anxiety during early pregnancy, but not other measures of prenatal stress, were associated with poorer infant cognitive

development at 12 months, but not earlier ages. The association remained after adjusting for covariates identified in preliminary analyses as variables that were associated with infant BSID-MDI, specifically gestational age at birth, maternal race and prenatal medical risk. Although preliminary analyses did not reveal significant associations between measures of postnatal maternal psychological state (state anxiety, depression and perceived stress) and the child outcome measure, Davis and Sandman (2010) tested their models with and without these possible confounding variables. Adjusting for these factors did not change the significance of their results.

Further support is provided by Henrichs, et al. (2011), assessing still another aspect of PNS, maternal prenatal family stress (measured using the General Functioning subscale of the Family Assessment Device, Epstein, Lawrence & Bishop, 1983) at 20 weeks gestation. Their analysis revealed prenatal family stress was associated with lower infant verbal cognitive function at 18 months and poorer non-verbal cognitive function at 2 years after adjusting for covariates. The covariates included in Henrichs et al.'s (2011) model were parental age, maternal education, alcohol consumption and smoking during pregnancy, parity, family income, birth weight, gestational age at birth, child age, gender, ethnicity, parental prenatal depressive symptoms and maternal postnatal depressive symptoms at 2 months. However, as Henrich's et al. (2011) did not assess family stress at postnatal follow-up, concurrent family stress cannot be ruled out as a confounder of the reported associations with infant verbal and non-verbal cognitive function.

Finally, not all reports are in agreement with the proposed negative association between PNS and offspring cognitive development. In Di Pietro, Novak, Costigan, Atella & Reusing's, (2006) sample, higher levels of prenatal anxiety (anxiety subscale of the Profile of Mood Scale) and depression (EPDS) were significantly positively

associated with BSID-MDI at two years. These authors suggest that moderate levels of PNS may actually be facilitative to the cognitive development of the child.

3.1.6. Studies examining exposure to stressors during pregnancy as a measure of prenatal stress. Others have attempted to replicate the methodologies used in the animal literature more closely by assessing purportedly more objective measures of PNS. One strand of research in this area has assessed PNS via report of the occurrence of stressful life events for example death of a partner or close family member, or experiencing a major financial problem, using validated questionnaires and interview measures.

In a study by Bergman et al. (2007), when infants were aged between 16 and 18 months of age, mothers reported the occurrence of stressful life events using a stressful life events questionnaire, which was adapted by the authors from the Inventory of Ranked Life events for Primiparous and Multiparous Women (Barnett, Hanna and Parker, 1983). The adapted questionnaire comprised 26 items, spanning events such having car or house burgled, experiencing a sudden drop in income, having a serious accident or illness, suffering from mental illness, or death of a partner. As well as stating whether or not these events had occurred, mothers were required to state whether they had occurred pre-or postnatally; and to subjectively appraise how much each event had affected them; either a little or a lot. Results revealed a significant negative correlation, both between the number of stressful life events in the prenatal period and infant BSID-MDI score at follow up, and between the perceived impact of these events and infant BSID-MDI score at follow up. There was no association between postnatal stressful life events and infant BSID-MDI score. This measure of PNS (stressful life events) significantly predicted infant cognitive development, independently accounting

for 17% of the variance in infant MDI scores. Furthermore, the proportion of variance explained by PNS was unchanged after accounting for potential pre- and postnatal confounders (specifically maternal education, smoking and alcohol use during pregnancy, child sex, child age, maternal age, postnatal maternal trait anxiety (STAI), postnatal maternal depressive symptoms (EPDS), social support and postnatal stressful life events.

Though the association between prenatal stressful life events and infant BSID-MDI score remained after accounting for postnatal stress symptomology (EPDS) assessed at 2 months' post-partum, maternal depression symptoms (EPDS) at the time of infant postnatal assessment (16-18m), were not assessed. Therefore, maternal stress concurrent with the assessment of infant cognitive development, or between these two time points, cannot be ruled out as a confounding factor. thus. Bergman et al.'s (2007) findings are somewhat further limited by the retrospective nature of the collection of PNS data.

However, Zhu et al. (2014), report similar findings with a prospective design. They administered the 19 item Prenatal Life Events Checklist (Zhu, Hao, Jiang, Huang and Tao, 2013) in late pregnancy (32 weeks) to collect information about PNS at different stages of pregnancy. Participants were asked to indicate whether each of the events from the checklist had occurred, and if so during which trimester it had occurred. They were also asked to rate the impact of the event on their wellbeing, ranging from 0 = no impact to 4 = extreme impact. Women were then categorised into an 'exposed' and 'non-exposed' group though the authors did not clearly outline the criteria for this. Women and their infants were then followed up from birth to 18 months' post-partum, with infant cognitive development assessed when infants were between 16 and 18 months of age. Zhu et al. (2014) reported that infants of mothers exposed to stressful

life events during the first trimester achieved lower BSID-MDI scores (on average 7 points lower) than children of mothers in the non-exposed group.

Unfortunately, Zhu et al.'s findings are also somewhat limited. First, they did not control for the presence or impact of postnatal stressful life events. Thus, the reported association between maternal prenatal stressful life events and infant cognitive development may be confounded by the mothers' experience of stressful life events in the postnatal period, or indeed the child's experience of these stressful life events and any associated changes to the child's environment. It has also been argued that stressful life events such as those assessed by Bergman and colleagues and Zhu et al. (2014) (e.g. job loss, being in trouble with the police) may not be independent characteristics of the prenatal environment; instead potentially being associated with a mother's poorer cognitive capacities, which may then be passed on genetically to the child (King & LaPlant, 2005). Zhu et al. (2014) did not take maternal cognitive abilities into account in their analysis to control for this. This raises an important consideration for future studies examining the association between maternal prenatal exposure to stressful life events and child cognitive ability such that measures of maternal cognitive ability should, where possible, be included in order to control for underlying genetic transmission of cognitive function in women reporting these kinds of stressors during pregnancy.

In addition to this, though Zhu et al. (2014) employed a measure assessing participants' exposure to stressors during pregnancy (rather than more subjective ratings of stress symptoms), Zhu et al., (2014) determined inclusion in the exposed vs non-exposed groups based not only on the presence of stressful life events but also the mother's subjective rating of the impact of these events. It is important to note that Zhu et al. (2014) did not set out to separate the influence of stressful life events, from the

subjective experience of these events, on infant cognitive development. However, given the differences across studies in this area of the literature regarding methods of PNS assessment, it is not clear whether Zhu et al.'s findings represent evidence for the importance of prenatal exposure to external stressors, or the presence of self-reported symptoms of stress in the prenatal period, for child cognitive development, or both. Few other studies in the literature to date have examined both maternal reports of prenatal exposure to external stressors and their subjective report of prenatal stress symptoms at multiple points during pregnancy and in the postnatal period. In the current study, exposure to life events during each trimester of pregnancy and self-reported prenatal anxiety symptoms in the second and third trimesters will be examined separately as predictors of cognitive outcomes in children. The same measures of stress (exposure to stressful life events and self-report of anxiety symptoms) will be applied at multiple time points in the postnatal period to control for the influence of postnatal stress on toddler cognitive outcome.

In a series of studies taking advantage of the occurrence of a natural disaster, the 1998 Ice Storms in Quebec, LaPlante and colleagues (2004-2008) have attempted to replicate more closely the objective nature of the experimental methodologies used in the animal literature in their investigation of the effect of PNS on cognitive development. Specifically, LaPlante et al., (2004 -2008) operationalised PNS as the severity of stress to which pregnant women were exposed due to the Ice storms and assessed infant cognitive development using the BSID-MDI. They reported that PNS was negatively correlated with infant cognitive development at 2 years of age, and accounted for a significant proportion of the variance (11.4%) in infant scores over and above that accounted for by potential confounding factors (gestational age, birth weight, infant sex, number of previous pregnancies, number of obstetric complications, and

postnatal depression, as measured by the EPDS). The authors also assessed mothers' subjective level of stress related to Ice Storm exposure, though no significant associations with infant cognitive ability were revealed. Subsequent reports from the same research group (King and LaPlante 2005) revealed that the time of exposure during pregnancy exerted significant moderating effects on the association between PNS and infant cognitive ability; implicating the first and second trimester as particular sensitive periods However, as conceded by the authors, the sample sizes available at child outcome assessment were relatively small (N=58). Therefore, replication would be required to provide confidence in the conclusions that can be drawn from these analyses but this is obviously challenging given the nature of the prenatal stressor examined.

3.1.7. PNS and child cognitive abilities later in development. A number of other groups have reported that the association between prenatal stress and child cognitive development is observable beyond infancy (>2 years), some employing maternal report of symptom of PNS and others assessing exposure to stressors. Thus suggesting an enduring nature of the impact of PNS for child cognitive development. Slykerman et al., (2005) for example, reported that high levels of mother-reported stress in late pregnancy, as assessed by the Perceived Stress Scale was inversely associated with children's cognitive ability at 3 ½ years, as measured by the Stanford Binet Intelligence scale. The analysis controlled for potential confounders, specifically gestational age, child sex, maternal age, education, marital status, parity, smoking during pregnancy and breastfeeding duration. Concurrent maternal stress, on the other hand, was not associated with children's cognitive ability. However, data regarding the mothers' appraisal of PNS was gathered retrospectively, albeit shortly after the birth of the child. Retrospective data may be considered to carry some concerns regarding

accuracy (e.g. Barusch, 2011).

Others have reported a detrimental impact of PNS on cognitive development even later in development. For example, LaPlante et al.'s group following the development of children exposed to a natural disaster (LaPlante, Brunet, Schitz, Ciampi & King, 2008) revealed that the association between PNS and cognitive development persisted into early childhood. At age 5 ½, children of mothers reporting exposure to high levels of objective stress had lower full scale IQs as measured by the Wechsler Pre-school and Primary Scale of Intelligence (WPPSI) compared with children of mothers exposed to low or moderate levels of PNS. Similarly, Niederhofer and Reiter (2004) reported a negative association between PNS and children's school grades at age 6. PNS was assessed during early pregnancy (16-20 weeks) using an author constructed and validated questionnaire, assessing the occurrence of stressful life events. Although the sample size for this study was relatively large (N=227), the authors did not employ a standardised or validated measure of child cognitive ability, nor did they adequately define the aspects of prenatal stress assessed. Furthermore, the authors did not control for potential pre and postnatal confounders.

In assessing the association at a similar point in development, Gutteling et al., (2006) reported that mothers' subjective rating of the negative impact of stressful life events in early pregnancy (but not mid or late pregnancy) was negatively associated with the children's attention/concentration at age six, after controlling for potential confounders. The potential confounders included in the analysis were child IQ, gender, maternal smoking and postnatal stress (STAI and Perceived Stress Scale, PSS) at 3 and 8 months. No significant associations were revealed between the child outcome measure and either frequency of daily hassles during pregnancy or pregnancy related anxiety (assessed in early, mid, and late gestation). The authors report, in their discussion, that

variations in the form of stress assessed by the different prenatal measures "may be reflected in different effects on maternal physiology that in turn could produce different effects on postnatal outcome" (p. 796). However, as the sample size was relatively small and multiple analyses were conducted, it cannot be ruled out that the reported result, being specific to one measure of prenatal stress at only one time point during pregnancy, may have been a chance finding. Replication with a larger sample sizes would increase confidence in the author's assertion.

A further limitation of Gutteling et al.'s (2006) finding is the absence of a control for postnatal stress concurrent with the child outcome measure. Though the STAI and the PSS were assessed at 3 and 8 months postpartum, maternal stress concurrent with the age 6 child outcome measure was not assessed. Therefore, concurrent maternal stress cannot be eliminated as a possible confounder of the reported results. Furthermore, though including measures of postnatal stress at 3 and 8 months postpartum, the authors did not examine the specific negative impact of these measures on child cognitive outcome, adding to the case that one cannot easily conclude that postnatal stress effects were adequately controlled. In prospective research, controlling for possible postnatal stress effects is key to the process of accurate identification of a true prenatal stress effect on child cognition.

In summary, there is accruing evidence suggesting that PNS influences the cognitive development of the unborn child. However, differences across studies in measures of PNS, specifically whether those measures reflect exposure to external stressors versus subjective reports of stress symptoms, mean that it is not clear whether these aspects of PNS are equally influential for child cognitive abilities. A few of the previously cited studies, examining exposure to stressful life events during pregnancy (Bergman et al., 2007; LaPante et al., 2004) also included a more subjective appraisal of

PNS, by asking participants to subjectively rate the impact of the events they had experienced, with discrepant findings. Specifically, Bergman's group found that both exposure to events and the subjective rating of the impact of these events were negatively associated with infant cognitive scores, whereas LaPlante et al.'s (2004) findings implicate only the level of exposure to events. Tarabulsy et al.'s (2014) meta-analysis provides some evidence for a greater sensitivity of child cognitive development to exposure to external stressors compared to more subjective measures of prenatal stress. Specifically, Tarabulsy et al. (2014) reported that studies examining such measures e.g. stressful life events yield greater effect sizes than those employing assessment of more subjective measures, such as report of symptoms of stress. However, Tarabulsy et al. (2014) point out that their meta-analysis is limited due to the number of studies examined, with only 11 studies meeting inclusion criteria for analysis. The current study therefore applies both measures of exposure to stressful life events and subjective rating of symptoms of stress, namely anxiety.

Although negative associations between measures PNS and indices of cognitive development have been replicated in infancy (age 0 -2) and early childhood (age 5 ½ to 6). The period between (aged 2 to 3 years) has received much less attention.
The current study aims to fill this gap in the literature, examining aspects of cognitive development in toddlers aged 2 ½ years whilst addressing some of the limitations of previous work.

A frequent limitation of the studies in this area of the literature has been the lack of, or inadequate, control for postnatal maternal stress. Controlling for the possible influence of postnatal stress is key to the accurate identification of a true prenatal stress effect on child cognition. As such, the current study applies repeated measurement of maternal stress (both exposure to stressful life events and subjective report of symptoms

of stress) across the postnatal period up to and including the point of child outcome assessment.

3.1.8. Sensitive periods in the association between prenatal stress and cognitive development. It has been proposed that there may be sensitive periods, during gestation for the implicated adverse effects of prenatal stress (e.g. Davis and Sandman, 2010). PNS has been variously assessed at different time points in pregnancy with PNS experienced at 20 weeks' gestation (Henrichs et al., 2011), at 32 weeks gestation (Brouwers et al., 2001), at 34-38 weeks gestation (Slykermnan et al., 2005) and 'during the third trimester' all being independently associated with poorer cognitive outcomes for the child. Additionally, Bergman et al.'s (2007) findings implicated the presence of PNS irrespective of the time point during gestation. It may therefore be suggested that there is no specific sensitive period for the proposed adverse influence of PNS, rather the unborn child may be vulnerable across the whole of gestation or at any time-point therein. However, the assessment of different samples; employing different measures of prenatal stress at different points in gestation across these studies (Bergman et al., 2007; Brouwers et al., 2001; Davids et al., 1963; Henrichs et al., 2011; and Slykerman et al., 2005) precludes confidence in this assertion.

A limited number of studies have attempted to resolve the issue of a possible sensitive period for the association between PNS and child cognitive development by assessing PNS at multiple gestational time-points within the same sample (Davis and Sandman, 2010; Gutteling et al., 2006; Huizink et al.,2003; LaPlant et al., 2005). However, the conclusions that can be drawn across these studies are far from clear. Davis and Sandman's (2010) findings implicate early pregnancy as a sensitive period for exposure to pregnancy specific anxiety (but not state anxiety or perceived stress) on

infant BSID-MDI at 8 months. Huizink et al.'s (2003) findings, on the other hand, implicate mid pregnancy as a sensitive period for exposure to pregnancy specific anxiety, and early pregnancy to be a sensitive period for exposure to daily hassles on infant BSID-MDI at 8 months. LaPlante et al.'s (2005) findings suggest both early and mid-gestation to be sensitive periods for exposure to external stressors on infant BSID-MDI, whereas Gutteling et al. (2006) report early pregnancy to be particularly important for the association between the negative impact of stressful life events and the attention/ concentration abilities of the children at 6 years of age.

Tarabulsy et al.'s (2014) meta-analysis (described in more detail in section 3.1.9 below) examined trimester of pregnancy in which prenatal stress was assessed as a potential moderator of the association between prenatal stress and child cognitive development. The analysis revealed no differences in effect sizes as a function of this. The authors concede, however, that this lack of a significant finding may be due to design related factors of the studies included in the analysis (specifically the relative lack of studies assessing prenatal stress in the first trimester) rather than true equivalence of effect sizes across samples completing prenatal stress assessments in different trimesters of pregnancy.

Therefore, it is not clear from the available literature whether there are particular sensitive periods during gestation for the influence of PNS on child cognitive development. However it is clear that this possibility should be taken into account in studies in this field. As the available literature has implicated different gestational periods when different measures of PNS have been employed, studies should seek to administer multiple measures of PNS at multiple points during pregnancy. The current study adopts this approach.

3.1.9. Meta-analytic approaches and the possibility of sex-specific effects.

There is accruing evidence that a child's cognitive development may be influenced by stress experienced by the mother during pregnancy. However not all findings are in agreement about the direction of effects. Many studies indicate adverse effects but at least one study, Di Pietro, Novak, Costigan, Atella and Reusing (2006), reports a positive association between prenatal stress and infant cognitive development. Variations in the methodologies employed across studies in this area of the literature make it difficult to integrate the findings into a succinct conclusion. In light of this, Tarabulsy, et al., (2014) conducted a meta-analysis of studies examining the association between prenatal stress and early child cognitive development published between 1970 and 2011. They report a small but significant inverse association between indices of prenatal stress and child cognitive development with an overall effect size of r = -.05, concluding that "there seems to be a consistent low-level association" between PNS and later child cognitive development, with stress during pregnancy being "meaningfully and negatively associated with later child cognitive outcome" (p. 41). However, Tarabulsy et al. (2014) point out that their meta-analysis is limited due to the number of studies examined, with only 11 studies meeting inclusion criteria for analysis.

One possible explanation for the mixed findings and low level association between PNS and cognitive outcomes may be rooted in findings suggesting that there may be differential effects of prenatal stress on cognitive outcomes in males and females. Such findings will be reviewed in the following section.

3.1.10. Sex differences in the association between PNS child cognitive abilities.

Though the majority of studies in animal models examining the effect of PNS on offspring learning ability were initially conducted solely in male offspring; more recent studies including both male and female offspring suggest that the association between PNS and offspring cognitive function may differ according to sex, with male offspring reportedly more vulnerable (See Weinstock, 2011, for review). Sierksma et al., (2013) for example, reported a PNS induced deficit in long-term spatial memory performance in male mice relative to controls, whereas female performance was not impaired by PNS. Similarly, Markham, Taylor, Taylor, Bell and Koenig, (2010) found that male but not female offspring of PNS dams were impaired relative to controls on a battery of tasks assessing both hippocampal and PFC mediated functions in adulthood. Mueller and Bale (2007) also reported a relative vulnerability of male offspring (compared to female offspring) of PNS dams in their spatial learning performance. These findings in the animal literature therefore suggest a male vulnerability to the effects of PNS on learning ability in the offspring (Weinstock, 2011).

Both Paris & Frye (2011) and Gué et al., (2004) however, found no sex differences in PNS-induced deficits in spatial working memory, with both male and female offspring of PNS dams impaired relative to controls. This null finding with regard to sex differences may reflect the younger age of Gué et al.'s (2004) and Paris & Frye's (2011) subjects (compared to the fore mentioned studies in which sex differences were reported). Indeed, Markham et al. (2010) report that, when tested in adolescence, both PNS males and females were indistinguishable from controls in their spatial working memory performance. However, in adulthood only males displayed impaired performance relative to controls. Markham et al. (2010) therefore suggest that the proposed sex-specific vulnerability to the impact of PNS on spatial working memory

performance may only become apparent late in development when it is 'unmasked' due to sex-specific patterns of structural maturation in the PFC (a brain region involved in the mediation of spatial working memory). Such sex specific PFC maturational patterns have been shown to be altered by PNS (Markham, Morris & Juraska, 2007; Markham and Koenig, 2009).

The examination of sex differences in the association between PNS and child cognitive development in human participants has lagged behind that in animal models, and the limited findings are conflicting. Bergman et al. (2007) and LaPlante et al. (2004) found no evidence that the association between PNS and child cognitive development was moderated by the sex of the child. However, Bergman et al.'s (2007) (N= 123) and LaPlante et al.'s (2004) (N=58) samples were relatively small and thus may have lacked the statistical power to detect such a moderating effect. Li et al. (2013) however, provide evidence supporting such sex differences with a sample of 1038 mother - child dyads. Specifically, they found that maternal exposure to stressful life events (at any point during pregnancy) was associated with lower reading scores in girls (N = 516) and higher reading scores in boys (N=521) at age 10. The interaction between sex and the PNS measure remained significant after adjusting for potential pre- and postnatal confounders (specifically maternal age, race, marital status, education, smoking in pregnancy, family income and maternal postnatal stress at 1 year and concurrent with the child outcome assessment at 10 years).

Despite the mixed results, such findings highlight the importance of examining possible sex differences in this area of the literature when mixed gender samples are studied. Specifically, non-significant main effects of PNS on child cognitive outcome may result when males and females are studied together, yet a non-significant main effect may mask significant associations between PNS and cognitive outcomes in

opposing direction in girls versus boys, or associations of different magnitudes. Failure to consider sex in the analysis of study data may lead to erroneous confirmation of the null hypothesis. Indeed, in Li et al's (2013) study, preliminary analysis of the pooled sample yielded no significant association between PNS and the outcome measures assessed. Similarly, in Buss, Davis, Hobel, and Sandman, (2011) report that high levels of PNS were associated with child executive function at 6 – 9 years of age, they found that high levels of pregnancy specific anxiety were associated with lower inhibitory control in girls only, and lower visuospatial working memory performance in both boys and girls. Such findings suggest that the impact of PNS on cognitive function may be different for boys and girls, in at least some aspects of cognition. Structural MRI assessments in Buss et al.'s sample also revealed that high levels of pregnancy specific anxiety were associated with reductions in grey matter volume in a number of neural regions, including the PFC, and that these reductions "were primarily observed in girls" (Sandman et al., 2012, p. 15).

Thus, there is currently some support for the assertion that the association between PNS and cognitive development in human participants may be different for male and female children, as evidenced in the animal literature. However, with the limited number of studies in human participants, this is rather speculative and warrants further research efforts. The possibility that PNS exerts differing effects on PFC structure and function according to gender represents an important area of research from a public health perspective given the striking sex differences reported for psychiatric conditions that are known to involve PFC dysfunction, such as schizophrenia, depression and risk for suicide (Aleman, Kahn & Selten, 2003; Kessler & Ronald, 2003; McGrath et al., 2004; Forum on child and family statistics, 2009).

3.1.11. How does PNS influence child cognitive development and could more specific cognitive functions be vulnerable? Research efforts in this area of the literature have also attempted to examine a mechanism for observed associations between PNS and the cognitive development of the child. It is suggested that associations between PNS and aspects of offspring development may be the result of 'foetal programming'. The foetal programming hypothesis states that the environment in-utero can shape the development of the foetus during particular sensitive periods. Specifically, it is suggested that the physiological, neuroendocrine or metabolic adaptations that enable the foetus to adapt to changes in the environment in utero result in permanent 'programming' of the developmental pattern of cell proliferation and differentiation within tissues and organ systems of the developing foetus. Such adaptations may be beneficial or deleterious to the developing offspring (Barker, 1998; Das, 2003).

Support for foetal programming as a mechanism underlying the association between PNS and offspring cognitive development can be drawn from animal literature indicating that PNS has adverse effects on the structural brain development of the foetus. Such structural alterations may reasonably result in functional deficits in the functions sub served by the neural regions affected and manifest in the offspring behaviourally during postnatal development. In rodents, Kawamura, Chen, Ichtani & Nakahara, (2006) demonstrated a 60% reduction in cell proliferation in the hippocampus at postnatal day 10 in offspring of PNS dams. PNS has also been reported to induce a 73% reduction in dendritic arborisation and 50% synaptic loss in areas of the hippocampus in rat offspring by Barros, Duhalde- Vega, Caltana, Brusco & Antonelli, (2006). Similarly Hayashiet al., (1998) reported a 32% reduction in synaptic density in the hippocampus of the offspring of PNS rats exposed to crowding and saline injection

during days 15 to 21 of pregnancy. Belnoue, Grosjean, Ladeveze, Abrous & Koehl, (2013) further reported reduced cell proliferation and neurogenesis in the hippocampus of adult offspring of PNS dams compared to their non-PNS counterparts. Furthermore, Barzegar, Sjjadi, Talaei, Hamidi & Salami, (2015) combined electrophysiological recordings with behavioural measures of hippocampal function in offspring of rats that had been exposed to sound stress prenatally, in order to directly examine the effect of PNS on offspring neural function. Their findings revealed that PNS negatively affected spatial working memory performance and synaptic activity in areas of the hippocampus.

However, since a substantial amount of neural and neuro-endocrine development occurs in rodents only after birth, the predictive utility of such findings to PNS in human participants may be somewhat limited. In light of this, Petit et al (2015) examined neural integrity in the offspring of prenatally stressed sheep asserting the greater predictive utility of this species due to their gyrencephalic cerebral cortex, i.e. displaying a convoluted surface (as in humans) as opposed to the lissencephalic (smooth) brains of rodents; as well as their greater similarity to humans in terms of brain organisation and prenatal brain development. Petit et al., (2015) reported neuro-morphological alterations (specifically increased dendritic spine density and greater numbers of stubby type spines) in the hippocampus of PNS lambs compared to controls. The authors suggest that such changes may reflect a less mature neural system with less efficient connectivity between neurons, in turn resulting in impairments in the cognitive functions sub-served by these neural regions.

Extending these findings further by examining the effect of PNS on the neural integrity of offspring in an animal model species more closely related to humans, Coe et al., (2003) applied an acoustic startle stressor to pregnant rhesus monkey dams. Offspring of PNS mothers displayed a reduction in neurogenesis and a 10% - 12%

reduction in hippocampal volumes in offspring at 3 -4yrs old (considered to be analogous to adolescence in humans). Taken together, such findings from the animal literature suggest that PNS alters processes in the development of new neurons and the fine-tuning of neural systems in the hippocampus, even into adulthood, resulting in a detrimental impact on both the structure and function of this neural region.

The specific mechanisms by which such programming may drive the adverse effects of PNS on offspring cognitive function are yet to be fully elucidated. However, the existing evidence indicates a role for the hypothalamic-pituitary-adrenal axis (HPA-axis) and its products. The HPA-axis is a major part of the body's neuroendocrine system and comprises a complex set of direct influences and interactions among the hypothalamus, the pituitary gland and the adrenal glands. The HPA-axis controls the body's reactions to stress and regulates many other bodily processes including digestion, mood and emotions, the immune system and energy storage and expenditure. In response to a stressor, a cascading release of hormones is initiated to mobilise an organism's resources in order to prepare them for action. Specifically, corticotropin releasing hormone (CRH) is released from the hypothalamus. CRH then stimulates the release of adreno-corticotropic hormone (ACTH) from the pituitary gland, which in turn stimulates the release of glucocorticoids (cortisol in humans and other primates, corticosterone in rats) from the adrenal cortex. The glucocorticoids (GCs) bind with glucocorticoid receptors in the brain, stimulating a negative feedback response, thus shutting off the stress response at the hypothalamus.

It is suggested that these gluco-corticoid end products of the biological stress response, (cortisol in humans and non-human primates and corticosterone in rats) may be intimately involved in the mechanism underlying the proposed adverse effects of PNS on offspring neural integrity and therefore cognitive development. Specifically, it

is proposed that maternal stress during gestation results in increased exposure of the developing foetus to excess levels of GCs and that this may have neurotoxic effects on the developing brain. In support of this assertion, Uno, Tahara, Else, Suleman & Sapolsky (1989) demonstrated a dose-dependent degenerative change, and a reduction of hippocampal neurons in 9 month old offspring of primates that were injected with a synthetic GC during pregnancy. Subsequent studies demonstrated that this hippocampal volume reduction was maintained at 2 years (Uno et al., 1994). Similar findings of impaired brain development, particularly in the hippocampus have been reported in rats (Bruschettini, Van Den Hove, Gazzalo, Steinbush,& Blanco, 2006) . Furthermore, the neurotoxic effect of prenatal GC exposure demonstrated by Bruschettini et al. (2006) was accompanied by a spatial learning deficit in exposed offspring compared to control offspring. Zagron and Weinstock (2006) provide further support in rodent models for the mediating role of foetal exposure to excess levels of GCs during gestation in the association between PNS and offspring cognitive development. Specifically, PNS-induced spatial learning deficits in rats were completely abolished in pups of dams that underwent adrenalectomy prior to exposure to PNS (thus preventing the increased foetal exposure to GCs during gestation that typically accompanies PNS).

Indirect evidence in human participants, supporting the role of foetal exposure to excess levels of GCs as a mediating mechanism for the association between PNS and cognitive development can be drawn from studies examining the administration of synthetic GCs to pregnant women and studies measuring levels of endogenous cortisol during pregnancy. A small number of studies have assessed the neurodevelopment of children of women who received synthetic GC treatment during pregnancy (given to women at risk for preterm delivery to support the maturation of the foetal lungs). Such studies have reported impaired memory in school age children and neurodevelopmental

impairments in toddlers (Spinillo et al., 2004; Wapner et al, 2007). Furthermore, Huizink et al., (2003) demonstrated that an increased level of endogenous maternal cortisol during the third trimester of gestation was associated with poorer BSID MDI scores at 3 months. Additionally, Davis and Sandman (2010) reported that elevated levels of maternal cortisol during early gestation was associated with a slower rate of cognitive development over the first year and lower BSID MDI scores at 12 months of age.

Of note is that studies of a possible neurotoxic effect of foetal exposure to excess levels of GCs have generally focussed on hippocampal integrity. The reasons for this are two-fold. First, the majority of the studies in the animal literature examining the effect of PNS on the cognitive ability of the offspring have been conducted in rodents, with robust findings of spatial learning deficits. Spatial learning abilities are known to be associated with hippocampal integrity (e.g. Eichenbaum, 1999). Secondly, the hippocampus is an integral structure in the HPA-axis, exhibiting a high concentration of glucocorticoid receptors (the binding sights for circulating GCs), and thus represents a target neural region for circulating GCs.

Like the hippocampus, the pre-frontal cortex (PFC) exhibits a high concentration of GC receptors therefore the PFC, like the hippocampus, represents a neural region that may be vulnerable to the neurotoxic effects of foetal exposure to excess levels of GCs. Indeed there is evidence that excess levels of endogenous GCs in human adult and adolescent participants are associated with indices of structural integrity in the PFC. Kremen et al. (2010) reported higher levels of salivary cortisol in adult males to be associated with reduced cortical thickness in the PFC. Similarly, Carrion, Weems, Richert, Hoffman & Reiss, (2010) reported a negative association between salivary cortisol levels in adolescent males and PFC volumes.

Furthermore, a negative association between PNS and PFC integrity (grey matter density) has been reported in 6 -9 year old girls (Buss et al., 2010). The pre-frontal cortex is known to be important for the mediation of tasks requiring executive function performance (Jurardo and Roselli, 2007). Therefore executive functions may be vulnerable to foetal exposure to PNS. Indeed, Van den Bergh and colleagues (2005) prospectively assessed PNS in pregnant women at different points during pregnancy (12-22 weeks, 23-31 weeks and 32-40 weeks) using the STAI and assessed the cognitive function of their children in adolescence. They found that adolescents (aged 15 – 17 years) of mothers reporting high state anxiety at 12-22 weeks gestation performed more poorly on tasks assessing executive functions, specifically, inhibitory control tasks and tasks requiring the integration and control of different task parameters. However, assessing the cognitive outcomes of these participants during adolescence affords the opportunity for the effects of non-specific environmental influences on the adolescents' executive function development to confound the reported results. Thus, prospective investigation of the association between maternal PNS and children's executive function from its earliest point of emergence is warranted.

3.1.12. Rationale for the current study. Despite the progress that has been made in the literature regarding the association between prenatal stress and child cognitive development, limitations of previous studies and gaps in the literature remain to be resolved:

(a) Findings that stress experienced by the mother during pregnancy may influence aspects of child cognitive development have been replicated in infancy (age 0-2) and school-age children (age 5 -6). However, the period between these ages (2 -3) has been somewhat neglected.

(b) There has been a heavy reliance on the BSID-MDI in the assessment of child cognitive abilities in this area of the literature. Though a well-validated and widely used clinical assessment tool, the BSID-MDI is a rather broad measure of cognitive development and does not allow assessment of specific structure function relations that may be influenced by PNS. The current study therefore assesses more specific measures of cognitive function.

As there are findings in older children and adults indicating that the PFC and the functions it sub-serves may be vulnerable to proposed neurotoxic effects of PNS, the current study assesses executive function as child cognitive outcome. The association between prenatal and executive function abilities in children prior to school age has not yet been examined in the previous literature in this area.

(c) There are studies reporting a significant negative association between mothers' report of subjectively felt PNS and child cognitive ability, and others reporting a negative influence of exposure to prenatal stressful life events on child cognitive ability. There are only a few that have examined both subjective measures of PNS and exposure to external stressors during pregnancy in the same sample, and they unfortunately give

discrepant findings. The current study employs both measures of prenatal exposure to stressful life events and subjective report of the experience of symptoms of stress (anxiety).

(d) The previous literature in this area has provided some, though inconclusive, evidence that the foetus may be differentially susceptible to the impact of PNS at different time points during gestation. Therefore, the current study applies repeated measures of PNS at a number of different time points during pregnancy.

(e) The extant studies examining the association between PNS and child cognitive ability are often limited by their lack of (or inadequate) control for mothers' experience of stress in the postnatal period. Therefore, the current study employs repeated measurement of postnatal stressful life events and stress symptoms (anxiety) across the postnatal period up to and including assessment of child cognitive outcome at 2 ½.

(f) There are well- replicated findings in the animal literature of sex differences in the association between PNS and offspring cognitive development, with male offspring reportedly more vulnerable. Few studies in the human literature have examined sex differences, and those that do report conflicting findings. Furthermore, failure to examine sex as a moderator of the association between PNS and cognitive ability may result in Type II error in studies focussing on main effects. Therefore, the current study examines sex as a moderator of the association between PNS and toddler executive function abilities.

3.1.13. Hypotheses.

(1) Higher levels of maternal prenatal stress, indexed by the experience of stress symptoms (anxiety) and exposure to stressful life events (SLEs), will be associated with poorer toddler executive function (indexed by measures of working memory and inhibitory control) at 2.5 years.

In line with the animal literature, the above association will evident in male infants rather than in female infants, as indicated by a significant statistical interaction between sex and prenatal stress exposure in the prediction of Executive Functioning at age 2 ½ years.

(2) The effects of maternal prenatal stress on infant cognitive outcomes will persist after controlling for demographic factors (maternal age, deprivation), gestational age at birth, smoking in pregnancy, breastfeeding, maternal IQ and exposure to maternal postnatal stress.

3.2. Method:

3.2.1. Design. The current study was embedded within the longitudinal Wirral Child Health and Development Study (WCHADS) and reports prospective findings from mother- infant dyads followed up from 20 weeks gestation to 2 ½ years.

The design and method of the WCHADS is described in more detail in section 2.2 of this thesis to provide a context for the sample and methods of the studies comprising the current thesis.

3.2.2. Participants. The current sample comprised those mother-infant dyads recruited into the Intensive Sample within the WCHADS who were eligible for postnatal follow-up (n=316) that provided complete maternal prenatal stress measures

collected at 20 weeks gestation and 32 weeks gestation and toddler executive function performance outcome data gathered at 2 ½ years of age (described in section 2.4.4.1) together with relevant confounding variables and covariates from intervening assessment points. Of the 254 toddlers providing complete executive function performance data at the 2 ½ year mother- infant observational assessment, 211 mother-infant dyads provided complete data for all the variables of interest for the current study. Mother –infant dyads that did not provide complete data across all variables of interest were excluded from the analysis. The demographic characteristics of the current sample of 211 mother- infant dyads are presented in Table 13

Table 13

Characteristics of sample included in the analysis predicting toddler EF from PNS (n= 211)

	Mean (SD)
Maternal age (Years)	31.7 (6.2)
Toddler age at assessment (Months)	31.5 (2.4)
Gestational age at birth (weeks)	40.2 (1.5)
	N (%)
IMD most deprived quintile	77 (36.5)
Ethnicity white British	201 (95.3)
M:F	98 (46.4) : 113 (53.5)

3.2.2.1. Characteristics of cases with missing data. As can be seen in Table 14*Table 13* complete data was available for the majority of the variables of interest. Missing data was minimal (< 1%) for the breastfeeding variable but somewhat more prevalent for maternal smoking during pregnancy (7%) and toddler Verbal Comprehension score (10%).

Comparison of the 211 dyads providing complete data and the 43 dyads providing partial data revealed that they did not differ in terms of maternal age (U = 3864; p = .13; d = 0.2), or in the proportion of participants in the most deprived quintile of the IMD; with 37% of those providing complete data being in the most deprived quintile compared to 40% of those providing partial data (χ^2 (1) = 0.14, p = .40; φ = 0.004).

The main source of missing data was for toddler verbal comprehension scores, with 26 toddlers having missing data for this variable. Missing data tended to result from toddler lack of engagement and/ or off task behaviour (n =18) during the course of administration of the task. This may have reflected toddlers' lack of understanding of experimenter requests, given the young age of the sample and the demands of the task. Comparison of the 228 toddlers who had valid verbal comprehension scores and the 26 toddlers for whom verbal comprehension score data were missing revealed those with missing scores were significantly younger (Mean = 30.25m, SD = 2.17), than those with valid scores (Mean = 31.5m, SD = 2.35) (U = 1981; p < .01; d = 0.03) and displayed significantly poorer inhibitory control factor scores (U = 1570; p < .01; 0.06). Working memory factor scores were also lower though this difference did not reach significance (U = 2341.52; p = .08; d = 0.01).

Due to the nature of the executive function tasks administered (see section 2.4.4.1) and their reliance on understanding the verbal instructions provided by the experimenter, data from these toddlers were excluded from the analysis. The poorer performance on the executive function tasks of these toddlers may be an artefact of a lack of understanding of the task demands rather than poor executive function ability per se.

Table 14

Proportion of the 254 dyads with toddler EF factor scores, from study 1, providing complete data for each variable of interest in the current analysis examining the prediction of toddler EF from measures of PNS (study 2).

Variable	% with complete data
Predictor Variables	
Prenatal stress (20 week and 32 week STAI)	100
Prenatal stressful life events (1st, 2nd and 3rd trimester)	100
Sex of toddler	100
Outcome Variables	
Toddler working memory factor score	100
Toddler inhibitory control factor score	100
Control Variables	
Postnatal stress (STAI / stressful life events)	100
Gestational age at birth	100
Potential Confounders and Covariates	
Risk stratification (psychological abuse)	100
Socioeconomic deprivation	100
Maternal FSIQ	100
Breastfeeding	99
Smoking during pregnancy	93
Toddler age at time of executive function assessment	100
Toddler Verbal Comprehension ability	90

3.2.3. Procedure. Data relevant to the current study were collected at 20 weeks' gestation, 32 weeks gestation, from birth records, 9 -12 weeks of age at age 6 months, age 12 months and when toddlers were 2 ½ (WCHADS study phase 1, 2, 3, 5, 6, 8 and 9 respectively). Written informed consent to take part in the overarching WCHADS study from which these measures were drawn was gained from mothers at phases 1 for phases 1, 3 and 5 of data collection, at phase 2 for phases 2, 4 and 8 of data collection, and at phase 9 for phase 9. See Figure 5 for an overview of participant progression through WCHADS study phases relevant to the current study.

3.2.3.1. Phase 1 procedure. At 20 weeks' gestation participants completed an interview and short questionnaire pack in the antenatal clinic with a research mid-wife. Of particular relevance to the current study, the questionnaire pack included collection of demographic information, the sample stratifying variable (intimate partner psychological abuse in the past year) and the Spielberger State-Trait Anxiety Inventory (STAI) (Spielberger et al., 1970).

3.2.3.2. Phase 2 procedure. At 32 weeks' gestation, participants completed a detailed investigator led interview and a questionnaire pack. Of particular relevance to the current study, and amongst measures related to the over-arching study design, the interview included the Life History Calendar (LHC) (Caspi, Moffitt, Thornton, Freedman, Ammell, Harrington et al., 1996), and a questionnaire pack that included the state anxiety subscale of the STAI (Spielberger et al., 1970). They also completed the WTAR (Holdnack, 2001).

3.2.3.3. Phase 3 procedure. Birth outcome data including feeding method on the postnatal ward was extracted from medical notes by research midwives.

3.2.3.4. Phase 5 procedure. At 9-12 weeks postpartum participants received a questionnaire in the post which included the STAI state anxiety subscale.

3.2.3.5. Phase 6 procedure. Participants completed the STAI state anxiety subscale as part of a larger face to face assessment when their infant was around 6 months.

3.2.3.6. Phase 8 procedure. Participants completed the STAI state anxiety subscale as part of a larger face to face assessment at around age 12 months.

3.2.3.7. Phase 9 procedure. The procedure followed at the 2 ½ year mother-toddler observational assessment during which measures of toddler executive function were administered is described in full in section 2.4.3.1. Toddlers also completed the measures of verbal ability, described in section 3.2.4.2. Following this face to face mother- toddler observational assessment, mothers were invited to return to the study base on their own to complete a detailed investigator- led interview which included, amongst measures relating to the over-arching study design, the LHC (Caspi et al., 1996) and the STAI state anxiety subscale (Spielberger et al., 1970).

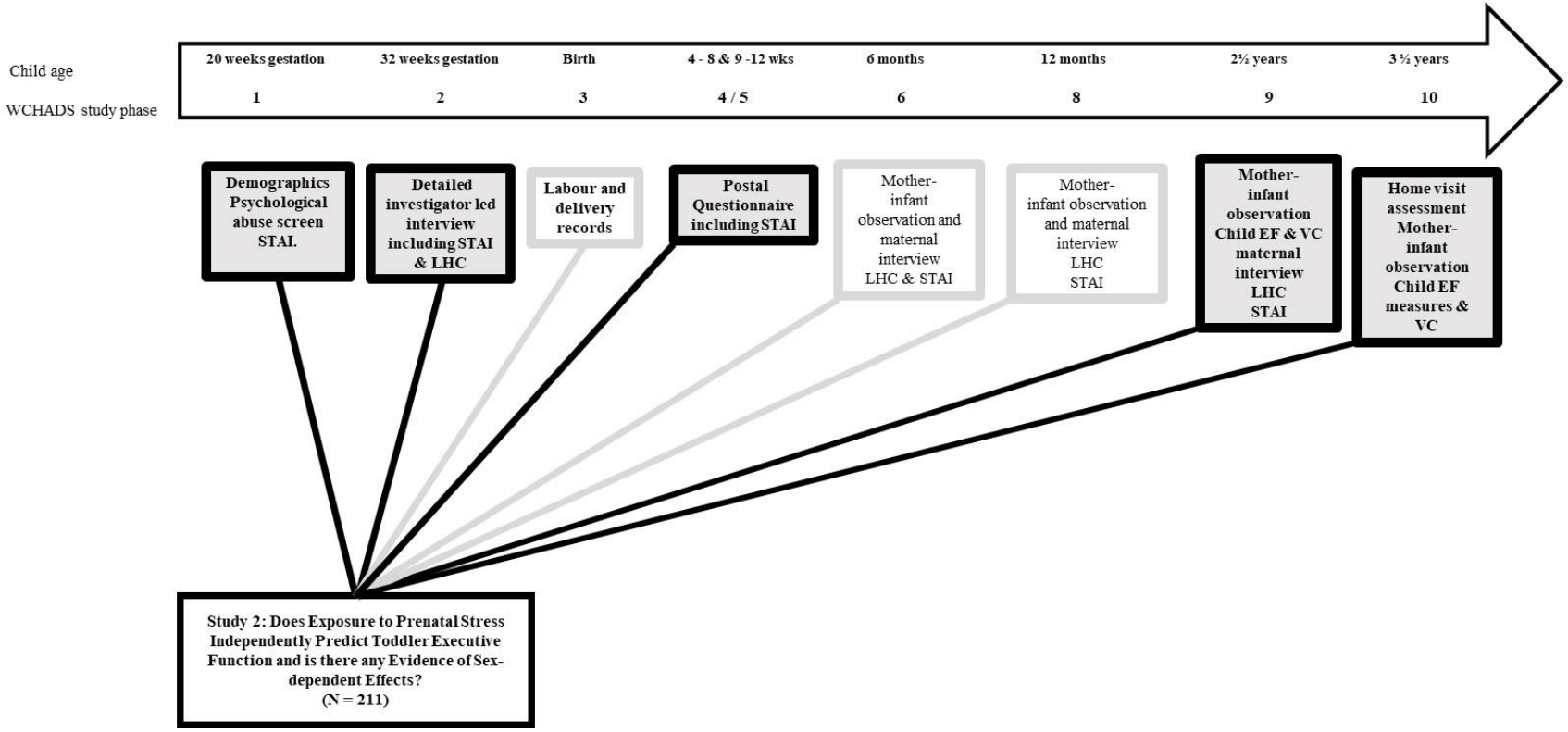


Figure 5

Measures employed in the current analysis and point of data collection within participant progression through the WCHADS study phases.

Note: measures with bold frame = main analyses measures, measures in greyscale frame = control/ confounding variable measures, LHC= Life history calendar; STAI = Spielberger State-Trait Anxiety scale (state anxiety subscale),EF = executive function, VC = Verbal comprehension.

3.2.4. Measures.

3.2.4.1. Maternal Measures of Prenatal and Postnatal Stress.

Spielberger State-Trait Anxiety Inventory (STAI) (Spielberger et al., 1970). The STAI is a brief self-report assessment tool with two subscales designed to measure trait anxiety and state anxiety respectively. The STAI state anxiety sub-scale was used in the current study which consists of 20 items asking participants to describe how they feel at the present time (e.g. calm, tense etc). Responses are rated on a four-point Likert scale, ranging from not at all to very much so (scored 1 to 4) and yielding a total score ranging from 20-80. The STAI is a widely used self-administered measure of anxiety symptoms and has been used for research purposes with both pregnant (Rini, Dunkel- Schetter, Whadwa & Sandman, 1999) and non-pregnant adults and has been shown to have good internal consistency with Cronbach's alpha co-efficient of 0.92 (Spielberger, 1983). The state-anxiety component of the questionnaire was completed prenatally by participants at 20 weeks gestation and 32 weeks gestation.

The STAI was also administered when infants were 4 - 8 weeks of age, 6 months old, 12 months old and at the 2 ½ year mother- child observational assessment. To control for exposure to postnatal maternal stress in the current analysis, a mean postnatal stress score was calculated as the mean score across these four administrations of the STAI.

The Life History Calendar (LHC) (Caspi et al., 1996): This structured interview is a calendar based tool designed to facilitate reliable retrospective recall of life trajectories and events. The measure consists of a large paper calendar with years and months represented by the columns and different categories of life activities (e.g. employment, residences and other life events) represented by the rows. The interview

begins by entering the estimated date of delivery (EDD) and from this calculating the estimated date of conception by tracking back 40 weeks from the EDD. In this way, the three trimesters are defined. Key events in participants' life are then marked on the calendar, such as birthdays. The interviewer then records information about participants' residences over the period of interest, and then moves to gather information regarding employment. Following this, the participant is asked about stressful life events (SLEs) that they may have experienced. A list of 30 SLEs was used with an additional open question allowing participants to report any other stressful life events they had experienced. Reliability studies have reported agreement with previously collected concurrent data of 90% for a period of three years (Caspi et al., 1996) and 81% over a period of five years (Freedman, Thornton, Camburn, Alwin & Young DeMarco, 1988).

The Life History calendar was administered at each intensive phase of the WCHADS study. LHC data from phases 2 (32 weeks gestation), 6 (6 months postnatal), 8 (12 months postnatal) and 9 (2 ½ years postnatal) were employed in the current study. Data from the 32 weeks' gestation administration of the LHC were used to calculate the total number of SLEs experienced in the first and second trimester of pregnancy. Data collected at both the 32 weeks' gestation and 6-month post-partum administration of the LHC were used to calculate the total number of SLEs experienced during the third trimester. Data from the postnatal administrations (6 month, 12 month and 2 ½ years) were used to calculate the number SLEs experienced in the postnatal period.

Variables measuring prenatal exposure to SLEs consisted of the total number of SLEs experienced within each trimester of pregnancy. Due to the small number of cases at the upper end of the range of scores the data were collapsed for each trimester, into values of 0, 1, 2 and 3 or more. The postnatal exposure variable was the mean number

of stressful life events experienced on average over each 12-week period between birth and the 2 ½ year administration of the LHC. That is, the total number of life events experienced between birth and the phase 9 mother interview divided by the number of 12 week periods between birth and the concurrent administration of the LHC). This was to make the rating periods comparable to the prenatal rating periods i.e. each trimester (12 week period) of pregnancy. This data was gathered from the 6 month, 12 month, and 2 ½ year administration of the LHC.

3.2.4.2. Child Outcome Measures.

Executive Function. Working memory (WM) and Inhibitory Control (IC) factor scores, derived from Confirmatory factor analysis of toddler performance on a battery of tasks assessing executive functions were employed as the toddler executive function outcome measures.

3.2.4.3. Child covariates. Data on individual child factors that might influence EF performance served as covariates in the current analysis. These were verbal comprehension ability and age at the time of EF assessment.

Verbal Comprehension ability: The British Ability Scales 2nd edition (BAS II, Elliot, 1996) was administered to all toddlers concurrently with the assessment of executive function ability at age the 2 ½ year mother- toddler assessment. The BAS-II Verbal Comprehension score exhibits moderate correlations with Wechsler Preschool and Primary Intelligence test revised (WPPSI-R, Wechsler,1967) verbal ability subtest scores (ranging from .50 to .52). The BAS II verbal comprehension subtest score was employed in regression analyses to control for toddler verbal ability due to the heavy

demands on toddler understanding of task instructions for successful executive function task performance.

Age at time of executive function assessment. Toddler age at the time of the phase 9 mother – toddler assessment was included in the regression models to control for its variation in the age at which toddler executive function abilities were assessed.

Gestational age at birth. As PNS has been associated with premature birth and in turn, this may be associated with poorer cognitive ability in offspring, gestational age at birth represents a possible mediator on a pathway from PNS to cognitive outcome. Tests of bi-variate correlations did not reveal any association between PNS and gestational age at birth. However, this variable was entered into the regression models as a last step as a conservative test of whether its addition to the models altered predictions from the PNS variables of interest.

3.2.4.4. Demographic variables, potential confounders and stratification variable.

In addition to postnatal maternal stress measures (outlined above), data for the following design- related factors, demographic background factors and other possible confounding variables identified in the literature were included in the analyses to account for their potential influence in the regression models.

Sample stratifier, Psychological abuse in intimate partner relationship during the past year. Full details regarding sample stratification are provided in section 2.2.4.3 of this thesis, a brief summary of this is provided here for ease of reference. The stratification variable was a dichotomous variable, representing whether or not mothers were

considered to be at higher psycho-social risk by virtue of them reporting psychological abuse in their intimate partner relationship (either mother to partner or vice versa). This was based on responses to the Dunedin Relationship scale at Phase 1 (20 weeks gestation) of data collection, which aimed to screen women for the presence of psychological abuse in their relationship over the previous twelve months.

Socioeconomic Deprivation, Index of Multiple Deprivation (Noble et al., 2004)

Socioeconomic deprivation was assessed from participant postcodes using the IMD (Noble et al., 2004). Data was collapsed for the purposes of analysis into two comparison categories (0,1) representing those living the socioeconomic circumstances equivalent to the most deprived quintile of the UK (code 1) versus those in the top four quintiles (coded 0).

Maternal IQ. Maternal IQ was assessed with the Wechsler Test of Adult Reading (WTAR; Holdnack, 2001) during the 32-week prenatal appointment. The WTAR displays high positive correlations with Wechsler Adult Intelligence test (WAIS III; Wechsler, 1997) verbal IQ scores (ranging from .66 to .80), and full scale IQ scores (ranging from.63 to .80), and moderate positive correlations with WAIS-III Performance IQ scores (ranging from.15 to .66). Mothers' predicted FSIQ score derived from the WTAR was used in the current study.

Smoking during pregnancy: At both prenatal assessments, women were asked about smoking habits during pregnancy. Responses were used to construct a binary variable recording whether mothers had or had not smoked at all during pregnancy, with a score

of 0 representing no smoking during pregnancy and a score of 1 representing any smoking during pregnancy.

Breastfeeding: Information was gathered from delivery and birth records at phase 3 as to whether mothers initiated any breastfeeding with their infants before discharge from the maternity unit. It was not possible to determine the duration of breastfeeding with accuracy therefore a binary variable was employed recording whether breastfeeding had (1) or had not (0) been initiated.

3.2.5. Statistical Methods: All analyses were conducted using SPSS version 22. Distributions of data for the variables of interest were first examined for deviations from normality. Skewnness/ kurtosis values and visual inspection of histograms indicated deviations from normality for all continuous predictor variables. See appendix 4. As all continuous variables exhibited deviations from normality non-parametric Spearman's rho was employed to examine correlations amongst variables of interest. Bi-variate associations between maternal prenatal stress predictor variables and child executive function outcome measures and between potential confounders, covariates and child executive function outcome measures were examined as a first step. A series of multiple linear regression analyses were then run to examine the relationship between each measure of prenatal stress at different points during pregnancy (1st and 2nd trimester STAI and 1st, 2nd and 3rd trimester SLEs) and each measure of toddler executive function ability (WM and IC) at 2 ½.

In all models tested, each variable was entered singly in consecutive blocks with forced entry. The sample stratification variable (psychosocial risk) was entered first in all models to account for the sampling strategy employed in the WCHADS. Following

this, the order of entry of subsequent control and confounder variables was guided by theoretical consideration of the importance of each variable in the prediction of toddler EF, with those considered most salient entered first. The regression models were run to examine the possible moderating effect of the role of toddler sex in this association. An a priori criterion of p- values < 0.05 was set to indicate significant findings.

For each analysis, the models were first specified to include a minimal set of essential control variables (part a). Where PNS made a significant contribution to the model, either as a main effect or in interaction with sex, a second model (part b) was run with a full set of confounders and covariates The model statistics are shown in Table 23 to Table 36. The top rows of each table display the results controlling for the minimal set of control variables: stratum membership, toddler age and verbal comprehension ability at the time of EF assessment. The lower rows of each table display the results after adjusting for the wider set of potential pre-and postnatal confounders All models were tested with gestational age at birth as a last step in the model building process in case of possible mediation of any observed association between PNS and EF by this variable.

Following Field (2009), diagnostic statistics were examined to ensure assumptions of multiple linear regression were not violated in the models tested. This involved (a) examination of bi-variate correlations amongst predictor variables, which were all < .8, indicating that there was no multi-collinearity within the data; (b) visual inspection of scatter plots, which suggested linearity and homoscedasticity (c) use of the Durbin-Watson test statistic, which was consistently < 2, thus confirming independence of residuals, (d) histograms and normal probability plots of residuals which all exhibited approximately normal distributions (e) case wise diagnostics which indicated that the models were not limited by the presence of outliers.

3.3. Results.

3.3.1. Descriptive statistics and bi-variate associations. Table 15 shows descriptive statistics of variables included in the regression analysis for the current sample of 211 mother-infant dyads. Statistics are displayed for the full sample and for male and female toddlers separately. Between-group comparisons revealed that boys and girls did not differ in the levels of exposure to PNS reported by mothers, either in prenatal stress symptoms, i.e. anxiety (20 week STAI, $U = 4827$; $p = .11$; $d = 0.22$; 32 week STAI, $U = 5353$; $p = .67$; $d = 0.06$) or exposure to stressful life events (1st trimester SLEs, $\chi^2 = 1.09$; $p = .78$; $\varphi = 0.01$; 2nd trimester SLEs, $\chi^2 = 4.90$; $p = .18$; $\varphi = 0.01$; 3rd trimester SLEs, $\chi^2 = 2.73$; $p = .44$; $\varphi = 0.01$). Furthermore, boys and girls did not differ in their EF abilities at age 2 ½ years, either in WM factor score ($U = 4972.50$; $p = .20$; $d = 0.18$) or IC factor score ($U = 9823.50$; $p = .16$; $d = 0.33$).

Bivariate associations (Spearman's rho) were examined amongst different maternal PNS predictor variables, and between these PNS predictor variables and toddler EF outcome measures. These were examined first for the whole sample (Table 16) and next for male and female toddlers separately (Table 17and Table 18). Women's reports of exposure to stressful life events (SLEs) during each trimester of pregnancy correlated significantly with their report of levels of stress symptoms (i.e. STAI state anxiety) both in mid pregnancy (20 weeks gestation) and late pregnancy (32 weeks gestation). The magnitude of association was small between the two different indices of PNS (SLEs and STAI) and moderate within-measure across time points.

Of the 5 indices of prenatal stress, only one indicator (STAI at 20 weeks' gestation) was significantly correlated with Working Memory. The magnitude of the

correlation was small (r_s = .14). Examination of the bivariate correlations for males and females separately revealed a significant positive association between 20 week STAI and WM factor score was evident in females only at r_s = .20. No significant correlations were observed between indices of PNS and toddler Inhibitory Control.

Next, bi-variate associations between toddler executive function outcome measures and potential confounders / covariates were examined to identify design related factors (stratification variable), sociodemographic factors (deprivation, maternal IQ), pregnancy related factors (smoking), perinatal factors (gestational age at birth, breastfeeding), maternal postnatal stress variables (postnatal STAI score and postnatal exposure to SLEs), and toddler related variables (sex, age at executive function assessment, and verbal comprehension ability) that might influence toddler executive function outcome measures. These can be seen for the full sample in Table 19, for males only in Table 20 and for females only in Table 21.

Across the whole sample, the only significant associations observed between toddler EF and the potential confounders and covariates examined were for toddler age at EF assessment and toddler verbal comprehension ability. This was observed in both WM and IC factor scores. The magnitude of association between verbal comprehension and EF factor scores was moderate (r_s = .30 for WM and r_s =.32 for IC), and between age at EF assessment and EF factor scores was small (r_s = .20, for WM and r_s = .22, for IC).

When males and females were examined separately, the significant associations between EF factor scores and verbal comprehension scores remained for males (r_s = .29 for WM and r_s = .24 for IC) and females (r_s = .30 for WM and r = .36 for IC). Significant associations between age and EF factors scores were evident in females only (r_s = .24 for WM and r_s = .30 for IC).

For a stringent examination of the prediction of toddler executive function from prenatal maternal stress measures, regression models were examined first with essential control variables (sample stratifier, age at EF testing and verbal comprehension at time of EF testing) and then examined with all potential confounding variables retained in the regression models tested.

Table 15

Descriptive Statistics for variables included in the regression analyses.

			Full sample				Males				Females			
Variable type	Measure		N	Mean	SD	%	N	Mean	SD	%	N	Mean	SD	%
Predictor	PNS	STAI 20wks	211	33.6	11.6	-	98	32.2	10.8	-	113	34.9	12.2	-
		STAI 32wks	211	33.2	9.6	-	98	32.6	8.4	-	113	33.8	10.6	-
	Exposure to SLEs	LHC 1st trimester	211	0.7	1.0	-	98	0.7	0.9	-	113	0.7	1.0	-
		LHC 2nd trimester	211	0.7	1.0	-	98	0.7	1.0	-	113	0.7	1.0	-
		LHC 3rd trimester	211	0.6	1.0	-	98	0.6	0.9	-	113	0.7	1.1	-
Outcome	WM factor score		211	0.2	2.3	-	98	-0.9	2.4	-	113	0.4	2.2	-
	IC factor score		211	0.1	0.9	-	98	0.2	0.9	-	113	0.2	1.0	-

Note. PNS = Prenatal stress; SLEs = Stressful life events; STAI = State trait anxiety inventory, State subscale score; LHC = Life history calendar; WM = working memory; IC = inhibitory control;

Table 15 (continued)

Descriptive Statistics for variables included in the regression analyses.

Covariates	Toddler VC	BAS VC	211	11.7	3.7	-	98	11.4	3.7	-	113	12.0	3.6	-
	Sex	Male	98	-	-	46.5	-	-	-	-	-	-	-	-
		Female	113	-	-	53.6	-	-	-	-	-	-	-	-
	Toddler age (months)		211	31.5	2.4	-	98	31.8	2.6	-	113	31.3	2.2	-
Confounders	Stratum	low risk	102	-	-	48.3	45	-	-	45.9	57	-	-	50.4
	membership	high risk	109	-	-	51.7	53	-	-	54.1	56	-	-	49.6
	Deprivation	Most deprived	77	-	-	36.5	32	-	-	32.7	45	-	-	39.8
	(IMD)	quintile												
		All other	134	-	-	63.5	66	-	-	67.3	68	-	-	60.2
		quintiles												
	Smoking	yes	56	-	-	26.5	29	-	-	29.6	27	-	-	23.9
		no	155	-	-	73.5	69	-	-	70.4	86	-	-	76.1

Note. VC = verbal comprehension; IMD = Indices of multiple deprivation.

Table 15 (continued)

Descriptive Statistics of variables included in regression analyses.

Breastfeeding	yes	170	-	-	80.6	79	-	-	80.6	91	-	-	80.5
	no	41	-	-	19.4	19	-	-	19.4	22	-	-	19.5
Maternal IQ	WTAR FSIQ	211	107.2	6.5	-	98	107.4	6.7	-	113	107.0	6.4	-
Gestational age at birth (days)		211	281.4	10.5	-	98	279.5	11.2	-	113	283.0	9.5	-
Postnatal Stress	STAI (mean 5-8wk, 6m, 12m and 2 ½ yr)	211	29.4	7.4	-	98	29.2	7.2	-	113	29.6	7.6	-
Postnatal SLEs	LHC (birth-2 ½ years)	211	5.4	4.3	-	98	5.4	4.7	-	113	5.4	4.0	-

Note. STAI= State trait anxiety inventory, state subscale score; SLEs, stressful life events; LHC = Life history calendar.

Table 16

Bi- variate associations (Spearman's rho) between PNS predictor measures and toddler EF outcome measures. Full sample.

	20 Wk STAI	32 Wk STAI	1st tri SLEs	2nd tri SLEs	3rd tri SLEs	WM	IC
20 Wk STAI	-	**.53****	**.17***	**.17***	**.16***	**.14***	.02
32 Wk STAI		-	**.14***	**.19****	**.20****	.00	.00
1st tri SLEs			-	**.39****	**.32****	.01	.00
2nd tri SLEs				-	**.44****	.11	-.01
3rd tri SLEs					-	.06	.12
WM						-	**.56****
IC							-

Table 17

Bi-variate associations (Spearman's rho) between PNS predictor measures and toddler EF outcome measures. Males only.

	20 Wk STAI	32 Wk STAI	1st tri SLEs	2nd tri SLEs	3rd tri SLEs	WM	IC
20 Wk STAI	-	**.55****	**.25***	.19	.15	.06	.00
32 Wk STAI		-	**.20***	.18	**.21***	-.13	-.03
1st tri SLEs			-	**.39****	**.34****	-.04	-.06
2nd tri SLEs				-	**.44****	.07	-.01
3rd tri SLEs					-	.10	.08
WM						-	**.53****
IC							-

Note. STAI= State trait anxiety inventory, state subscale score, SLEs = Stressful life events; tri = trimester; WM = working memory factor score, IC = inhibitory control, factor score; * $p < 0.05$, ** $p < 0.01$.

Table 18

Bi-variate associations (Spearman's rho) between PNS predictor measures and toddler EF outcome measures. Females only.

	20 Wk STAI	32 Wk STAI	1st tri SLEs	2nd tri SLEs	3rd tri SLEs	WM	IC
20 Wk STAI	-	**.51****	.12	.18	**.19***	**.20***	.02
32 Wk STAI		-	.09	**.19***	**.20***	.10	.03
1st tri SLEs			-	**.39****	**.31****	.06	.07
2nd tri SLEs				-	**.44****	.14	.02
3rd tri SLEs					-	.02	.18
WM						-	**.58****
IC							-

Note. STAI= State trait anxiety inventory, state subscale score, SLEs = Stressful life events; tri = trimester; WM = working memory factor score, IC = inhibitory control, factor score; * $p < 0.05$, ** $p < 0.01$.

Table 19

Bi-variate associations (Spearman's rho) between EF outcome measures and potential covariate and confounder measures. Full sample.

	WM	IC	Risk stratif-ication	Depri vation (IMD)	Mater nal FSIQ	Smoking	Breast-feeding	GA	VC	Toddler age	Postnatal STAI	Postnatal SLEs
WM	-	**.56****	-.05	-.05	-.07	.01	-.00	.06	**.30****	**.20****	.07	.12
IC	-	-	.02	-.02	.05	.00	-.02	.13	**.32****	**.22****	.01	.04
Risk stratification	-	-	-	.08	-.09	**.22****	-.12	.10	-.01	.07	**.20****	**.23****
Deprivation (IMD)	-	-	-	-	**-.31****	.21**	-.23**	-.02	**-.20****	-.02	.01	**.17***
Maternal FSIQ	-	-	-	-	-	-.45**	**.32****	.09	**.15***	.06	-.06	-.13
Smoking	-	-	-	-	-	-	**-.41****	-.03	-.04	.02	.11	**.26****
Breastfeeding	-	-	-	-	-	-	-	-.04	.08	.00	-.06	-.05
GA	-	-	-	-	-	-	-	-	.08	-.01	.09	.04
VC	-	-	-	-	-	-	-	-	-	**.30****	-.02	.09
Toddler age	-	-	-	-	-	-	-	-	-	-	.11	.12
Postnatal STAI	-	-	-	-	-	-	-	-	-	-	-	**.14***
Postnatal SLEs	-	-	-	-	-	-	-	-	-	-	-	-

Note. WM = working memory factor score, IC = inhibitory control, factor score; GA = gestational age at birth, VC = toddler verbal comprehension score; STAI= State trait anxiety inventory, state subscale score. * $p < 0.05$, ** $p < 0.01$.

Table 20

Bi-variate associations (Spearman's rho) between EF outcome measures and potential covariate and confounder measures. Males.

	WM	IC	Risk strat-ification	Depriv-ation (IMD)	Maternal FSIQ	Smoking	Breast-feeding	GA	VC	Toddler age	Postnatal STAI	Postnatal SLEs
WM	-	**.53****	.03	.00	-.16	.06	-.04	-.06	**.29****	.17	.09	.15
IC	-	-	-.01	.05	-.02	.04	**-.20***	.14	**.24***	.15	-.03	-.01
Risk stratification	-	-	-	.07	**-.21***	**.28****	-.19	.16	.01	.09	.13	**.26****
Deprivation (IMD)	-	-	-	-	**-.37****	**.26****	**-.26****	-.06	**-.21***	**.23***	.11	**.31****
Maternal FSIQ	-	-	-	-	-	-.44**	**.36****	.05	.15	-.06	-.18	-.15
Smoking	-	-	-	-	-	-	**-.42****	.02	-.07	.15	**.22***	**.30****
Breastfeeding	-	-	-	-	-	-	-	.01	-.02	-.09	-.11	-.09
GA	-	-	-	-	-	-	-	-	-.02	-.11	-.06	.02
VC	-	-	-	-	-	-	-	-	-	**.31****	-.10	.12
Toddler age	-	-	-	-	-	-	-	-	-	-	.11	.14
Postnatal STAI	-	-	-	-	-	-	-	-	-	-	-	.14
Postnatal SLEs	-	-	-	-	-	-	-	-	-	-	-	-

Note. WM = working memory factor score, IC = inhibitory control, factor score; GA = gestational age at birth, VC = toddler verbal comprehension score; STAI= State trait anxiety inventory, state subscale score. * $p < 0.05$, ** $p < 0.01$.

Table 21

Bi-variate associations (Spearman's rho) between EF outcome measures and potential covariate and confounder measures. Females.

	WM	IC	Risk stratif-ication	Depriv-ation (IMD)	Maternal FSIQ	Smoking	Breast-feeding	GA	VC	Toddler age	Postnatal STAI	Postnatal SLEs
WM	-	**.58****	-.12	-.11	.02	-.03	.04	.14	**.30****	**.24***	.06	.08
IC	-	-	.07	-.06	.01	-.02	.12	.09	**.36****	**.30****	.04	.09
Risk stratification	-	-	-	.10	.01	.15	-.05	.05	-.04	.03	**.25****	**.21***
Deprivation (IMD)	-	-	-	-	**-.26****	.18	**-.19***	-.01	**-.21***	-.18	-.07	.04
Maternal FSIQ	-	-	-	-	-	**-.46****	**.28****	.13	.15	.15	.05	-.11
Smoking	-	-	-	-	-	-	**-.41****	-.05	-.01	-.10	.02	**.22***
Breastfeeding	-	-	-	-	-	-	-	-.09	.17	.06	-.01	-.01
GA	-	-	-	-	-	-	-	-	.13	.09	**.22***	.03
VC	-	-	-	-	-	-	-	-	-	**.31****	.04	.05
Toddler age	-	-	-	-	-	-	-	-	-	-	.13	.11
Postnatal STAI	-	-	-	-	-	-	-	-	-	-	-	.15
Postnatal SLEs	-	-	-	-	-	-	-	-	-	-	-	-

Note. WM = working memory factor score, IC = inhibitory control, factor score; GA = gestational age at birth, VC = toddler verbal comprehension score; STAI= State trait anxiety inventory, state subscale score. * $p < 0.05$, ** $p < 0.01$.

Table 22

Bivariate correlations (Spearman's rho) between PNS predictor variables and potential confounder variables and covariates.

	32 Wk STAI	1st tri SLEs	2nd tri SLEs	3rd tri SLEs	Risk stratif-ication	Depri-vation (IMD)	Maternal FSIQ	Smoking	Breast-feeding	GA	VC	Toddler age
20 Wk STAI	**.53****	**.17***	**.17***	**.16***	**.15***	-.03	.01	.07	-.11	.07	.13	.09
32 Wk STAI	-	**.14***	**.19****	**.20****	.11	-.05	-.01	.09	-.06	.07	.06	-.02
1st tri SLEs	-	-	**.39****	**.32****	**.18***	.07	-.01	.05	-.04	.07	.05	-.03
2nd tri SLEs	-	-	-	**.44****	**.17***	**.15***	-.04	.13	-.07	.00	.09	**.15***
3rd tri SLEs	-	-	-	-	**.16***	.07	-.10	.12	-.10	-.07	**.17***	.09
Risk stratification	-	-	-	-	-	.08	-.09	**.22****	-.12	.10	-.01	.07
Deprivation (IMD)	-	-	-	-	-	-	**.31****	**.21****	**-.23****	-.02	**-.20****	-.02
Maternal IQ	-	-	-	-	-	-	-	**.45****	**.32****	.09	**.15***	.06
Smoking	-	-	-	-	-	-	-	-	**-.41****	-.03	-.04	.02
Breastfeeding	-	-	-	-	-	-	-	-	-	-.04	.08	.00
GA	-	-	-	-	-	-	-	-	-	-	.08	-.03
VC	-	-	-	-	-	-	-	-	-	-	-	**.30****

Note STAI= State trait anxiety inventory, state subscale score; SLEs = stressful life events; tri = trimester; VC = toddler verbal comprehension; GA= gestational age at birth. * $p < 0.05$, ** $p < 0.01$.

3.3.2. Bi-variate associations between PNS variables and potential confounder variables and covariates.

As can be seen in **Table 22**, there were no significant associations between prenatal stress symptoms (STAI) experienced in 3rd trimester and any of the specified confounder variables or covariates. There was a small positive association between prenatal stress symptoms (STAI) experienced in the 2nd trimester and risk stratification, such that mothers considered to be at higher psychosocial risk reported higher levels of prenatal stress symptoms. Similarly, exposure to stressful life events in the 1st trimester, 2nd trimester and 3rd trimester were all positively associated with risk stratification, such that mothers considered to be at higher psychosocial risk reported increased exposure to stressful life events. Exposure to stressful life events in the 2nd trimester was also positively associated with deprivation and toddler age at the time of EF assessment, while exposure to stressful life events in the 3rd trimester was positively associated with toddler verbal comprehension.

3.3.3. Prediction of toddler EF (working memory and inhibitory control) from prenatal stress symptoms (STAI).

3.3.3.1. Prediction of toddler working memory (WM) from prenatal stress symptoms (STAI).

In regression Model 1 a (Table 23) the association was examined between trimester 2 (20 weeks gestation) STAI score as the index of prenatal stress and toddler working memory. The overall model was significant ($F(6, 204) = 5.92$; $p < 0.01$), and accounted for approximately 12% of the variance in working memory performance. Neither the main effect of 2nd trimester STAI score nor the interaction between 2nd trimester STAI and sex of toddler made a significant contribution to the model, though the interaction term accounted for 1 % of the variance in outcomes.

As expected, toddler age at the time of EF testing and verbal comprehension ability made a significant contribution.

Table 23

Model 1a Regression Model Predicting WM from 2nd Trimester (20 week) STAI score, showing Main Effects and Sex x PNS (STAI) Interaction.

			95 % CI						
Model 1a	β	*p*-value	lower	upper	Adjusted R^2	ΔR^2	ΔF	df	*p* - value
Risk stratification	-0.08	.24	-0.98	0.24	.00	.00	0.66	1, 209	.42
Toddler age	**0.12**	**.08**	**-0.02**	**0.26**	**.05**	**.05**	**11.22**	**1, 208**	**.00**
VC	**0.29**	**.00**	**0.09**	**0.27**	**.12**	**.08**	**18.24**	**1, 207**	**.00**
Sex	0.08	.25	-0.25	0.91	.12	.01	1.56	1, 206	.21
2nd tri STAI	-0.20	.38	-0.13	0.05	.12	.00	1.07	1, 205	.30
Sex X 2nd tri STAI	0.28	.21	-0.02	0.09	.12	.01	1.57	1, 204	.21

Note VC = Verbal Comprehension, GA = Gestational age at birth.

Next the prediction of toddler working memory was tested using STAI score in trimester 3 (at 32 weeks' gestation) as the index of prenatal stress in models 2a and 2b. The overall models were each significant ($F(6, 204) = 6.40$, $p < 0.01$) and ($F(12, 198) = 3.80$, $p < 0.01$) respectively, and accounted for approximately 14% of the variance in working memory performance. As can be seen in Table 24, toddler age at testing and verbal comprehension ability contributed significantly to the model. The sex by PNS interaction term significantly predicted toddler working memory after controlling for the initial set of pre-and postnatal confounders (Model 2a), therefore the analysis was re-run with the full set of co-variates (Model 2b) and the magnitude of the effects were observed. The sex by PNS interaction term remained significant ($p = .02$) and was not diminished in magnitude. Adding gestational age at birth into the model made almost no difference to the estimated interaction co-efficient). The

interaction term accounted for approximately 2% of the variance in working memory performance

Table 24

Model 2a and 2b- Regression Models Predicting WM from 3rd Trimester (32 week) STAI score, showing Main Effects and Sex x PNS (STAI) Interaction.

			95% C.I.						
Model 2a	β	*p*-value	lower	upper	Adjusted R^2	ΔR^2	ΔF	df	*p* - value
Risk stratification	-0.06	.34	-0.90	0.31	.00	.00	0.66	1, 209	.42
Toddler age	**0.13**	**.07**	**-0.01**	**0.26**	**.05**	**.05**	**11.22**	**1, 208**	**.00**
VC	**0.30**	**.00**	**0.10**	**0.28**	**.12**	**.08**	**18.24**	**1, 207**	**.00**
Sex	0.09	.17	-0.18	1.02	.12	.01	1.56	1, 206	.21
3rd tri STAI	-0.51	.03	-0.23	-0.01	.12	.00	0.02	1, 205	.90
Sex x 3rd tri STAI	**0.52**	**.02**	**0.01**	**0.14**	**.13**	**.02**	**5.12**	**1, 204**	**.02**
			95% C.I.						
Model 2b	β	*p*-value	lower	upper	Adjusted R^2	ΔR^2	ΔF	df	*p* - value
Risk stratification	-0.09	.18	-1.05	0.20	.00	.00	0.66	1, 209	.42
Deprivation	-0.01	.84	-0.73	0.60	-.01	.00	0.29	1, 208	.59
Maternal IQ	-0.14	.06	-0.11	0.00	.00	.01	1.76	1, 207	.19
Smoking	0.02	.76	-0.68	0.94	-.01	.00	0.03	1, 206	.86
Breastfeeding	0.03	.71	-0.69	0.99	-.01	.00	0.00	1, 205	1.00
Toddler age	**0.13**	**.07**	**-0.01**	**0.27**	**.04**	**.05**	**11.71**	**1, 204**	**.00**
VC	**0.32**	**.00**	**0.11**	**0.30**	**.12**	**.08**	**19.33**	**1, 203**	**.00**
Postnatal STAI	0.05	.50	-0.03	0.06	.12	.00	0.58	1, 202	.45
Sex	0.07	.29	-0.28	0.94	.12	.01	1.26	1, 201	.26
3rd tri STAI	-0.57	.02	-0.25	-0.03	.11	.00	0.28	1, 200	.60
Sex x 3rd tri STAI	**0.55**	**.02**	**0.01**	**0.14**	**.13**	**.02**	**5.55**	**1, 199**	**.02**
GA	0.09	.18	-0.01	0.05	.14	.01	1.85	1, 198	.18

Note VC = Verbal Comprehension, GA = Gestational age at birth

Standardised predicted values of working memory factor scores from the regression model (2b) shown in table Table 24 are plotted in Figure 6 to show the nature of the underlying relationships between PNS and working memory in girls and boys separately. The 2-way interaction arose from the fact that increased 3rd

trimester STAI score was associated with a lower working memory factor score in boys and a higher working memory factor score in girls.

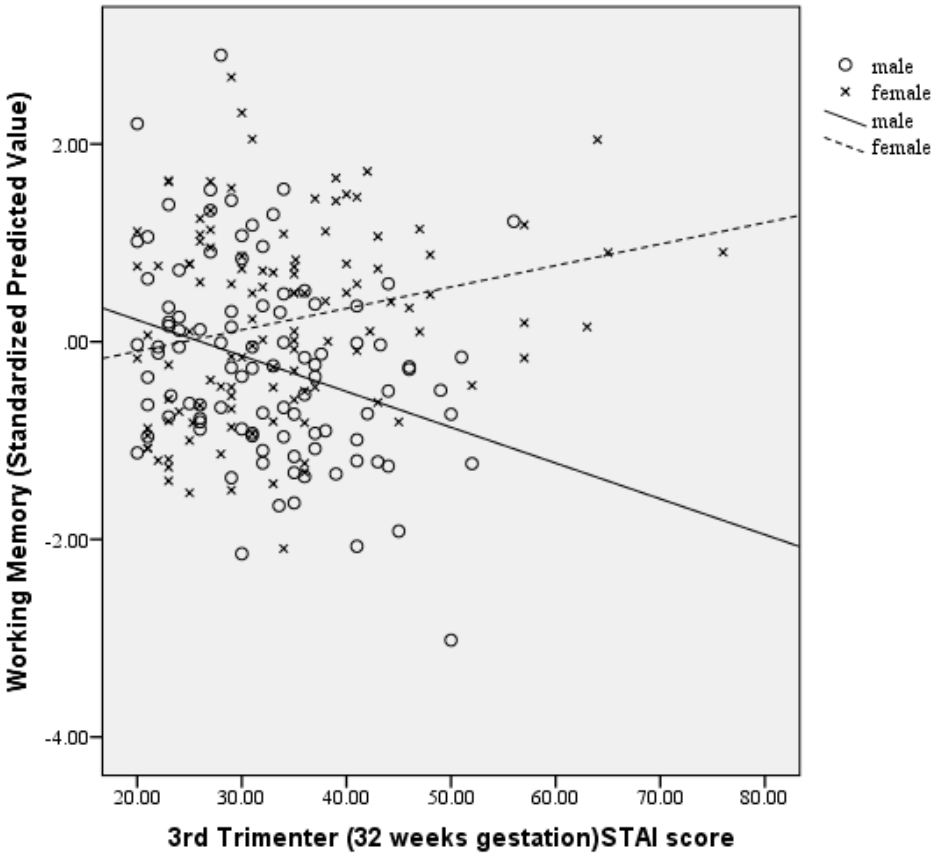


Figure 6

Predicted Values of toddler WM as a function of Sex and Prenatal Stress reported in the 3rd trimester (32 week STAI score)

In order to examine the magnitude and statistical significance of the associations observed in Figure 6 between 3rd trimester STAI score and working memory, the regression models were next examined in male (models 3a and 3b) and female toddlers separately (models 4a and 4 b). Table 25 (males) and Table 26 (females) show the model statistics from these regression models. In male toddlers, after controlling for confounders, there was a significant negative association ($\underline{\beta}$ = -

0.30, $p < .01$) between 3rd trimester STAI score and working memory factor score, with 3rd trimester STAI score accounting for an additional 7% in the variance in toddler working memory.

In contrast, in females there was a marginal positive association ($\beta = 0.20$, $p = .07$) between 3rd trimester STAI score and working memory which accounted for an additional 2% of the variance in working memory performance.

Table 25

Models 3a and 3b - Regression Model Predicting WM Factor score from 3rd Trimester (32 Week) STAI score. Males

			95% CI						
Model 3a	β	*p*-value	lower	upper	Adjusted R^2	ΔR^2	ΔF	df	*p* - value
Risk stratification	0.04	.66	-0.73	1.14	-.01	.00	0.12	1, 209	.73
Toddler age	**0.06**	**.55**	**-0.14**	**0.26**	**.02**	**.04**	**3.97**	**1, 208**	**.05**
VC	**0.35**	**.00**	**-0.09**	**0.36**	**.09**	**.08**	**8.49**	**1, 207**	**.00**
3rd tri STAI	**-0.19**	**.05**	**-0.11**	**0.00**	**.12**	**.03**	**3.85**	**1, 205**	**.05**

			95% CI						
Model 3b	β	*p*-value	lower	upper	Adjusted R^2	ΔR^2	ΔF	df	*p* - value
Risk stratification	-0.02	.86	-1.06	0.89	-.01	.00	0.12	1, 209	.73
Deprivation	0.03	.81	-0.98	1.26	-.02	.00	0.03	1, 208	.85
Maternal IQ	-0.15	.20	-0.14	0.03	-.01	.02	1.48	1, 207	.23
Smoking	0.08	.47	-0.78	1.66	-.02	.00	0.09	1, 206	.76
Breastfeeding	0.07	.51	-0.88	1.77	-.04	.00	0.00	1, 205	.95
Toddler age	**0.00**	**1.00**	**-0.22**	**0.22**	**.00**	**.04**	**4.03**	**1, 204**	**.05**
VC	**0.44**	**.00**	**0.14**	**0.43**	**.09**	**.09**	**9.99**	**1, 203**	**.00**
Postnatal STAI	0.20	.07	-0.01	0.14	.08	.01	0.55	1, 202	.46
3rd tri STAI	**-0.30**	**.01**	**-0.15**	**-0.03**	**.15**	**.07**	**7.78**	**1, 200**	**.01**
GA	0.01	.88	-0.04	0.05	.14	.00	0.02	1, 198	.88

Note VC = Verbal Comprehension, GA = Gestational age at birth

Table 26

Models 4a and 4b - Regression Models Predicting WM Factor score from 3rd Trimester (32 Week) STAI score. Females.

			95% CI						
Model 4a	β	*p*-value	lower	upper	Adjusted R^2	ΔR^2	ΔF	df	*p* - value
Risk stratification	-0.17	.07	-1.53	0.05	.01	.02	2.04	1, 209	.16
Toddler age	**0.19**	**.05**	**0.00**	**0.38**	**.08**	**.07**	**8.91**	**1, 208**	**.00**
VC	**0.26**	**.01**	**0.05**	**0.28**	**.13**	**.06**	**8.03**	**1, 207**	**.01**
3rd tri STAI	0.14	.13	-0.01	0.07	.14	.02	2.29	1, 205	.13

			95% CI						
Model 4b	β	*p*-value	lower	upper	Adjusted R^2	ΔR^2	ΔF	df	*p* - value
Risk stratification	-0.16	.09	-1.54	0.11	.01	.02	2.04	1, 209	.16
Deprivation	0.00	1.00	-0.86	0.85	.01	.01	1.15	1, 208	.29
Maternal IQ	-0.13	.21	-0.12	0.03	.00	.00	0.12	1, 207	.73
Smoking	0.00	.97	-1.14	1.09	-.01	.00	0.01	1, 206	.94
Breastfeeding	0.05	.63	-0.86	1.40	-.02	.00	0.02	1, 205	.88
Toddler age	**0.22**	**.03**	**0.03**	**0.42**	**.05**	**.07**	**8.11**	**1, 204**	**.01**
VC	**0.24**	**.02**	**0.03**	**0.27**	**.10**	**.06**	**7.74**	**1, 203**	**.01**
Postnatal stress	-0.12	.27	-0.10	0.03	.10	.00	0.04	1, 202	.85
3rd tri STAI	**0.20**	**.07**	**0.00**	**0.09**	**.11**	**.02**	**2.92**	**1, 200**	**.09**
GA	**0.18**	**.05**	**0.00**	**0.08**	**.14**	**.03**	**3.77**	**1, 198**	**.05**

Note VC = Verbal Comprehension, GA = Gestational age at birth.

3.3.3.2. Prediction of toddler inhibitory control (IC) from prenatal stress symptoms (STAI).

Regression models (Models 5a and 6a) were then specified to test the prediction of toddler inhibitory control from STAI scores in the 2nd and 3rd trimesters respectively, and for a possible interaction between sex of toddler and prenatal stress in that prediction.

First, model 5a examined the relationship using 2nd trimester (20 weeks' gestation) STAI score as the prenatal stress index. The overall model was significant ($F(6, 204) = 6.23$, $p < 0.01$) accounting for approximately 13% of the variance in inhibitory control performance. However, toddler age at the time of testing and verbal comprehension ability were the only significant predictors in the model. As can be seen from Table 27, neither the main effect of 2nd trimester STAI nor the specified interaction term made a significant contribution to the model.

Table 27

Model 5a Regression Model Predicting IC from 2nd Trimester (20 week) STAI score showing main effects and Sex x PNS (STAI) interaction.

			95% CI						
Model 5a	β	*p*-value	lower	upper	Adjusted R^2	ΔR^2	ΔF	df	*p* - value
Risk stratification	0.00	.99	-0.24	0.24	.00	.00	0.00	1, 209	.97
Toddler age	**0.14**	**.05**	**0.00**	**0.11**	**.05**	**.06**	**13.50**	**1, 208**	**.00**
VC	**0.31**	**.00**	**0.05**	**0.12**	**.14**	**.09**	**21.38**	**1, 207**	**.00**
Sex	0.07	.27	-0.10	0.38	.14	.00	1.14	1, 206	.29
2nt tri STAI	-0.10	.64	-0.04	0.03	.13	.00	0.16	1, 205	.69
Sex X 2nd tri STAI	0.08	.72	-0.02	0.03	.13	.00	0.13	1, 204	.72

Note VC = Verbal Comprehension, GA = Gestational age at birth.

Next, the relationship was tested using 3rd trimester (32 weeks' gestation) STAI score (Model 6a see Table 28Table 28) as the index of prenatal stress. The overall regression model was significant ($F(6, 204) = 6.20$, $p < 0.01$) and accounted for around 13% of the variance in inhibitory control performance. However, neither the main effect of 3rd trimester STAI nor the specified interaction term made a significant contribution to the model. As expected, toddler age at time of EF assessment and verbal comprehension scores made significant contributions to the model.

Table 28

Model 6a Regression Model Predicting IC from 3rd Trimester (32 week) STAI score. Main effects and Sex x PNS (STAI) Interaction.

			95% CI						
Model 6a	β	*p*-value	lower	upper	Adjusted R^2	ΔR^2	ΔF	df	*p* - value
Risk stratification	0.00	.97	-0.25	0.24	.00	.00	0.00	1, 209	.97
Toddler age	**0.14**	**.05**	**0.00**	**0.11**	**.05**	**.06**	**13.50**	**1, 208**	**.00**
VC	**0.31**	**.00**	**0.04**	**0.12**	**.14**	**.09**	**21.38**	**1, 207**	**.00**
Sex	0.07	.28	-0.11	0.37	.14	.00	1.14	1, 206	.29
3rd tri STAI	-0.09	.70	-0.05	0.04	.13	.00	0.05	1, 205	.82
Sex X 3rd tri STAI	0.08	.74	0.02	0.03	.13	.00	0.11	1, 204	.74

Note VC = Verbal Comprehension, GA = Gestational age at birth.

3.3.4. Prediction of toddler working memory and inhibitory control from prenatal stressful life events (SLEs).

Next, a series of regression models (Models 7 to 14) tested the relationship between exposure to stressful life events in each trimester of pregnancy separately, to toddler working memory performance and inhibitory control in turn.

3.3.4.1. Prediction of toddler WM from exposure to SLEs. Model 7a (see Table 29) examined the prediction of toddler working memory using exposure to SLEs during the first trimester as the index of PNS. The overall model was significant ($F(6, 204) = 5.65$ $p < .01$). As expected, toddler age at the time of testing and verbal comprehension ability were significant predictors of working memory. However, neither the main effect of PNS nor the PNS x sex interaction term were significant.

Table 29

Model 7a Regression Model predicting WM Factor Score from 1st trimester SLEs with Main effects and Sex x 1st trimester SLEs interaction.

			95% CI						
Model 7a	β	*p*-value	lower	upper	Adjusted R^2	ΔR^2	ΔF	df	*p* - value
Risk stratification	-0.07	.31	-0.92	0.30	.00	.00	0.66	1, 209	.42
Toddler age	**0.14**	**.05**	**0.00**	**0.27**	**.05**	**.05**	**11.22**	**1, 208**	**.00**
VC	**0.29**	**.00**	**0.10**	**0.27**	**.12**	**.08**	**18.24**	**1, 207**	**.00**
Sex	0.08	.21	0.22	0.99	.12	.01	1.56	1, 206	.21
1st tri SLEs	-0.22	.31	-1.75	0.55	.12	.00	0.01	1, 205	.92
Sex X 1st tri SLEs	0.23	.27	-0.31	1.13	.12	.01	1.25	1, 204	.27

Note VC = Verbal Comprehension, GA = Gestational age at birth

Model 8a (Table 30) examined the prediction of toddler working memory using exposure to stressful life events (SLEs) during the second trimester as the index of PNS. The overall model was significant; F (6, 204) = 5.88 (p< 0.01), accounting for around 13% of the variance in toddler working memory. Neither the main effect of PNS nor the interaction between sex of toddler and 2nd trimester SLEs made a significant contribution to the model. Toddler age at the time of assessment and toddler verbal comprehension ability each made a significant contribution to the model.

Table 30

Model 8a Regression Model Predicting WM from 2nd trimester SLEs with Main Effects and Sex x PNS (SLEs) interaction.

			95% CI						
Model 8a	β	*p*-value	lower	upper	Adjusted R^2	ΔR^2	ΔF	df	*p* - value
Risk stratification	-0.08	.24	-0.97	0.25	.00	.00	0.66	1, 209	.42
Toddler age	**0.12**	**.09**	**-0.02**	**0.25**	**.05**	**.05**	**11.22**	**1, 208**	**.00**
VC	**0.28**	**.00**	**-0.09**	**0.27**	**.12**	**.08**	**18.24**	**1, 207**	**.00**
Sex	0.08	.20	-0.21	1.00	.12	.01	1.56	1, 206	.21
2nd tri SLES	0.01	.95	-1.01	1.08	.13	.01	2.24	1, 205	.14
Sex X 2nd tri SLEs	0.09	.68	-0.50	0.77	.12	.00	0.18	1, 204	.68

Note VC = Verbal Comprehension, GA = Gestational age at birth

Table 31 displays regression model 9a, examining the prediction of toddler working memory from exposure to stressful life events in the 3rd trimester. The overall model was significant, (F (6, 204) = 5.55, $p < .01$), and accounted for around 12 % of the variance in toddler working memory performance. Toddler age at the time of testing and verbal comprehension ability were significant predictors of working memory. Neither the main effect of 3rd trimester SLEs nor the PNS x sex interaction term made a significant contribution to the model.

Table 31

Model 9a - Regression Model Predicting WM from 3rd Trimester SLEs with Main Effects and Sex x 3rd Trimester SLEs Interaction.

			95% CI						
Model 9a	β	*p*-value	lower	upper	Adjusted R^2	ΔR^2	ΔF	df	*p* - value
Risk stratification	-0.06	.37	-0.90	0.33	.00	.00	0.66	1, 209	.42
Toddler age	**0.13**	**.07**	**-0.01**	**0.26**	**.05**	**.05**	**11.22**	**1, 208**	**.00**
VC	**0.28**	**.00**	**0.09**	**0.27**	**.12**	**.08**	**18.24**	**1, 207**	**.00**
Sex	0.08	.22	-0.22	0.98	.12	.01	1.56	1, 206	.21
3rd tri SLEs	0.18	.42	-0.70	1.67	.12	.00	0.27	1, 205	.60
Sex x 3rd tri SLEs	-0.15	.50	-0.96	0.47	.12	.00	0.46	1, 204	.50

Note VC = Verbal Comprehension, GA = Gestational age at birth

3.3.4.2. Prediction of toddler IC from exposure to (SLEs). Next the prediction of toddler inhibitory control was tested using exposure to stressful life events in the 1st trimester as the index of prenatal stress in models 10a and 10b (Table 32). The overall models were each significant, (F (6, 204) = 5.14, p < .01) and (F (12, 198) = 3.96, p < .01) respectively, and accounted for around 13 % of the variance in toddler inhibitory control. Again, toddler age at testing and verbal comprehension ability contributed significantly to the model. The Sex x PNS

interaction term made a significant contribution to the model, accounting for 2% variance in inhibitory control scores after controlling for the initial set of pre-and postnatal confounders (Model 10a). Therefore, the analysis was re-run with the full set of covariates (Model 10b) and in this model the sex by PNS interaction term remained significant and was not diminished in magnitude. Adding gestational age at birth as a last step made almost no difference to the estimated interaction co-efficient, which remained significant.

Table 32

Regression Model 10a and 10b - Predicting IC from Exposure to SLEs in the 1st Trimester, with Main Effects and Sex x 1st Trimester SLEs Interaction.

			95% CI						
Model 10a	β	*p*-value	lower	upper	Adjusted R^2	ΔR^2	ΔF	df	*p* - value
Risk stratification	-0.01	.86	-0.26	0.22	.00	.00	0.00	1, 209	.97
Toddler age	**0.16**	**.02**	**0.01**	**0.12**	**.05**	**.06**	**13.50**	**1, 208**	**.00**
VC	**0.32**	**.00**	**0.05**	**0.12**	**.14**	**.09**	**21.38**	**1, 207**	**.00**
Sex	0.07	.29	-0.11	0.37	.14	.00	1.14	1, 206	.29
1st tri SLEs	-0.41	.05	-0.90	0.00	.13	.00	0.01	1, 205	.93
Sex X 1st tri SLEs	**0.42**	**.04**	**0.01**	**0.58**	**.15**	**.02**	**4.17**	**1, 204**	**.04**

			95% CI						
Model 10b	β	*p*-value	lower	upper	Adjusted R^2	ΔR^2	ΔF	df	*p* - value
Risk stratification	-0.03	.63	-0.31	0.19	.00	.00	0.00	1, 209	.97
Deprivation	0.01	.85	-0.24	0.29	-.01	.00	0.10	1, 208	.76
Maternal IQ	-0.01	.92	-0.02	0.02	-.01	.00	0.48	1, 207	.49
Smoking	0.00	.95	-0.31	0.33	-.02	.00	0.20	1, 206	.66
Breastfeeding	-0.02	.80	-0.38	0.29	-.02	.00	0.17	1, 205	.68
Toddler age	**0.17**	**.02**	**0.01**	**0.12**	**.04**	**.06**	**12.73**	**1, 204**	**.00**
VC	**0.31**	**.00**	**0.04**	**0.11**	**.12**	**.09**	**21.15**	**1, 203**	**.00**
Postnatal SLEs	0.03	.71	-0.27	0.39	.12	.00	0.11	1, 202	.74
Sex	0.04	.52	-0.16	0.32	.12	.00	0.99	1, 201	.32
1st tri SLEs	-0.44	.04	-0.95	-0.03	.11	.00	0.05	1, 200	.83
Sex x 1st tri SLEs	**0.45**	**.04**	**0.02**	**0.60**	**.13**	**.02**	**3.90**	**1, 199**	**.05**
GA	**0.15**	**.02**	**0.00**	**0.03**	**.14**	**.02**	**5.17**	**1, 198**	**.02**

Note VC = Verbal Comprehension, GA = Gestational age at birth

Figure 7 displays the predicted values of toddler inhibitory control from this model. The significant interaction appears to represent an opposing pattern of PNS effects in male and female infants. In girls, increasing inhibitory control is seen in the context of prior exposure to higher numbers of stressful life events during the 1st trimester of pregnancy. In contrast, in boys decreasing inhibitory control is associated with increased levels of exposure to stressful life events during the 1st trimester pregnancy.

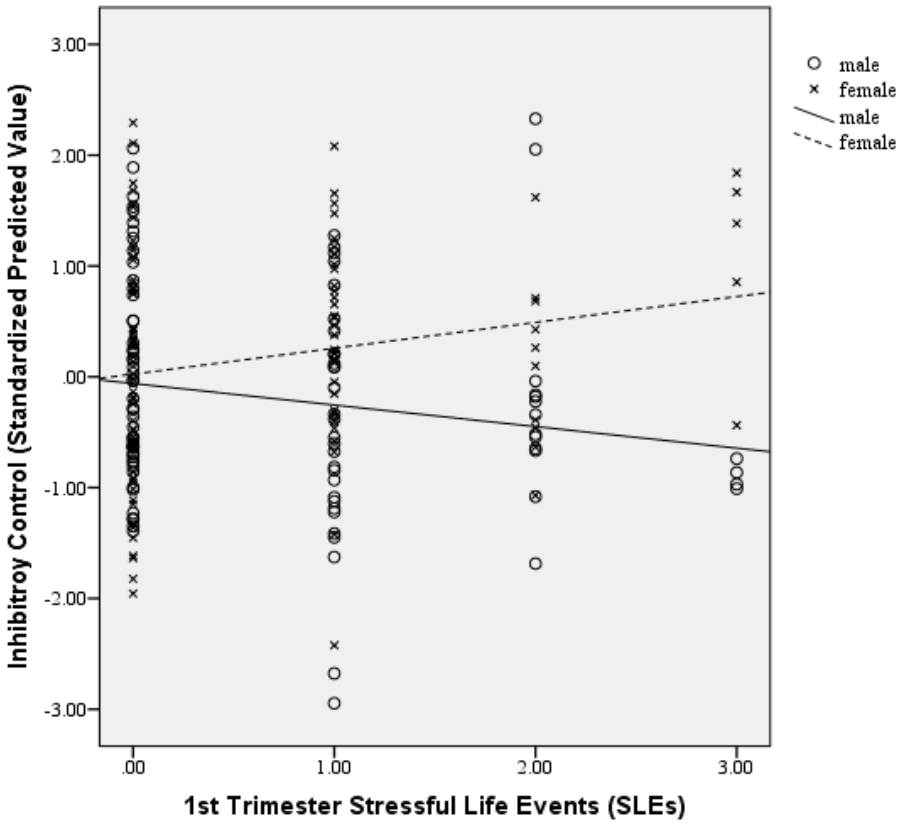


Figure 7

Predicted Values of toddler IC Factor Score as a function of Sex and exposure to SLEs in the 1st Trimester

In order to inspect the pattern and magnitude of associations underlying the interaction, a further regression model predicting inhibitory control from first trimester stressful life events was tested in male toddlers (models 11a and 11b) and female (Models 12a and 12b) toddlers separately. The model statistics for these analyses can be seen in Table 33 (males) and

Table 34 (females). As expected, verbal comprehension made a significant contribution to the models in both males and females. Toddler age at time of EF assessment also contributed significantly to the model in females and whereas gestational age at birth contributed in males. The association between PNS and inhibitory control performance was non- significant in girls and boys, which suggests that the significant interaction term in the whole group analysis arose from the opposing pattern of associations, that is, the negative association in boys and positive association in girls. The direction of effects appeared to mirror that observed with the STAI. Inspection of the effect sizes in the models, indexed by the change in R^2 for PNS, suggested that 2% of the variance in IC was accounted for by PNS in males ($\underline{\beta}$ = -.17) and 1% ($\underline{\beta}$ = .12) in females.

Table 33

Regression Model 11a and 11b - Regression Models Predicting IC from Exposure to SLEs in the 1st Trimester, Males.

			95% CI						
Model 11a	β	*p*-value	lower	upper	Adjusted R^2	ΔR^2	ΔF	df	*p* - value
Risk stratification	-0.02	.88	-0.38	0.32	-.01	.00	0.03	1, 209	.86
Toddler age	0.08	.48	-0.05	0.10	.01	.03	2.67	1, 208	.11
VC	**0.26**	**.02**	**0.01**	**0.11**	**.05**	**.05**	**5.08**	**1, 207**	**.03**
1st tri SLEs	-0.13	.21	-0.34	0.08	.05	.02	1.58	1, 205	.21
			95% CI						
Model 11b	β	*p*-value	lower	upper	Adjusted R^2	ΔR^2	ΔF	df	*p* - value
Risk stratification	-0.08	.47	-0.50	0.24	-.01	.00	0.03	1, 209	.86
Deprivation	-0.04	.76	-0.51	0.38	-.02	.00	0.03	1, 208	.87
Maternal IQ	-0.05	.68	-0.04	0.02	-.03	.00	0.02	1, 207	.88
Smoking	-0.01	.96	-0.46	0.44	-.04	.00	0.18	1, 206	.67
Breastfeeding	-0.15	.20	-0.82	0.17	-.02	.03	2.65	1, 205	.11
Toddler age	0.11	.37	-0.04	0.12	-.01	.02	2.18	1, 204	.14
VC	**0.25**	**.04**	**0.00**	**0.11**	**.03**	**.05**	**4.72**	**1, 203**	**.03**
Postnatal SLEs	0.05	.68	-0.36	0.55	.02	.00	0.00	1, 202	.95
1st tri SLEs	-0.17	.12	-0.39	0.05	.03	.02	1.69	1, 200	.20
GA	**0.20**	**.05**	**0.00**	**0.03**	**.06**	**.04**	**3.89**	**1, 198**	**.05**

Table 34

Regression Model 12a and 12b - Regression Models Predicting IC from Exposure to SLEs in the 1st Trimester, Females.

			95% CI						
Model 12a	β	*p*-value	lower	upper	Adjusted R^2	ΔR^2	ΔF	df	*p* - value
Risk stratification	-0.01	.91	-0.35	0.31	-.01	.00	0.07	1, 209	.79
Toddler age	**0.24**	**.01**	**0.03**	**0.19**	**.10**	**.12**	**14.58**	**1, 208**	**.00**
VC	**0.36**	**.00**	**0.05**	**0.15**	**.21**	**.12**	**16.69**	**1, 207**	**.00**
1st tri SLEs	0.13	.13	-0.05	0.36	.22	.02	2.36	1, 205	.13
			95% CI						
Model 12b	β	*p*-value	lower	upper	Adjusted R^2	ΔR^2	ΔF	df	*p* - value
Risk stratification	-0.02	.79	-0.40	0.30	-.01	.00	0.07	1, 209	.79
Deprivation	0.06	.49	-0.24	0.49	-.01	.00	0.43	1, 208	.52
Maternal IQ	0.03	.77	-0.03	0.04	-.01	.01	1.13	1, 207	.29
Smoking	0.03	.78	-0.41	0.55	-.02	.00	0.17	1, 206	.68
Breastfeeding	0.06	.57	-0.34	0.61	-.02	.01	0.69	1, 205	.41
Toddler age	**0.25**	**.01**	**0.03**	**0.19**	**.08**	**.11**	**13.59**	**1, 204**	**.00**
VC	**0.35**	**.00**	**0.04**	**0.15**	**.19**	**.11**	**15.12**	**1, 203**	**.00**
Postnatal SLEs	0.00	.96	-0.50	0.52	.19	.00	0.25	1, 202	.62
1st tri SLEs	0.12	.21	-0.08	0.36	.19	.01	1.64	1, 200	.20
GA	0.08	.35	-0.01	0.03	.19	.01	0.89	1, 198	.35

Next the prediction of inhibitory control was tested using stressful life events in the 2nd trimester as the index of PNS in model 13a. The overall model was

significant (F (6, 204) = 6.34, $p < .01$), accounting for around 13% of the variance in toddler inhibitory control. As can be seen in Table 35, toddler age at testing and verbal comprehension ability made a significant contribution to the model. Neither the main effect of PNS nor the interaction between 2nd trimester SLEs and sex of toddler made a significant contribution.

Table 35

Model 13a Model Predicting IC from Exposure to SLEs in the 2nd Trimester with Main Effects and Sex x 2nd Trimester SLEs Interaction.

			95% CI						
Model 13a	β	*p*-value	lower	upper	Adjusted R^2	ΔR^2	ΔF	df	*p* - value
Risk stratification	0.00	.95	-0.23	0.25	.00	.00	0.00	1, 209	.97
Toddler age	0.15	.04	0.00	0.11	.05	.06	13.50	1, 208	.00
VC	0.31	.00	0.05	0.11	.14	.09	21.38	1, 207	.00
Sex	0.07	.30	-0.11	0.36	.14	.00	1.14	1, 206	.29
2nd tri SLEs	-0.06	.78	-0.47	0.36	.14	.00	0.90	1, 205	.34
Sex x 2nd tri SLEs	0.00	.99	-0.26	0.25	.13	.00	0.00	1, 204	.99

Note VC = Verbal Comprehension, GA = Gestational age at birth

Finally, the prediction of toddler inhibitory control was tested using exposure to stressful life events in the 3rd trimester as the index of prenatal stress in model 14a. The overall model was significant (F (6, 204) = 6.92 $p < .01$) and accounted for around 14% of the variance in toddler inhibitory control. As can be seen from Table 36, toddler age at testing, and verbal comprehension contributed significantly to the model. Neither the main effect of 3rd trimester SLEs nor the PNS x Sex interaction term made a significant contribution to the model.

Table 36

Model 14a *Regression Model Predicting IC from Exposure to SLEs in the 3rd Trimester with Main Effects and Sex x 3rd Trimester SLEs Interaction.*

			95% CI						
Model 14a	β	*p*-value	lower	upper	Adjusted R^2	ΔR^2	ΔF	df	*p* - value
Risk stratification	-0.03	.70	-0.29	0.19	.00	.00	0.00	1, 209	.97
Toddler age	**0.14**	**.04**	**0.00**	**0.11**	**.05**	**.06**	**13.50**	**1, 208**	**.00**
VC	**0.29**	**.00**	**0.04**	**0.11**	**.14**	**.09**	**21.38**	**1, 207**	**.00**
Sex	0.07	.29	-0.11	0.37	.14	.00	1.14	1, 206	.29
3rd tri SLEs	-0.12	.58	-0.60	0.34	.14	.01	1.43	1, 205	.23
Sex X 3rd tri SLEs	0.21	.34	-0.15	0.42	.14	.00	0.92	1, 204	.34

Note VC = Verbal Comprehension, GA = Gestational age at birth

3.3.5. Power analysis. Post- hoc power analyses were conducted using the software package G-Power (Faul et al., 2007). Following Cohen (1988), the recommended effect sizes were: small ($f^2 = .02$), $f^2 = .15$ (medium) and $f^2 = .35$ (large). A threshold of > 0.80 was employed to indicate sufficient power.

Post-hoc power analyses were conducted for the analyses testing the prediction of toddler WM and IC by PNS (STAI and SLEs). Power analyses for models tested in the pooled sample of male and female toddlers and including only the minimal set of essential confounder variables and covariates, used a 6 predictor variable equation and a sample size of n = 211. The power to detect large effects was 0.99 to detect medium effects was 0.99 and to detect small effects was 0.28. Therefore, there was more than sufficient power to detect moderate to large effects but the power to detect small effects was inadequate.

Power analyses for the models tested in male and female toddlers separately and including only the minimal set of essential confounder variables and covariates, employed a 4 predictor variable equation. A sample size of n = 113 was used for the models tested in females only and n = 98 for the models tested in males only. In the female only sample, the post -hoc power analysis revealed the power to detect large

effects was 0.99, to detect medium effects was 0.86, and to detect small effect was 0.15. In the male only sample, the power to detect large effects was 0.97, to detect medium effects was 0.80 and to detect small effects was 0.14. Therefore, when the sample was split by sex, the power remained sufficient to detect moderate to large effects and inadequate to detect small effects.

Post-hoc power analyses for models testing the PNS prediction of toddler IC and WM in the pooled sample of male and female toddlers, including the comprehensive set of confounder variables and covariates, employed a 12 predictor variable equation and a sample size of n = 211. The power analysis revealed the power to detect large effects was 0.99, to detect medium effects was 0.98 and to detect small effects was 0.20. Thus, there was more than sufficient power to detect moderate to large effects but the power to detect small effects was inadequate.

Power analyses for the models tested in male and female toddlers separately and including the comprehensive set of confounder variables and co-variates employed a 10 predictor variable equation. Power analyses for models tested in females used a sample size of n = 113, and for those tested males, used a sample size of n =98. In the female only sample, the power to detect large effects was 0.99, to detect medium effects was 0.74 and to detect small effects was 0.11. In the male only sample, the power to detect large effect sizes was 0.98, to detect medium effects was 0.65 and to detect small effects was 0.10. Thus when the sample was split by sex, and the analysis included the comprehensive set of confounder variables and covariates, the power to detect small effects remained inadequate and the power to detect large effects remained adequate. The power was no longer sufficient to detect moderate effects.

3.4. Discussion

The current study found that two relatively independent measures of prenatal stress, exposure to stressful life events (during the first trimester) and experience of stress (anxiety symptoms during the third trimester) were each associated with indices of executive function in toddlerhood but in a sex-dependent manner. These prenatal stress effects persisted even after controlling for a broad set of pre- and postnatal confounders including exposure to postnatal stress. Single sex analyses revealed that associations operated in opposing directions in males and females. No significant prediction was evident from either measure of prenatal stress in the second trimester of pregnancy to later toddler executive function.

In this study, mothers' increasing experience of stress symptoms (STAI score) during the third trimester was associated with poorer working memory scores in male toddlers (p = .01; accounting for 7% variance in outcome) and better working memory scores (marginal) in female toddlers (accounting for 2% variance in outcome). This interaction between third trimester anxiety symptoms and sex of toddler was significant. A similar pattern was observed in the association between mothers' experience of stressful life events (SLEs) during the first trimester of pregnancy and toddler inhibitory control. Specifically, increasing exposure to SLEs

was associated with poorer inhibitory control in male toddlers and better inhibitory control in females. Overall, the interaction between first trimester SLEs and sex accounted for 2% of the variance in toddler inhibitory control. However, the associations between first trimester SLEs and inhibitory control were non-significant when examined in males and females separately which might reflect reduced power to detect more subtle (small) effects when the sample was split by sex. The fact that a similar pattern of findings was observed for third trimester anxiety symptoms in the prediction of toddler working memory strengthens confidence in the sex- dependent pattern of findings.

Our findings that 1st trimester PNS predicted IC only and 3rd trimester PNS predicted WM only was unexpected. As both of these indices of EF are thought to be mediated by the PFC, one may expect that vulnerability to the proposed detrimental effects of PNS would be observed at the same point during gestation for each of them. It could be argued that the two indices may be localised to differing sub-regions of the PFC and that these sub-regions may have differing sensitive periods to PNS. On the other hand, both conceptually and anecdotally from administration of EF tasks, those tasks loading onto the IC factor score from study 1 of this thesis were generally more arousing to toddlers than those loading onto the WM factor score. This was likely due to the food rewards involved in the snack delay task and the novelty and aesthetics of the equipment of the detour reaching task. It is plausible that poor regulation of arousal systems affects performance on the IC tasks administered, with higher levels interfering with inhibitory control ability. Arousal levels are associated with the functioning of subcortical brain structures (Pribram & McGuinness, 1975) such regions begin to develop earlier than PFC structures and thus may be vulnerable to PNS earlier in gestation.

3.4.1. Study Findings in the Context of Previous Research and Novel Contributions to the field.

This study makes a novel contribution to the existing literature through its examination of the association between prenatal stress and cognitive outcome at an age than has previously been largely neglected by such research. Indeed, at the time of study planning there were no such studies apparent in the literature and a recent search of the literature revealed only one (very recently published) study in this age group. Lin, Xu, Huang, Jia, Zhang, Yan and Zhang, (2017) reported that prenatal maternal emotional stress was inversely associated with 32-month-old toddlers' general cognitive development. However, Lin et al.'s (2017) study exhibits a number of limitations, which are improved upon in the current study

First, maternal prenatal stress was assessed via a stressful life events scale and with the Self- Report Symptom-Checklist-90-Revised scale (SCL-90-R, Schauenburg and Strack, 1999). The SCL-90-R is a 90 item self-report inventory assessing the level of symptoms on a 5-point scale (ranging from 'not at all' to 'extremely') across nine dimensions, specifically somatization, obsessive-compulsive, interpersonal sensitivity, anxiety, depression, hostility, phobic anxiety, paranoid ideation, and psychoticism, yielding a total 'Global Severity Index' score. Lin et al., (2017) reported that prenatal maternal emotional stress (SCL-90-R score) was inversely associated with toddlers' general cognitive development. However, as the SCL-90 is a rather broad measure of symptoms of varying psychopathologies, the reported association may have been predominantly driven by symptoms of any one or more of these 9 dimensions rather than symptoms of stress specifically. The current study employs a more specific measure of stress symptoms, namely anxiety,

therefore the reported associations are more clearly attributable to stress per se as opposed to other aspects of psychological well-being.

Second, though Lin et al. (2017) reported no association between prenatal stressful life events and toddler cognitive outcome; they assessed life events at a single time point, during the third trimester, but assessed the occurrence of life events across the whole of pregnancy as opposed to per trimester. Previous findings suggest that the foetus may be differentially susceptible to the effects of prenatal stress at different points during pregnancy, and that possible sensitive periods may differ according to the type of stress experienced (i.e. exposure to stressful life events compared with experience of symptoms of stress). Thus, a significant impact of SLEs during one particular trimester, such as that reported in the current study, may have been masked by lack of associations for SLEs experienced during other trimesters (when the foetus may be less susceptible) in Lin et al.'s (2017) study. The current study employed repeated measurement of both exposure to stressful life events and experience of symptoms of stress at different points during pregnancy in order to assess the specificity of any associations detected.

We also add to the field by assessing both exposure to external stressors in the prenatal period and experience of subjectively felt PNS, few previous studies have applied measures of both. Tarabulsy et al. (2014) however conducted a meta - analysis of studies examining the association between PNS and infant cognitive development and included studies employing both these forms of PNS measure. They reported a larger effect size for exposure to external stressors than for more subjective measures of PNS. In contrast to Tarabulsy at al. (2014), we did not find that prenatal exposure to external stressors (SLEs) had a larger effect size than more subjective measures of PNS (in our case STAI score). We found a similar effect size

for the 3rd trimester STAI score by sex prediction of toddler WM and the 1st trimester SLE by sex prediction of IC ($\beta = 0.55$ and $\beta = 0.45$ respectively). Differences in methodologies amongst the studies included that employed more subjective measures of stress (N=8) compared with greater consistency in methodologies amongst the studies that examined exposure to external stressors (N=3) in Tarabulsy et al.'s (2014) report limits confidence in a true difference in effect sizes. For example, in those studies examining more subjective measures, measures employed included the STAI, the pregnancy specific anxiety scale (PSA), a diagnostic interview for anxiety disorder, family stress and the perceived stress scale (PSS). These measures may exhibit subtle differences in the aspects of stress they assess during the prenatal period. The studies examining prenatal exposure to external stressors, on the other hand all assessed exposure to SLEs. The assessment of both exposure to external stressors (SLEs) and experience of stress (anxiety, STAI) in the same sample in our study, allows a more direct comparison of effect sizes for these two indices of PNS in the prediction of child cognitive outcome. However, the assessment of indices of EF at 2 ½ as our outcome measures compared to the studies included in Tarabulsy et al.'s (2014) analysis, that all employed more general measures of cognitive development, makes comparison of our findings with those of Tarabulsy et al. (2014) difficult.

The current study further adds to the existing literature by employing measures of executive function as a more specific form of cognitive outcome. Previous studies in this area of the literature have predominantly employed the Bayley Scales of Infant Development in assessing child cognitive outcome. Although this is a well validated tool, frequently used in the clinical assessment of infant and early child development, it is a broad measure of cognitive development and

therefore does not aid in the identification of specific structure-function relations that may be influenced by PNS. Previous studies examining the relationship between PNS and cognitive abilities in adolescent and older child samples have employed measures of more specific cognitive domains, such as executive functions. An inverse relationship between PNS and aspects of EF abilities, like that seen in males in the current study, has been reported in adolescents aged 15 – 17 years (Van den Bergh et al., 2005) and school age children (6 -9 years; Buss et al., 2011). To our knowledge, ours is the first study to examine the association between PNS and executive functions in the early preschool period.

Though the animal literature examining the association between PNS and offspring cognitive abilities has reported sex differences, with a reported relative male vulnerability; sex differences in studies of the association between PNS and child cognitive ability in human participants have not routinely been examined. In the few studies that have examined this issue, conflicting findings have been reported. Specifically, in Bergman et al.'s (2007) infant sample and LaPlante et al.'s (2004) school age sample, the authors reported that the associations between PNS and cognitive ability were not moderated by the sex of the child. Li et al. (2013), on the other hand reported a relative vulnerability in girls such that maternal prenatal exposure to stressful life events (across the whole of the prenatal period) was associated with lower reading scores in girls and higher reading scores in boys. In a study of executive functioning, Buss et al. (2011) reported that high levels of pregnancy specific anxiety were associated with lower inhibitory control in girls only, and with higher visuospatial working memory in both boys and girls in their school age sample.

Although the current study assessed similar aspects of executive function to Buss et al. (2011), we report an opposite pattern of findings with regards to sex differences. Analyses revealed a pattern of decreasing working memory ability following exposure to increasing maternal prenatal anxiety (STAI score) in male toddlers and a pattern of increasing working memory abilities in females (though the association in females was only marginal ($p = 0.07$). In relation to inhibitory control ability, we found the same directional pattern of decreasing ability associated with increasing prenatal exposure to prenatal stressful life events in male toddlers and increasing ability with increasing exposure in female toddlers. These effects were non-significant when male and female toddlers were examined separately, possibly due to the smaller sample size when split by sex.

Discrepancies between the current findings and those of Buss et al. (2011) may be explained by a number of methodological differences across studies. Not least is the large difference in the age of children at outcome assessment. Though Buss et al., (2011) controlled for postnatal maternal stress via assessment of symptoms of depression, this was assessed at 8 weeks' post-partum and concurrent with child EF assessment (6 to 9 years) only. It may be argued that Buss et al.'s study did not include adequate control of the possible impact of postnatal maternal stress on the developing child's EF abilities in the intervening period. Such a large window of opportunity for the impact of postnatal processes due to the age of the children at outcome assessment may contribute to the observed pattern of EF abilities in their sample as opposed to a specific impact of prenatal stress. Furthermore, although the studies assessed similar aspects of executive function, the measures employed to assess these were rather different. Additionally, measures of PNS were administered at different time points during pregnancy across studies.

However, the pattern of a relative male vulnerability to the influence of PNS across these two aspects of EF observed in the current study does reflect numerous findings in the animal literature of a male vulnerability to the influence of PNS on cognitive ability. The direction of effects in the current study are also in line with Sandman, Glynn and Davies (2013) report, in which they conducted supplementary analyses of data from previously published studies (by their own research group) examining the association between biological markers of PNS and neurodevelopment. Specifically, supplementary analysis of the data from a study examining the association between maternal prenatal cortisol levels and infant psychomotor and mental development on the BSID, revealed sex differences in the observed association. The original study (Davis & Sandman 2010) reported impaired psychomotor and mental development at 1 year associated with elevated maternal cortisol in early gestation. When sex differences were subsequently examined the analysis revealed that the negative association was significant only in males. Similarly, reanalysis of the data of another study by this group (Ellman et al., 2008) also revealed sex differences. The original study reported that elevated maternal cortisol in early pregnancy and increased corticotropin releasing hormone (CRH) in late pregnancy were significantly associated with decreased physical and neuromuscular maturation. Upon supplementary analysis of the data to examine sex differences, Sandman et al. (2013) found that male foetuses exposed to elevated maternal cortisol in early pregnancy and elevated CRH in late pregnancy displayed delayed physical and neuromuscular development in early infancy. In females, on the other hand, there was a trend to suggest that elevated maternal cortisol in late pregnancy was associated with increased physical and neuromuscular maturation.

Such findings, together with those of the current study, are consistent with suggestions that exposure to prenatal adversity results in increased risk for morbidity in males, whereas "the female foetus adjust and adapts to environmental challenge" (Sandman et al., 2013, p. 330).

Such findings may also be interpreted drawing on the influential evolutionary sex biased preferential maternal investment hypothesis (e.g. Trivers & Willard, 1973). Specifically, the Trivers and Willard model asserts that reproductive strategies under conditions of maternal adversity during pregnancy, such as the presence of PNS, involve less investment in male foetus' and greater investment in females, resulting in less resources for the optimal development of male foetus' compared to females. According to such hypotheses, any environmental adversity in early development will therefore always affect males more than females, as observed in our findings of a significant negative association between indices of PNS (anxiety and exposure to stressful life events) and indices of EF (WM and IC respectively) in males only. The observed marginal ($p = 0.07$) positive association between PNS (STAI) and WM in females in the current study may suggest that this preferential investment in female foetus' allows them to adapt to the environmental challenge of exposure to PNS as suggested by Sandman et al. (2013).

3.4.2. Methodological strengths. Previous findings reporting negative associations between infant cognitive ability and PNS have often employed different measures of PNS. Studies variously operationalise PNS as exposure to stressful life events or as the experience of stress symptoms. Yet, there are findings to suggest that the foetus may be differentially susceptible to the impact of PNS at different points during pregnancy. Furthermore, there is the possibility that there are different sensitive periods for the influence of different aspects of PNS on child cognitive

abilities so the timing of measurement in pregnancy is also important. Few other studies have examined both exposure to stressful life events *and* self-report of stress symptoms at multiple time points across gestation. One of the strengths of the current study is its repeated measurement of both exposure to stressful life events and experience of anxiety symptoms, at multiple points during gestation. This allowed us to explore timing effects for these two, relatively independent, aspects of PNS whilst controlling for parallel forms of postnatal stress exposure.

A further methodological strength of the current study lies in the rigorous set of potential confounders and co-variates included in the regression analyses. Previous studies in this area of the literature have often lacked in adequate control for exposure to postnatal stress. Yet, such control measures are a requisite in prospective research if one is to be confident of a true prenatal effect. We included repeated administration of the same measures of stress across the postnatal period and, as such, controlled for postnatal stress robustly in all analyses.

Notably, we also included maternal IQ as a potential confounder in the regression analyses as a possible means of controlling for genetic transmission of cognitive abilities. Measures of PNS such as exposure to SLEs (e.g. job loss, imprisonment, major financial difficulties) may not assess independent characteristics of participants and may be related to cognitive abilities which may be passed on genetically from mother to child (LaPlante et al., 2004). Employing a well validated and standardised measure of maternal IQ (WTAR), we improve on previous work in the field which has largely employed proxy measures of maternal cognitive abilities, such as education level/ years in education or annual occupational earnings.

Finally, the current study is further strengthened by its approach to the assessment and analysis of executive function performance. Applying CFA to toddler performance on a battery of tasks considered a priori to tap working memory and inhibitory control processes (as reported in chapter 2) allowed us to parse out shared variance in performance that was attributable to extraneous characteristics of the tasks (such as lower order cognitive functioning), leaving only that which was attributable to the latent abilities under study (working memory and inhibitory control).

3.4.3. Methodological Limitations. Despite the current study's novel contributions to the field and its clear strengths, the study exhibits a number of limitations. Although the overall sample size was adequate to detect a statistical interaction, the examination of sex differences in the underlying observed associations using single group analyses reduced sample sizes for these analyses considerably. Following Green (1991), a sample size of at least 146 should be used to detect small effects in the overall regression models of the current study and in testing the contribution of their individual predictors. This is based on the number of predictors specified in the models. This may account for the observed associations between PNS and toddler inhibitory control being small in magnitude but non-significant when examined for boys and girls separately. A larger sample size may have allowed requisite statistical power to detect small effects. Given the novelty of the study findings, and the importance of understanding the impact of prenatal stress on development, replication of the study findings with larger sample sizes would be an obvious focus for future research.

Additionally, multiple comparisons were conducted to assess effects of type of stress experienced (STAI and SLEs) and timing of stress experienced (1^{st}, 2^{nd} and

3rd trimester) on the two outcomes (WM and IC), resulting in five sets of analyses for each outcome. It may be argued that with only two of these analyses showing a significant PNS effect on outcome, this could be the result of type-I error arising from the multiple comparisons conducted. However, the direction of the observed effects (sex by 32 weeks STAI on WM and sex by 1st trimester SLEs on IC) mirrored each other with poorer outcome associated with increasing PNS in males and enhanced EF outcome associated with increasing PNS in females. The consistency in the direction of effects increases confidence that the observed interactions were real effects. Consistency of the findings with evolutionary hypotheses of sex biased preferential maternal investment (e.g. Trivers & Willard, 1973) also increases confidence in the reported associations.

Adjustment of *p* values, such as Bonferroni correction is a common approach to guard against erroneous rejection of the null hypothesis when multiple comparisons are conducted. We decided not to adopt such adjustment procedures for a number of reasons. First, these adjustments carry the cost of increasing the frequency of Type-II error (Rothman, 1990). Indeed, Rothman argues that the often mechanistic application of such adjustment procedures, "to the extent that...[it]….shields some observed associations from more intensive scrutiny by labelling them as chance findings, defeats the purpose of scientists."

Second, testing the 2-way interaction (sex by PNS) prediction of toddler EF reduced the power in the analysis to detect small but potentially important effects. Adjustment of *p*- values, due to the multiple analyses conducted would further limit the ability to detect smaller effects that may be significant in larger samples. Due to the novelty of the investigations comprised in the current thesis, we were cautious to

avoid overlooking smaller effects. However, the results should be interpreted with caution and require replication with larger samples.

The repeated assessment of maternal pre- and postnatal stress is a clear strength of the current study; however repeated administration of the same self-report measure (STAI) across the pre and postnatal phases of study may have reduced reliability of data regarding experience of stress symptoms. Specifically, participants may have become overly accustomed to the questions asked, biasing their responses by prior response styles. Additionally, it was not possible to administer the STAI measure in the 1st trimester of pregnancy, which would have allowed for a more thorough examination of the association between first trimester PNS and EF abilities. Life event data was available at this early time point in pregnancy, not because there was a trimester 1 assessment, but because the Life History Calendar (Caspi et al., 1996) methodology built into the study allowed for the timing of events to be identified.

Although maternal stress measures were, for the most part collected prospectively, an exception strictly applied to the measures of stressful life events used. Data from the 32 weeks' gestation maternal interview were used to calculate the total number of events experienced in the 1st and 2nd trimester, data from the 32 weeks' gestation maternal interview and 6-month post-partum maternal interview were used to calculate the total number of events in the 3rd trimester. Retrospective data has been suggested to carry some concerns regarding accuracy (e.g. Barusch, 2011). However, the relatively short time frame between the experience of events and data collection regarding them, together with the objective nature of the events being recalled may reduce such concerns (e.g. Henry et al., 1994). Also, the Life History Calendar methodology adopted was used to minimise the chance of

erroneous dating of events as far as possible.

Additionally in relation to the SLE data, we assessed only the frequency of exposure to SLEs, without assessing the severity of the events experienced. Analysis of mothers' appraisal of the severity of the events they experienced may have enriched the current findings. Individual variation in the extent to which different mothers find the same event as stressful likely results concomitant differences in the activation of their physiological stress response systems. This may therefore impact upon the relationship between PNS (as measured by SLEs) and toddler EF.

3.4.4. Conclusions, Implications and Directions for future Research. The findings reported here are consistent with well replicated findings in animal models of a negative association between PNS and offspring cognitive development, particularly in males. These findings in males are consistent with a proposed mechanism of the negative association between PNS and cognitive development. Specifically, a proposed detrimental impact on the structure and function of the developing brain due to increased exposure to glucocorticoids (GCs) associated with PNS, together with the high concentration of gluco-corticoid receptors in the PFC (a neural region underlying EF performance) making it a target region for these circulating GCs. Our findings are consistent with previous reports of negative associations between PNS and EF abilities in older child and adolescent samples and extend these findings by examining this association very early in the development of EF abilities.

The current study is the first to report moderation of prenatal stress effects on early EF by sex in humans, with effects in boys and girls being evident in opposite directions in toddlerhood. Though novel, the fact that this same pattern of findings

was observed when PNS was measured in the first and third trimester of pregnancy using differing indices and in relation to different aspects of toddler executive function strengthens the findings. Opposite patterns have been observed previously (e.g. Li et al., 2013 and Buss et al., 2011) but later in childhood and with differing cognitive outcomes. Importantly, some have evidenced male vulnerability and others female vulnerability to prenatal stress effects on cognitive outcomes, and some have reported enhanced effects similar to those observed in females in the current study.

Evidence of an inverse association between aspects of PNS and toddler executive function has important clinical implications, such that it suggests that children of mothers experiencing increased levels of prenatal stress may be at risk of sub optimal EF development. Executive dysfunction has been reported in a range of developmental disorders in young children (e.g. Sergeant et al., 2002) and EF abilities have also been implicated in the development of later academic abilities (e.g. Welsh et al., 2010). Early identification of pregnant women experiencing increased PNS may offer an opportunity to reduce suboptimal child EF abilities and its associated risks through psychological interventions to reduce stress in pregnancy. Furthermore, given that much of the development of the human brain and specifically the pre-frontal cortex occurs postnatally, early identification of children at risk of sub optimal EF development (due to their mothers' experience of stress during pregnancy) may provide opportunity for postnatal interventions focused on optimising aspects of the early environment that enhance EF abilities over time. Future research should aim to investigate potential prenatal interventions aimed at reducing maternal PNS as well as postnatal influences that may minimise or reverse the negative impact of PNS that has been reported for child EF abilities. A number of studies have indicated that high quality parenting can support optimal cognitive

development in childhood, so interventions to support cognitive development may be warranted where difficulties in early EF are identified.

4. Study 3: Are Prenatal Stress Effects on Toddler Executive Function Abilities Moderated by the Early Postnatal Caregiving Environment?

4.1. Background

The identification of aspects of the postnatal environment that may moderate the impact of prenatal stress on child cognitive development offers the opportunity for early intervention aimed at improving outcomes for children. Yet the literature examining postnatal processes that may impact upon the association between prenatal stress and cognitive development is somewhat lacking (Tarabulsy et al. 2014).

A frequently proposed mechanism for the negative association between prenatal stress and child cognitive development is the detrimental impact of changes to the foetal environment, resulting from the mothers' exposure to stress, on foetal neural development. This proposed mechanism is outlined in more detail in 3.1.11 of this thesis. Given that the development and fine tuning of neural systems continues beyond birth; there is much opportunity for postnatal environmental influences to exert an influence on later child neural development and, as such, the potential to moderate the association between prenatal stress and child cognitive development.

The first few years of life are characterised by substantial neural plasticity accompanying important cognitive developmental processes. For example, through processes such as synaptogenesis and synaptic pruning, efficient neural networks are created and maintained. Since synaptic contacts require repeated use and reinforcement to ensure their survival, these processes are largely guided by experience. The specific patterns of neural activation and neural processing that are determined by the child's interactions with the environment will, to a large degree,

determine which synaptic connections persist and which are pruned due to lack of use (e.g. Benefiel & Greenough, 1998). Indeed, it has been shown that such neural developmental processes are highly sensitive to environmental influences (Nelson and Bosquet, 2000).

4.1.1. The Early Care-Giving Relationship and Development. In early development, parental care-giving is one of the foremost features of the environment determining an infant's survival. The parent-infant relationship represents a fundamental, all-encompassing and long enduring aspect of the child's environment particularly in the first few years of life. As such, it represents an important candidate for the investigation of potential moderators of the association between maternal prenatal stress and early developmental processes. The importance of aspects of this relationship, in early development, for later outcomes has been implicated across a number of domains of functioning including social competence, academic skills, emotional regulation, aggression and behavioural problems (Fraley, Roisman, and Haltigan, 2013; Jaffari- Bimmel, Juffer, van IJzendorn, Bakermans- Kranenburg and Mooijaart, 2006; Sroufe, 2000).

Of particular relevance to the current thesis are findings demonstrating the influence of aspects of the mother - offspring relationship on cognitive development and neural function. A positive influence of maternal care-giving behaviours on offspring cognitive function and markers of efficient neural processing/ neural integrity has been robustly demonstrated in rodents. Such findings will be reviewed first along with a proposed mechanism for such effects before turning to a review of work conducted in humans.

4.1.2. Evidence Demonstrating the Benefits of Increased Maternal Care-Giving Behaviours for Offspring Cognitive Development in Animal Models.

Evidence of a positive influence of maternal care-giving behaviour on offspring cognitive function and markers of efficient neural processing/ neural integrity can be drawn from studies employing the 'neonatal handling' procedure with mother–pup dyads in rodents. The neonatal handling procedure involves briefly separating pups from their mother and exposing them to a new environment. Specifically, mothers are removed from the home cage and placed in a holding cage. Pups are then removed from the home cage and placed in a small clean container for 15 minutes. Following this, pups are replaced to the home cage before reintroducing their mother. Comparison groups of untreated dams and their pups are left undisturbed in their home cages (Meaney, Aitken, Berkel, Bhatnagar, Sapolsky, 1988). A number of research groups have demonstrated improved hippocampal dependent learning and memory in handled compared with non- handled pups (Bredy, Lee, Meaney and Brown, 2004; Kosten, Lee and Kim, 2007; Meaney, Aitken, Bhatnagar and Sapolsky, 1991; Meaney et al., 1988; Zaharia, Kulczycki, Shanks, Meaney and Anisman, 1996).

It is of note that Kosten et al. (2007) reported the effect in both male and female rats whereas Bredy et al. (2004) found the effect evident only in males when the subjects were a breed of mouse. Others have included only one sex of subject and therefore the presence of sex differences cannot be determined from their findings (Meaney et al., 1991; Zaharia et al., 1996). Nonetheless, there are findings to indicate that brief periods of separation from the mother are associated with improved cognitive performance in pups. Such findings, perhaps somewhat counterintuitively,

seem to indicate a beneficial impact of reduced mother- pup contact on cognitive development in the offspring.

However, researchers have discovered that the neonatal handling procedure is also associated with increased levels of the expression of specific maternal care-giving behaviours, outside of the brief separation periods involved.

"Mother – pup contact in the rat occurs primarily within the context of a nest bout, in which the mother approaches the litter, gathers the pups under her, licks and grooms her pups and nurses while continuing to occasionally lick and groom the pups; the bout terminates when the mother leaves the nest" (Liu, Diorio, Day, Francis and Meaney, 2000, p. 799).

Increased levels of these two characteristic maternal care-giving behaviours, arched back nursing (ABN) and licking and grooming (LG) have been demonstrated in mothers of handled pups compared with those of non-handled pups (Bredy, et al., 2004; Lee and Williams, 1974; Liu, et al., 1997). It is this increase in maternal caregiving behaviours to which the cognitive advantage demonstrated in handled versus non-handled pups is attributed. Indeed, in their comprehensive study, Bredy et al. (2004) demonstrated both that handled pups were exposed to increased levels of maternal LG and ABN compared with non-handled pups; and that male handled pups (but not females) displayed improved cognitive performance (spatial learning) compared to their non- handled counterparts. Together, such findings implicate increased levels of maternal caregiving behaviour as a mechanism for the effects of neonatal handling on offspring cognitive development, at least for male offspring.

Longer periods of separation however (180 minutes per day as opposed to the 15 minutes of the neonatal handling procedure) have been associated with poorer cognitive performance in the offspring (Frankola, Flora, Torres, Grissom, Overstreet

and Dohanich, 2010). Specifically, Frankola et al. (2010) compared spatial memory performance in pups separated from their mothers for 180min per day with that of pups undergoing a handling procedure (i.e. separated for 15 minutes per day). All other conditions were kept constant across groups. Male rats (but not females) that experienced the longer period of daily separation were impaired in their spatial memory performance compared with those experiencing the 15 minutes daily separation of the handling procedure. These findings therefore suggest that though brief periods of maternal separation in rodent mother- infant dyads appear to increase the level of certain maternal caregiving behaviours, which in turn have a beneficial impact on cognitive ability in male offspring, longer periods of separation may not induce this positive behaviour in dams. However, this is speculative since Frankola et al., (2010) did not measure levels of maternal caregiving behaviours around the separation periods.

It is of note that female offspring undergoing the longer separation period of Frankola et al.'s (2010) study however were not impaired, and in some cases displayed better cognitive performance than females receiving the shorter separation periods of the early handling procedure. Thus, as in Bredy et al.'s (2004) study, the advantage of maternal LG and ABN behaviour for offspring cognitive ability may be sex-specific, with male offspring only benefiting.

Further evidence of the impact of maternal LG and ABN on offspring cognitive development comes from studies examining naturally occurring individual differences (i.e. no experimental interference of mother-pup dyadic behaviour) in the frequency/ duration of these behaviours on offspring cognitive performance. The two behaviours are commonly examined jointly as a single variable (LG/ABN) due to the strong correlation between them, which has been reported to be as high as $r = .94$

(Liu, et al., 1997). Studies in this area have demonstrated that offspring of high LG/ABN dams (usually operationalised as 1SD above the sample mean level of LG/ABN) exhibit enhanced hippocampal dependent spatial learning and memory compared with those of low LG/ABN dams (operationalised 1SD below the sample mean of LG/ABN). Thus, providing further evidence that this form of maternal caregiving behaviour exerts a significant beneficial effect on offspring cognitive development (Bredy, et al., 2004; Bredy, Humpartzoomian, Cain and Meaney, 2003; Liu et al. 2000; and Toki, Morinobu, Imanaka, Yamamoto, Yamawaki and Honma, 2007). The presence of sex differences in the observed effect cannot be determined from these findings since either only male rats were studied (Bredy, et al., 2004; Bredy, et al., 2003; Toki, et al., 2007) or, in the case of Liu et al. (2000), although male and female offspring were included, the authors simply did not examine sex differences directly.

Despite the convincing evidence of a beneficial effect of increased levels of maternal LG/ABN behaviour for offspring cognitive performance cited above (Bredy, et al., 2004; Bredy, et al., 2003; Toki, et al., 2000; Liu et al., 2000), there are also opposing findings in this area of the literature. Barha, Pawluski, and Galea (2007), for example, reported that female offspring of low LG mothers displayed enhanced working memory compared with female offspring of high LG dams, and male offspring of both high and low LG dams. In their discussion, Barha et al. (2007) suggest that such discrepancies between their findings and previous research may be related to a number of differences in research design, such as the strain of rat and the cognitive tasks employed. However, within offspring of high LG dams and irrespective of sex they did report a significant negative association between the number of reference memory errors and maternal LG, such that increased levels of

maternal LG were associated with fewer reference memory errors. Thus, their findings may still be considered to provide some evidence that increased levels of this maternal caregiving behaviour is associated with better cognitive outcomes in offspring, but perhaps only for some cognitive abilities and above some critical level of LG.

Other groups have extended the findings suggesting a beneficial effect of maternal LG/ABN on offspring cognitive performance to demonstrate changes at the neural level, which may underlie such performance benefits. Specifically, Liu et al. (2000) and Champagne, et al. (2008) demonstrated relative benefits in performance on hippocampal-dependent cognitive tasks, and markers of improved neural structure and function in the hippocampus, in offspring of high versus low LG/ABN mothers.

Champagne et al. (2008), for example, reported that offspring of low LG/ABN mothers displayed decreased dendritic spine length and spine density in the CA1 region of the hippocampus as well as impaired long term potentiation (LTP). Additionally, Liu et al. (2000) reported that offspring of high LG/ABN dams showed increased expression of two independent synaptic markers in the hippocampus (synaptophysin and neural cell adhesion molecule) indicating either increased levels of hippocampal synaptogenesis or increased levels of synaptic survival in these offspring compared with those of low LG/ABN dams. Such differences at the neural level may be considered indicative of more efficient neural architecture for appropriate new learning and memory (Moser, Trommald and Andersen, 1994) in high LG/ABN offspring compared with low LG/ABN offspring.

Furthermore, Liu et al.'s (2000) cross fostering design provides support for a causal role of individual differences in maternal LG/ABN behaviours on these measures of offspring cognitive and neural function. Specifically, biological

offspring of low LG/ABN dams reared by high LG/ ABN dams were indistinguishable from the biological offspring of high LG/ ABN dams (reared by these high LG/ABN dams) both in terms of their spatial memory and learning abilities and in terms of these markers of hippocampal synaptogenesis/ synaptic survival.

Others have approached the examination of the impact of this form of maternal care-giving behaviour on offspring cognitive development through direct experimental manipulation of the levels of LG/ABN to which pups are exposed. D'Amato, et al. (2011) employed a double - mothering procedure, i.e. placing a second female in the cage with the mother and her pup from birth until weaning. For comparison, control group pups were reared by their biological mother alone. The authors reported that pups reared with an additional female received more LG/ABN than those reared by their biological mother alone. Furthermore, these pups exhibited improved performance on hippocampal dependent cognitive tasks as well as increased dendritic spine length and spine density in the CA1 region of the hippocampus.

4.1.3. A Mechanism for the Effects of Maternal Care-Giving Behaviours on Offspring Cognitive Development in Animal Models: Tactile Stimulation. A fundamental feature of rodent LG/ABN behaviour is the tactile stimulation of the pups involved. Pups deprived of this form of tactile stimulation, through being reared away from the dam, display poorer performance in at least some aspects of cognition than offspring of mother-reared rats (Burton, Lovic and Fleming, 2006; Lomanowska, Lovic, Rankine, Mooney, Robinson and Kraemer, 2011; Lovic and Fleming, 2004; Lovic, Keen, Fletcher and Fleming, 2011). Artificially mimicking this form of tactile stimulation through gentle stroking of the pup with a paint brush has been shown to, at least partially; reverse these effects (see Lomanowska and Melo, 2016 for a review).

Similar findings have also been reported at the neural level. For example, Chatterjee, Chatterjee-Chakraborty, Rees, Cauchi, de Medeiros and Fleming, (2007) reported that male pups reared away from the dam display decreased expression of several protein markers that are related to neuron number, neuronal plasticity and neuronal functionality in a range of neural regions. Furthermore, the artificial tactile stimulation procedure described above (gentle stroking of the pup with a paint brush) was found to reverse the decreased expression of these protein markers, as well as the observed behavioural deficits in offspring reared away from the dam (Chatterjee et al., 2007). Corresponding or opposing effects in female offspring cannot be determined from Chatterjee et al.'s study since only male rats were examined. Nevertheless, these findings suggest that, in male rats at least, the tactile stimulation component of maternal LG/ABN behaviour plays a crucial role in its beneficial effects on the neural and cognitive development of rat offspring.

4.1.4. Research Examining the Impact of Maternal Caregiving Behaviours on Child Cognitive Development in Human Participants.

Corresponding findings suggesting a beneficial influence of caregiving behaviours on child cognitive development have been reported in human participants who have experienced naturally occurring variation in the caregiving environment. One strand of research has focused on the detrimental impact of markedly inadequate caregiving experiences, such as neglect, abuse (e.g. Curtis and Cichetti, 2007; De Bellis, 2001; Teicher, Tomoda and Anderson, 2006), or a lack of a parental caregiving relationship altogether, through being raised in an institution (Chugani et al., 2001; Marshall and Fox, 2004; Rutter and O'Conor, 2004). Findings suggest that such unfavourable parent-child relationship experiences in early development can exert a profound detrimental impact on brain development, both at the neural level and the functional level.

Of particular relevance to the current thesis, however, is research examining naturally occurring variation within the normal range of care-giving behaviours and its association with child cognitive development. In western societies, a child's primary care-giver is most likely to be the mother therefore research in this area is predominantly focussed on mother – child relationship functioning. Such research indicates that higher levels of (or higher quality) maternal interactive behaviours are associated with improved cognitive development in children. Multiple aspects of the mother-child relationship have been examined in this area of the literature including maternal sensitivity and/ or responsiveness, attachment classification, scaffolding behaviours and amount of time spent in interaction with the child; all of which have been implicated by different research groups. Similarly, varying outcome measures of child cognitive ability have been employed across studies, with relative benefits

demonstrated in general cognitive ability, language development, academic skills, attention and executive function. Table 37 illustrates the range of methods used to assess aspects of the mother-child relationship and those employed to assess cognitive development in this area of the literature.

As can be seen from Table 37, better cognitive outcomes have consistently been associated with more positive features of the mother- child relationship despite the varying methodologies employed. Furthermore, this has been reported in a range of cognitive developmental outcomes including general cognitive development (BSID MDI), IQ, language development, academic-related skills and attention and of particular relevance to the current thesis; executive function abilities. Thus, there is convincing evidence that better quality mother- infant relationship functioning or increased levels of positive mother-infant interactive behaviours are associated with improved cognitive development, in agreement with findings from the animal literature.

Others have demonstrated that the benefits in cognitive development associated with such positive features of the mother- child relationship are accompanied by advantages at the neural level. For example, in their prospective longitudinal study of 127 children across pre-school to school age, Luby, Belden, Harms, Tillman and Barch (2016) reported that greater levels of maternal support assessed in the pre-school period (age 3 years, 11 months to 6 years, 11 months, mean age not reported) during child completion of a delay of gratification task were associated with faster hippocampal volume growth across pre-school to school age. Similarly, in another prospective study, Bernier, Calkins and Bell, (2016) examined the association between maternal interactive behaviour during free play and markers of frontal brain development in 215 infants at five months, ten months and 24

months. Higher quality mother-infant interactions were operationalised as those scoring higher on maternal sensitivity, positive affect and physical stimulation, and lower on intrusiveness. A high low contrast was set as +/- one standard deviation of the mean level of maternal interactive behaviour. Bernier et al., (2016) reported that infants of mothers that displayed higher quality interactive behaviour at infant age five months exhibited advanced frontal brain development at ten and twenty-four months of age, as well as greater age-related increases in markers of frontal brain development, than infants of mothers demonstrating lower quality mother-infant interactive behaviour.

Thus, despite the varying methodologies employed, these prospective longitudinal studies have repeatedly reported that positive mother- child relationship features and better quality mother- child interactive behaviours are associated with benefits in general measures of child cognitive and language development and enhanced neural development. Such findings are comparable to those in the animal literature demonstrating the beneficial effect of increased levels of specific maternal caregiving behaviours for offspring cognitive and neural function. However, the aspects of maternal care examined and the timing of measurement of these differ starkly in the animal and the human literature, with studies in rodents examining maternal care-giving behaviours that occur pre-weaning. As can be seen Table **37** on the other hand, very few studies in the human literature have examined the association between aspects of the mother- child relationship and aspects of cognitive development in the first few months of life. Nevertheless, such findings of an association between the mother- infant relationship and aspects of cognitive development in human participants converge with findings in the animal literature of an association between levels of specific maternal care-giving behaviours (licking

and grooming and arched back nursing) and improved cognitive development to suggest that aspects of maternal care are associated with cognitive benefits in the offspring. As such, the mother- offspring relationship may offer an opportunity for intervention aimed at improving outcomes in those at risk of poor cognitive development.

Table 37

Prospective Research Examining the Association between Maternal Caregiving Behaviours and Child General Cognitive and Language Development.

Authors	N (M:F)	Age of child at M-C interaction assessment	Features of caregiving behaviour/ parent - child relationship assessed	Age of child at cognitive outcome assessment	Child cognitive domain assessed.	Main findings
Bornstein and Tamis-LeMonda (1989)	20 (not reported)	5m	Maternal responsivity to distress Maternal responsivity to non - distress	4yr	IQ	Four year olds with mothers who were rated as more responsive (at 4m) obtained higher IQ scores at 4yrs.
Lugo-Gil and Tamis-LeMonda (2008)	2089 (1069: 1020)	14m, 24m and 36m	**Parenting Quality:** (Sensitivity, Positive regard, Responsiveness to distress cues, Cognitive growth fostering, Socio-emotional growth fostering, Supportive presence, Quality of Assistance	14m, 24m and 36m	General Cognitive ability	Parenting quality composite scores were significantly positively associated with general cognitive ability scores at each age and across ages. Parenting quality uniquely contributed to cognitive performance at each age.
Lemelin et al. (2006)	62 (31:31)	15m and 18m	**Maternal Sensitivity:** Appropriate and consistent responses to infant's signals.	36m	General Cognitive ability	Maternal sensitivity was significantly (positively) associated with general cognitive ability scores.

Table 37 (continued)

Prospective Research Examining the Association between Maternal Caregiving Behaviours and Child General Cognitive and Language Development.

Authors	N (M:F)	Age of child at M-C interaction assessment	Features of caregiving behaviour/ parent - child relationship assessed	Age of child at cognitive outcome assessment	Child cognitive domain assessed	Main findings
Mermelshtine and Barnes (2016)	400 (199: 201)	10m	'Responsive Dydactic Care-Giving' (RDC): Contingent responses, Cognitively stimulating language, Autonomy promoting language	18m	General cognitive Ability	Maternal RDC accounted for a significant 8% of the variance in 18m cognitive ability.
Stams et al.(2002)	146 (65: 81)	12m	Attachment security	7 yrs	Cognitive development composite score from a standardised intelligence test and academic Performance.	Higher quality mother- child relationships (attachment security and maternal sensitivity) predicted better cognitive development.
Belsky et al. (2015)	695 (not reported)	6m, 15m, 24m	Sensitivity to non-distress	Kindergarten, Grades 1, 2, 3, 4, 5	Academic skills	Higher levels of maternal sensitivity assessed at 6 -36 months (averaged across time points) predicted better academic skills in primary school.
		36m	Supportive presence	54m, Grade 1, 3, 5	Academic skills	

Table 37 (continued)

Prospective Research Examining the Association between Maternal Caregiving Behaviours and Child General Cognitive and Language Development.

Authors	N (M:F)	Age of child at M-C interaction assessment	Features of caregiving behaviour/ parent - child relationship assessed	Age of child at cognitive outcome assessment	Child cognitive domain assessed.	Main findings
Estrada et al. 1987	47 (24:23)	4yrs	Affective relationship rating: Responsiveness, Flexibility, Warm Concern, Emotional displays of affect, Punitiveness.	3yrs 8m and 4yrs 5yrs and 6yrs 6yrs 12yrs	General mental ability (composite measure derived from performance at 3yr 8m and at 4yrs). School Readiness IQ School achievement	Affective rating of the mother child relationship at age 4 was significantly (positively) associated with general mental ability at age 4, with school readiness at age 5 to 6, IQ at age 6 and school achievement at age 12.
Kelly et al. (1996)	53 (30: 23)	13m 20m	Infant positive tone Infant Negative tone Mother Positive affect Dyadic verbal reciprocity. Infant positive tone Infant Negative tone Mother Positive affect Dyadic verbal reciprocity Infant engagement Maternal sensitivity Dyadic interactional fit.	3yrs 5yrs	Language development IQ	Mothers positive affect at 13 months and dyadic reciprocity at 13 months were both significant predictors of language development at 5yrs. Maternal sensitivity at 20 months was a significant predictor of child IQ at 5 yrs.

Table 37 (continued)

Prospective Research Examining the Association between Maternal Caregiving Behaviours and Child General Cognitive and Language Developments.

Authors	N (M:F)	Age of child at M-C interaction assessment	Features of caregiving behaviour/ parent - child relationship assessed	Age of child at cognitive outcome assessment	Child cognitive domain assessed.	Main findings
Landry et al. (2001)	103 (52:51)	6m, 12m, 24m, 3½yrs, 4½yrs	Maternal warm responsiveness: Warm Acceptance Flexibility/ responsiveness	6m, 12m and 24m 3½yrs, 4½yrs	General Cognitive ability Language skills	Warm responsive parenting was associated with better cognitive outcomes in children. Longitudinally, children showed faster cognitive development when mothers showed consistently greater levels of warm responsiveness (across time points).
Hirsh-Pasek and Burchinal (2006)	1097 (560: 537)	6m, 15, and 24 m 36m and 54m	Maternal stimulation, Maternal sensitivity to child non-distress, Intrusiveness (reversed), Positive regard. Maternal stimulation, Supportive presence, Hostility (reversed), Respect for autonomy.	4.5 yrs Assessed during first Grade (mean age not reported)	Language development Pre-academic skills. Pre-academic skills Sustained Attention	Children scored higher on language and academic tests when they experienced more sensitive caregiving on average over time (i.e. from 6m to first grade). Children scored higher on language, academic and attention outcomes when mothers became increasingly more responsive and stimulating over time (i.e. from 6m to 54m).

Table 37 (continued)

Prospective Research Examining the Association between Maternal Caregiving Behaviours and Child General Cognitive and Language Development.

Authors	N (M:F)	Age of child at M-C interaction assessment	Features of caregiving behaviour/ parent - child relationship assessed	Age of child at cognitive outcome assessment	Child cognitive domain assessed.	Main findings
Jaffee (2007)	1720 (912: 808)	1st assessment at 3-24m (mean age not reported)	Quality and quantity of cognitive stimulation received in the home. Emotional Support.	21m - 36m (18m after first research visit, mean age not reported)	Language development	Children who experienced improvements in the amounts of sensitive and stimulating caregiving they received over time had more positive language development outcomes.
Moss and St Laurent (2001)	108 (48:60)	6yrs	Attachment Security. Affective quality of mother-child interaction: Coordination Communication Appropriate parent-child roles Emotional expression Responsivity-Sensitivity Tension vs relaxation Mood Enjoyment Overall quality	6yrs	Cognitive engagement during a joint planning task (extent to which child participated in the operations needed to solve the task, task planning, item selection and retrieval, monitoring of operations needed to solve the task.	Securely attached children (age 6) showed a higher level of cognitive engagement during mother-child joint problem solving (age 6) and higher school age academic performance (age 8). Affective quality of mother-child interactions (age 6) mediated the association between attachment classification and child academic functioning at age 8.
				8yrs	Mastery Motivation pertaining to academic learning: Academic Performance	

Table 37 (continued)

Prospective Research Examining the Association between Maternal Caregiving Behaviours and Child General Cognitive and Language Development.

Authors	N (M:F)	Age of child at M-C interaction assessment	Features of caregiving behaviour/ parent - child relationship assessed	Age of child at cognitive outcome assessment	Child cognitive domain assessed.	Main findings
Olsen et al. (1984)	121 (69:52)	6m	Baby happy and active, Baby fussy, Baby look and reach toward mother, Baby frown. Maternal close contact, Mother object stimulation, Mother look at and tickle infant, Mother approaches/ leaves infants view	24m	Child competence composite (general cognitive ability and Language development combined)	6m Maternal close contact, Object stimulation and Maternal Involvement, were positively associated with the 24m child cognitive and language competence composite.
		13m	Infant social demandingness, Infant object communication, Infant persists (after prohibition), Infant speech-type vocalisation, Mother teaching, Mother behaviour management. Maternal response to infant speech, Mother affection and caregiving.			13m mother teaching and infant object communication (combined), maternal responsiveness to infant speech and maternal affection were positively correlated with the child cognitive and language competence composite at 24m.
Stein et al. (2008)	999 (not reported though sample included M and F)	10m and 36m	Maternal Responsivity Instructional/ Teaching Qualities of Maternal Care	36m	Language development	Maternal responsivity and opportunities for learning at 10m were positively associated with infant language at 10m and 36 months. Measures of maternal caregiving at 10m and 36m contributed to language at 36m

Table 37 (continued)

Prospective Research Examining the Association between Maternal Caregiving Behaviours and Child Executive Function Development.

Authors	N (M:F)	Age of child at M-C interaction assessment	Features of caregiving behaviour/ parent - child relationship assessed	Age of child at cognitive outcome assessment	Child cognitive domain assessed.	Main findings
Bernier et al. (2010)	80 (36:44)	12m to 15m	Maternal sensitivity Maternal mind - mindedness Maternal autonomy support	18m	EF: Working Memory, Categorisation.	Higher levels of maternal autonomy support (but not other measures of care-giving) predicted higher infant working memory (but not categorisation) at 18m, and higher infant inhibition of a prepotent response (but not delay of gratification) at 24m
				24m	EF: Delay of gratification, Inhibition of a prepotent response.	
Bernier et al. (2012)	62 (24:38)	7m	Positive parenting Negative Parenting	3yr	EF: PCA derived scores for: Conflict EF factor (tapping working memory, set-shifting and inhibition of a prepotent response) and Impulse Control factor (finger tapping task, delay of gratification)	Children exposed to higher quality parenting and those more securely attached had higher Conflict EF factor scores at 3yrs. Parenting Quality and attachment security accounted for 18% of the variance in Conflict EF.
		12m	Maternal sensitivity Maternal mind mindedness			
		15m	Maternal autonomy support Attachment security			
		18m	Quality of interaction (communication, cooperation and emotional ambience).			
		24m	Positive parenting Negative Parenting Attachment security			

Table 37 (continued)

Prospective Research Examining the Association between Maternal Caregiving Behaviours and Child Executive Function Development.

Authors	N (M:F)	Age of child at M-C interaction assessment	Features of caregiving behaviour/ parent- child relationship assessed	Age of child at cognitive outcome assessment	Child cognitive domain assessed / assessment used.	Main findings
Bridgett et al. (2011)	69 (not reported)	6m	Duration of time spent in interactive caregiving behaviours with infant.	18m	Effortful Control	Maternal time spent in interactive care-giving with infants at 6 months significantly predicted infant effortful control at 18m
Cuevas et al. (2014)	62 (25:37)	10m	Maternal Negative Caregiving Composite (Failure to facilitate attention, physical stimulation, intrusiveness).	Composite across ages assessed (24m, 36m and 48m)	Executive Function	Higher maternal negative caregiving composite scores (average across 10m, 24m and 36m) were associated with lower child EF composite scores. Maternal negative caregiving behaviours accounted for unique variance in child EF.
		24m and 36m	Maternal Negative Caregiving Composite (Failure to facilitate attention, negative affect, intrusiveness)			
Hammond et al. (2012)	82 (44:38)	2 yrs 3 yrs	Scaffolding during a problem solving puzzle	2yrs 3yrs 4yrs	EF: working memory factor and conflict inhibition factor.	Scaffolding at 3yrs was a significant predictor of age 4 EF. Scaffolding at age 2 had an indirect effect on age 4 EF through age 3 verbal ability.
Rhoades et al. (2011)	1155 (reported as approximately half male)	7m	Maternal Positive Engagement Maternal Negative Intrusiveness	36m	Executive Function (Composite of tasks assessing Working Memory, Conflict Inhibition, and Attentional Flexibility).	Children exposed to greater Maternal Positive Engagement and children exposed to Lower levels of Negative Intrusiveness at 7m were more likely to have higher EF skills at 36m.

Table 37 (continued)

Prospective Research Examining the Association between Maternal Caregiving Behaviours and Child Executive Function Development.

Authors	N (M:F)	Age of child at M-C interaction assessment	Features of caregiving behaviour/ parent- child relationship assessed	Age of child at cognitive outcome assessment	Child cognitive domain assessed / assessment used.	Main Findings
Towe-Goodman et al. (2014)	620 (329:291)	7m	Sensitive Parenting Composite: Sensitivity/ Supportive Presence; Detachment/ Disengagement; Stimulation of Cognitive Development; Positive Regard; Animation in Interacting with the Child.	3 yrs	Executive Function Composite score: from tasks of Working Memory, Inhibitory Control, Attention Shifting	Greater maternal Sensitive Parenting at 7m significantly predicted higher levels of EF at 3 years.
		24m				Greater maternal Sensitive Parenting at 24m significantly predicted higher levels of EF at 3 years.
Kraybill et al. (2012)	56 (30:26)	10m	Maternal Positive Affect (based on facial expressivity and vocal affect.	4yrs	EF: Average score across tasks requiring working memory, inhibitory control and cognitive flexibility.	Maternal positive affect was a significant unique predictor of the age 4 EF composite, accounting for 8% of the variance in EF composite scores
				6yrs	EF: Maternal report (Behavior rating inventory of executive function – preschool version).	Maternal positive affect was a significant unique predictor of age 6 EF scores, accounting for 8% of the variance in scores.

4.1.5. Bringing Together Findings in the Animal Literature and Human Participants. Though frequently drawing on findings in the animal literature in their rationales for study, the fore mentioned research in human mother- child dyads summarised Table 37, differs starkly in the aspects of maternal care examined and the timing of these, compared with that in the animal literature. This is a common encumbrance of research attempting to translate findings in animal research to human participants. However, evidence implicating tactile stimulation as the crucial feature driving the demonstrated effects of maternal-caregiving behaviours on offspring cognitive and neural development in the rodents, may offer an opportunity to approach bridging this gap. At present, the impact of variations in maternal tactile stimulation on child cognitive development in typically developing children is a relatively unstudied area.

A related strand of research in high risk infants, however, has reported benefits of a form of tactile stimulation (infant massage) in the early post-partum period for a number of aspects of child development. Massage therapy for neonates typically constitutes stroking of the head, back, arms and legs with moderate pressure; and flexion and extension of the extremities (Field 2003). Predominantly examined in preterm and low birth weight infants, neonatal massage has been associated with improvements in a range of outcomes, such as weight gain, shorter durations of neonatal hospital stay, increases in length, head circumference and body temperature and improved cognitive development compared with control infants receiving no massage therapy (Diego, Field, and Hernandez –Reif, 2008; Dieter, Field, Hernandez- Reif, Feldman and Redzepi, 2003; Ferber, Kuint, Weller, Feldman, Dollberg, Arbel, and Kohelet, 2002; Field, 1988; Kuhn, Schandberg, Field, Symansky, Zimmerman, Scafidi and Roberts, 1991; Procianoy, Mendes and Silveira,

2010; Scafidi, Field, Schanberg, Bauer, Tucci, Roberts, Morrow and Kuhn, 1990; Vickers, Ohlsson, Lacy and Horsely, 2004). In their meta- analysis of 19 studies examining tactile stimulation in pre-term infants, Ottenbacher et al. (1987) estimated 72 % of the infants who received a form of tactile stimulation therapy showed greater weight gain and development relative to control groups receiving standard treatment without additional tactile stimulation.

Field et al. (1996) investigated the therapeutic potential of infant massage in full-term infants, though in a sample of infants that may still be considered at-risk. Their sample comprised 40 healthy, full-term one to three-month-old infants, selected for inclusion based on mothers being depressed adolescent mothers that were categorised as low SES. Field et al. (1996) compared infants receiving 15 minutes of either massage or rocking, for two days per week over a six-week period on a range of developmental outcomes. They reported that compared to rocked infants, massaged infants gained more weight, spent more time in active alert and active awake states, had lower salivary cortisol levels (suggesting lower stress levels), and improved emotionality, sociability and soothability.

Similar investigation of the influence of infant massage on development is limited in healthy full term infants from community samples. However, in one such study, Inal and Yildiz (2012) reported beneficial effects of this form of tactile stimulation for developmental outcome. Specifically, they randomly allocated a sample of 104 heathy full term new-borns to either a massage therapy group or a no intervention group. The massage therapy group received 15 minutes of daily massage over a six-month period. Mental and motor development scores on the Ankara Developmental Screening Inventory (ADSI) were then compared across groups. The

authors reported that mental and motor development scores of the massaged infants were significantly higher than that of the control group infants.

In summary, there is some, albeit limited evidence suggesting that tactile stimulation may be associated with improved cognitive development in human infants, as has been demonstrated in animal models. Tactile stimulation may therefore represent an opportunity for intervention aimed at improving outcomes for infants at risk of poor cognitive development. Given the reported negative impact of maternal prenatal stress on child cognitive development (as reviewed in sections 3.1.4 to 3.1.7 109and demonstrated in study 2 of this thesis), infants of mothers that were stressed during pregnancy represent a group that may benefit from this form of mother- infant interactive behaviour.

4.1.6. Harnessing the Benefits of Maternal Care-Giving Behaviours for Intervention: Tactile stimulation in PNS rat offspring. There is some evidence from animal models to support the position that maternal tactile stimulation may represent an opportunity for intervention aimed at improving outcomes for infants of PNS mothers. Specifically, from findings indicating that the neonatal handling procedure, which has been demonstrated to induce increased levels of maternal tactile stimulation of handled pups reverses or prevents the negative impact of PNS on offspring cognitive outcomes.

One of the earliest reports suggesting a protective effect of maternal tactile stimulation for the cognitive development of the offspring of PNS mothers was offered by Vallée, Maccari, Dellu, Simon, Le Moal and Mayo (1999). The authors assessed the cognitive performance of three groups of rats longitudinally during adulthood, during middle age, and in later life. The groups consisted of (a) offspring

of prenatally stressed (PNS) rats with no postnatal manipulation of the pups (PNS); (b) offspring of non- PNS rats that were administered the neonatal handling procedure postnatally (H); and (c) control offspring whose mothers had not experienced PNS and the pups did not receive the postnatal handling procedure (C). The authors report that "prenatal stress and postnatal handling induced opposite effects on offspring cognitive function" (p. 2096). Specifically, their data revealed that normative age-related cognitive impairments were observed in controls (C) and these age-related impairments were exacerbated in PNS offspring. Whereas, in postnatally handled offspring (H) no age related cognitive deficits were observed, with handled (H) and control (C) offspring exhibiting similar cognitive performance across development.

The authors also report that their results "strongly suggest" that the exacerbated age-related learning and memory deficits observed in PNS offspring "can be prevented by postnatal handling" (p.2914). Although Vallée et al. (1999) provide strong evidence of opposing effects of PNS and postnatal handling on offspring cognitive abilities, the lack of a group of rats to which both PNS and postnatal handling of pups was administered precludes supposition of a moderating effect of this postnatal manipulation on offspring cognitive function.

More robust findings were reported by De Los Angeles, Del Carmen, Wendy and Socorro, (2016) who directly applied a tactile stimulation manipulation to pups, rather than inducing increased maternal LG/ABN via application of the neonatal handling procedure. The tactile stimulation procedure applied consisted of a researcher stroking the backs of rat pups with their fingers for 60 seconds daily. The study comprised 4 groups of rat offspring; (a) control offspring, pups of non-prenatally stressed mothers that received no postnatal tactile stimulation (C), (b) PNS

only offspring, pups of prenatally stressed mothers that received no postnatal tactile stimulation manipulation (PNS), (c) tactile stimulation only offspring, pups of non-prenatally stressed mothers that received the postnatal tactile stimulation manipulation (TS) and (d) prenatal stress plus tactile stimulation offspring, pups of prenatally stressed mothers that received the postnatal tactile stimulation manipulation (PNS+TS). Each group was further split according to sex, in order to detect sex differences in any effects on offspring cognitive performance observed. Groups were compared in spatial learning and spatial memory performance, as well as markers of hippocampal neurogenesis (neural cell survival and differentiation).

De Los Angeles et al.'s (2016) results revealed that both male and female PNS offspring displayed poorer spatial learning and memory performance compared to controls. Furthermore, PNS+TS offspring of both sexes displayed levels of performance that were similar to controls, indicating that the postnatal tactile stimulation manipulation protected pups against the cognitive impairments associated with prenatal stress, irrespective of sex. The protective effects of tactile stimulation were also reflected in markers of hippocampal neurogenesis. Specifically, PNS+TS offspring (again of both sexes) exhibited increased differentiation of immature neural precursor cells into neurons compared to other groups. The authors report that this finding may indicate that TS supports the quick maturation of cells in the hippocampus, allowing them to integrate into existing neural networks more quickly. Such neural changes may be the mechanism for improving the spatial learning and memory performance of the PNS+TS offspring beyond performance levels of PNS offspring resulting in similar performance to controls.

Others have reported similar findings of a protective effect of postnatal tactile stimulation against the detrimental impact of PNS on neural structure/ function in the hippocampus. Applying the neonatal handling procedure to induce increased levels of LG/ABN from the dam, Lemaire, Lamarque, Le Moal, Piazza and Abrous (2006) reported that PNS prompted decreases in hippocampal cell proliferation, decreased cell survival rate and decreased number of both immature neural precursor cells and differentiated neurons in the hippocampus in the offspring. Application of the neonatal handling procedure however counteracted all these detrimental effects on hippocampal neural structure. Since only male offspring were retained for study, sex differences could not be examined.

Similarly, Bock, Murmu, Weinstock and Braun (2011) reported a protective effect of neonatal handling in offspring of PNS dams. Furthermore, including both male and female offspring allowed them to examine sex differences in this effect. In the CA1 region of the hippocampus, PNS induced a decrease in spine density and complexity (reflecting less favourable neural architecture for the establishment and maturation of efficient neural networks) that was significantly more pronounced in female offspring than male offspring. In the dentate gyrus region of the hippocampus PNS induced opposite neuromorphological effects in male and female offspring. Specifically, in male offspring PNS induced an increase in spine density and dendritic length and complexity allowing for more efficient neural networks whereas in females PNS induced a decrease in these morphological dendritic features. PNS abolished the usual sex differences evident in these measures of dendritic structural integrity. I.e. in control offspring (non PNS non-handled), females had significantly higher spine densities and dendritic length and complexity than males, whereas in PNS offspring, these dendritic features were similar in males and females.

Addressing the posited protective effect of tactile stimulation on the detrimental impact of prenatal stress, Bock et al. (2011) reported that some of the PNS induced neural alterations were reversed by the neonatal handling procedure and that this effect was particularly evident in males. More specifically, the PNS induced increase in spine density in males was not evident in those males exposed to the neonatal handling procedure. In females, the PNS induced reduction in spine density was augmented in those exposed to the neonatal handling procedure. As such neonatal handling restored the sex differences in dendritic morphology evident in control offspring (i.e. significantly higher spine densities and dendritic length and complexities in females compared to males).

4.1.7. Moderation of the Association Between PNS and Child Cognitive Development by Maternal Caregiving: Human Participants. As in the animal literature, research examining the moderating effect of maternal care-giving on the association between PNS and child cognitive function has been reported in human participants. Though such studies are few, (with a search of the literature revealing only two such investigations), the findings suggest that positive mother- infant relationship features may protect against the poorer cognitive outcomes that have been associated with PNS.

For example, in a sample of 125 pregnant women recruited sequentially from an amniocentesis clinic Bergman, Sarker, Glover and O'Connor (2010) assessed a biological marker of prenatal stress (amniotic fluid cortisol levels) in mid pregnancy, and the cognitive development (BSID-MDI) and attachment classification of their infants at 17 months. Amniotic fluid cortisol levels were examined on the basis of evidence from animal models that exposure to gluco- corticoids (of which cortisol is

one) may be a mechanism for the effects of prenatal stress on offspring cognitive development. Bergman et al. (2010) observed a negative association between amniotic fluid cortisol levels and infant cognitive development. Furthermore, their findings revealed a moderating effect of infant attachment classification on this association. Specifically they reported that prenatal cortisol levels strongly predicted infant BSID MDI score in children with an insecure attachment classification (r (54) = -.47, $p< 0.01$), with essentially no association between prenatal cortisol levels and BSID-MDI score in those classified as securely attached (r (70) = -0.05, ns). The authors concluded that these findings provide evidence that "cortisol level in utero predicts infant cognitive development and that this effect is eliminated by a sensitive early rearing environment" (p. 1030). Though providing some of the first evidence of a moderating effect of features of the mother-infant relationship on the association between (a biological marker of) prenatal stress and infant cognitive development in human participants, the level of sensitivity of the care-giving environment was inferred from infant attachment classification rather than observed / assessed directly and the level of PNS experienced by the mother inferred from amniotic fluid cortisol levels rather than assessed directly.

Assessing PNS and sensitivity of the caregiving environment more directly, Grant, McMahon, Reilly and Austin (2010) compared BSID scores of 7 month old infants of mothers that had reached DSM-IV diagnostic criteria for anxiety disorder during pregnancy with those of mothers that had not reached this criterion. Maternal sensitivity to infant distress and non- distress at 7 months was also assessed. Grant et al.'s (2010) results revealed that infants of PNS mothers did not differ from those of non PNS mothers in their BSID-MDI score, however the number of mothers

reaching criteria for anxiety disorder during pregnancy was small (*n*=14) and may have limited statistical power to detect this main effect.

When the impact of maternal care-giving behaviours on the association between PNS and infant cognitive ability were taken into account, the analysis revealed a significant moderating effect of maternal sensitivity to distress. Specifically, there was a significant positive association between maternal sensitivity to distress and infant BSID-MDI score in infants of PNS mothers, whereas sensitivity had little impact on BSID-MDI scores in infants of non PNS mothers. As such, Grant et al.'s (2010) findings suggest that maternal caregiving behaviours may offer an opportunity for intervention aimed at improving outcomes in infants at risk of poor cognitive development as a function of maternal PNS.

Unfortunately, a number of limitations are acknowledged by Grant et al. (2010). For example, the demographic characteristics of the sample (largely middle class and well-educated) may limit generalisability of the findings to women of different socioeconomic backgrounds and their focus on a clinical sample means that it is not clear whether this moderating effect is also present in community samples of women experiencing symptoms of PNS across the sub-clinical range.

In summary, despite some promising findings approaching the translation of observations in animal models of a moderating effect of postnatal maternal caregiving behaviour on the association between PNS and infant cognitive development, the number of studies are few and those available are not without methodological limitations. Additionally, in the animal literature maternal care-giving behaviours were examined very early in development specifically pre-weaning; whereas in the few corresponding studies in the human literature maternal caregiving behaviour was examined beyond this period (i.e. at infant age 17 months

in Bergman et al.'s 2010 study and at 7 months in Grant et al.'s, 2010 study). Furthermore, the caregiving processes examined in these human studies differ markedly from those of the animal literature. This may be particularly salient given findings that it is the tactile stimulation component of maternal LG/ABN behaviour that plays the crucial role in its beneficial effects on offspring cognitive and neural development in the rat.

To our knowledge only one study has specifically examined the moderating role of human tactile stimulation early in life as a possible mediator of effects of PNS on infant development and this work was not focussed on cognitive outcomes. Using data from the Wirral Child Health and Development Study, maternal tactile stimulation was shown to moderate the association between maternal prenatal psychological state and infant development at 7 months of age (Sharp, Pickles, Meaney, Marshall, Tibu and Hill (2012). Specifically, findings revealed that the frequency with which mothers stroke their infant in the first few weeks of life (assessed by self-report) moderated the effect of prenatal symptoms of depression (EPDS) on infants' physiological response (heart-rate variability) to a social stressor and mother-reported infant negative emotionality (fearfulness and a frustration prone temperament). Maternal tactile stimulation has not previously been examined as an aspect of maternal caregiving that may moderate the association between PNS and infant cognitive development.

Finally, previous investigations of a moderating effect of maternal care-giving behaviours on the association between PNS and cognitive development in human samples (i.e. Bergman et al., 2010 and Grant et al., 2010) have employed measures of general cognitive ability (BSID-MDI). The current study aimed to extend these findings by examining a more specific cognitive function, namely

Executive Function (EF) which is associated with the functioning of the pre-frontal cortex, a region that may also be vulnerable to PNS and is important in supporting the development of later academic skills and EF deficits have been implicated in a number of developmental disorders (as outlined in section 2.1.4).

4.1.8. Aims of the Current Study. The current study aims to add to the existing research in human participants of a maternal caregiving moderation of the association between PNS and infant cognitive ability, in four ways (a) directly assessing PNS symptoms and the occurrence of stressful life events during pregnancy in a community sample of pregnant women; (b) employing a socioecomically diverse sample of women oversampled for psychosocial risk (c) examining a more specific aspect of cognitive function than has previously been examined in this area of the literature, namely Executive Function and (d) examining both a broad aspect of maternal care that has previously been implicated in this area of the literature (maternal sensitivity at 7 months) as well as a more specific measure of maternal caregiving more closely related to that observed in the animal literature than has previously been used in the human literature (i.e. maternal tactile stimulation of the infant) assessed very early in the postnatal period.

Specifically, the current research examines whether the association between PNS (state anxiety and stressful life events) and EF ability (working memory and inhibitory control) in toddlerhood is moderated by the frequency with which mothers stroke their infants (following Sharp et al., 2012) during the first 5 to 8 weeks and/ or by maternal sensitivity assessed at 7 months of age.

4.2. Method.

4.2.1. Design. The current research was embedded within The Wirral Child Health and Development Study (WCHADS); a prospective longitudinal study funded by the Medical Research Council. The design of the WCHADS was outlined in section 2.2 in order to provide a context for the studies comprising the current thesis. The current analysis examining the moderation of the association between maternal PNS and child EF reports data from the WCHADS intensive sample at phases 1,2,3,4,5 and 9. Participant progression through WCHADS study phases at which data was collected for the current study, together with the measures employed at each, can be seen in *Figure 8Figure 9*.

The current study examined the moderation of the prenatal stress prediction of toddler working memory and inhibitory control (reported in chapter 3 of this thesis), by indices of early maternal caregiving. The indices of maternal caregiving employed were (a) a measure of maternal tactile stimulation of the infant (maternal self-report of the frequency with which they stroked their infants over the first few weeks of life) and (b) maternal sensitivity during play, assessed at 7 months.

4.2.2. Participants

The sample for the first analysis of the current study, examining moderation of the association between PNS and toddler EF by maternal tactile stimulation, comprised WCHADS intensive sample mother-infant dyads that were included in the analysis of the PNS prediction of toddler EF ability (reported in chapter 3) for whom complete data regarding maternal tactile stimulation of the infant were available. Complete data regarding maternal postnatal tactile stimulation of the infant in the

early postnatal period were available for 207/ 211 of these mother- infant dyads. Demographic characteristics of these 207 dyads are presented in Table 38.

Table 38

Demographic Characteristics of the Sample included in the Analysis Testing Moderation by Maternal Tactile Stimulation (N = 207)

		Mean (SD)	N (%)
Toddler age at EF assessment (Months)		31.5 (2.4)	
Gestational age at birth (weeks)		40 (1.5)	
Sex	Male		96 (46)
	Female		111 (54)
Maternal age at toddler EF assessment (Years)		31.7 (6.2)	
Maternal age at leaving fulltime education	16 or below		43 (21)
	Post 16		159 (77)
	Missing		5 (2)
Deprivation: IMD Quintile	1 (most deprived)		74 (36)
	2		42 (20)
	3		57 (28)
	4		16 (8)
	5 (least deprived)		18 (9)
Psychosocial risk	High Risk		106 (49)
	Low risk		101 (51)
Ethnicity white British			197 (95)

The sample for the second analysis of the current study, examining moderation of the association between PNS and toddler EF by maternal sensitivity, comprised WCHADS intensive sample mother-infant dyads that were included in the analysis of the PNS prediction of toddler EF ability (reported in chapter 3) for whom complete data regarding maternal sensitivity at 7 months were available. Complete

data regarding maternal sensitivity were available 198 of these mother- infant dyads. Demographic characteristics of these 198 dyads are presented in Table 39.

Table 39

Demographic Characteristics of the Sample Included in the Analysis Testing Moderation by Maternal Sensitivity (N = 198)

		Mean (SD)	N (%)
Toddler age at EF assessment (Months)		31.5 (2.4)	
Gestational age at birth (weeks)		40 (1.5)	
Sex	Male		90 (45.5)
	Female		108 (54.5)
Maternal age at toddler EF assessment (Years)		31.7 (6.2)	
Maternal age at leaving fulltime education	16 or below		40 (20)
	Post 16		158 (80)
Deprivation: IMD Quintile	1 (most deprived)		71 (36)
	2		42 (21)
	3		52 (26)
	4		16 (8)
	5 (least deprived)		17 (9)
Psychosocial risk	High Risk		105 (53)
	Low risk		93 (47)
Ethnicity white British			190 (96)

4.2.3. Procedures

The current analysis employs data collected at the WCHADS phase 1 (psychosocial risk stratification, socioeconomic deprivation), phase 2 (PNS, smoking during pregnancy, maternal IQ), phase 3 (breastfeeding, gestational age at birth) phases 4 and 5 (maternal tactile stimulation of the infant), phase 6 (maternal sensitivity), and 6, 8 and 9 (maternal postnatal stress) and phase 9 (toddler EF and verbal comprehension). This is displayed in Figure 8

4.2.3.1. Phase 2 procedure. The procedure followed at the WCHADS phase 2 (32-week gestation) mother interview, at which data for measures of prenatal stress were collected is described in full in chapter 3, section 3.2.3.2, of this thesis.

4.2.3.2. Phase 4 procedure. When infants were around 4 weeks old, mothers completed a questionnaire pack at the study base during the WCHADS phase 4 mother- infant observational assessment. This involved, amongst measures related to the over-arching study design and of relevance to the current study, the maternal tactile stimulation measure.

4.2.3.3. Phase 5 procedure. When infants were between 9 and 12 weeks old, mothers received a postal questionnaire pack with a cover letter and a freepost return envelope from the WCHADS study team. This pack also contained the maternal tactile stimulation measure. Mothers were asked to complete the questionnaire and return it to the study base in the freepost envelope that was provided.

4.2.3.4. Phase 6 procedure. When infants were approaching 6 months, mothers were contacted and invited to attend the study base with their infants to complete the WCHADS phase 6 mother-infant assessments.

Upon arriving at the study base mothers and infants were shown to a seating

area and made comfortable. Procedures for the assessment were then explained in full and the mother was given an information sheet to read. Any questions mothers had were then answered before written consent was taken (copies of the phase 6 information sheet can be found in appendix 2). Mothers and infants then completed around an hour of further assessment tasks, with intermittent breaks for naps, changing, feeding and other care-taking needs as required.

As part of the WCHADS phase 6 (6 month) mother-infant assessment, dyads completed a 15-minute standard laboratory based semi-structured free play task (NICHD Early Child Care Research Network, 1999). Mothers were asked to bring a favourite toy of the infant with them to the assessment at booking in. During the assessment, they were then asked to play alone with the infant for a total of 15 minutes. They were asked to play with their infant as they would do at home with the toy they had brought along. They were also instructed to put this toy away and play with one of a selection of toys provided by the research team when they heard a knock on the door. Mothers and infants were left alone to play with the toy they had brought for 7 minutes. The researcher then knocked on the door to signify the change to playing with the tasks provided by the research team. They were left to play with these toys for 8 minutes. At the end of 15 minutes, researchers re-entered the room and answered any questions the mothers had.

4.2.3.5. Phase 9 procedure. The procedure followed at the 2 ½ year mother-toddler assessments (during which measures of toddler Executive Function were administered), and detailed investigator led maternal interviews (during which the Life History Calendar (Caspi et al., 1996) and the STAI state anxiety subscale (Spielberger et al., 1970) were administered) is described in full in chapter 3, section 3.2.3.7.

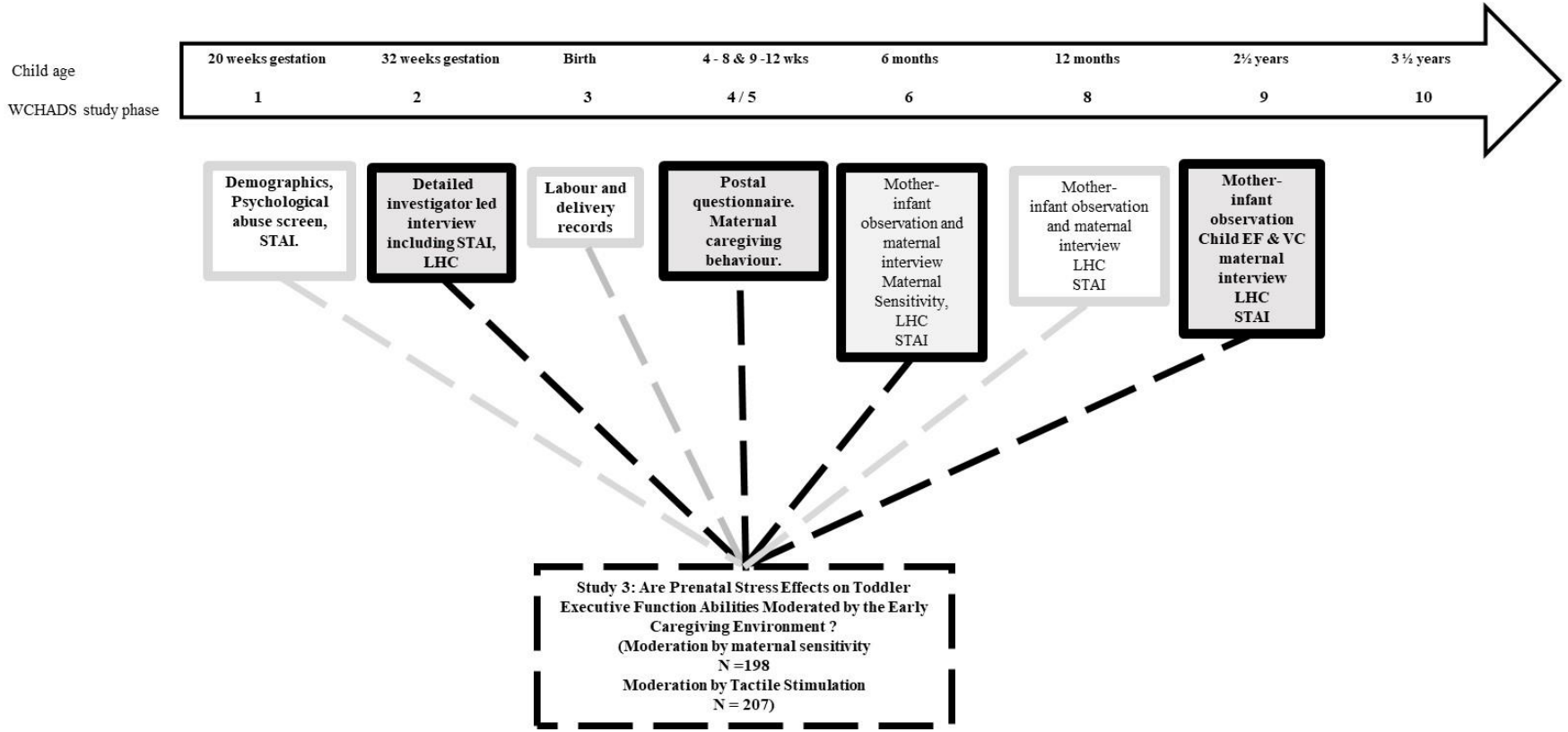


Figure 8

Measures employed in the current analysis and point of data collection during participant progression through WCHADS study phases.

Note: Measures with bold frame = main analyses measures, measures with greyscale frame = confounding variables/ covariates, LHC= Life history calendar; STAI = Spielberger State-Trait Anxiety scale (state anxiety subscale), EF = Executive Function, VC = Verbal comprehension.

4.2.4. Measures.

4.2.4.1. Maternal prenatal and postnatal stress. Details of the measures employed to assess maternal prenatal and postnatal stress (STAI and LHC) are described in full in Chapter 3, section 3.2.4.1 of this thesis.

In the current analysis SLEs data was further collapsed in to three categories of 0 = no life events experienced in the relevant period, 1 = one life event experienced in relevant the period, 2 = two or more life events experienced in the relevant period. This was due to the smaller number of cases who had experienced higher numbers of life events and an awareness of the need to avoid small cell sizes in the 3 way interactions included in the models tested.

4.2.4.2. Toddler Executive function. Toddlers completed a battery of EF tasks at the phase 9 mother- toddler observational assessment. The EF tasks administered are described in full in chapter 2 section 2.4.4.1 of this thesis. Confirmatory factor analysis was applied to toddler performance on these tasks, yielding two EF factor scores, namely working memory (WM) and inhibitory control (IC). The CFA yielding these factor scores is described in full in chapter 2 of this thesis.

4.2.4.3. Maternal Care-giving behaviours.

The parent infant care-giving scale (Sharp, Pickles, Meaney, Marshall, Tibu, & Hill, 2012). Maternal tactile stimulation of the infant was assessed using the Parent-Infant Caregiving Scale (Sharp et al., 2012). This scale consists of four items asking mothers to report how often (1 = never, 2 = rarely, 3 = sometimes, 4 = often, 5 = a lot) they currently stroke their babies' face, back, tummy and legs. The four items have previously been shown to assess a common latent 'stroking' construct and

postulated to be analogous to the tactile stimulation of pups observed in rodents (Sharp et al., 2012). The mean score across the two administrations of the measure (phase 4 and phase 5) was used to summarise stroking behaviour as a first step following Sharp et al. (2012). Mothers were subsequently categorised into high or low stroking mothers using a median split to define high (2) or low (1) levels of stroking.

Maternal Sensitivity. Maternal sensitivity was rated from video recordings of mothers playing with their infants during a 15-minute standard play procedure (NICHD Early Child Care Research Network). Sensitivity was rated on a global 5-point scale, ranging from 1 (not at all characteristic) to 5 (highly characteristic reflecting mothers' appropriate, supportive and warm responding to infant communications, playful bids or distress. Three raters blind to the aims of the current study coded sensitivity. Raters achieved good inter-rater reliability for the maternal sensitivity measure on a subset of 30 video recordings (ICCs .85 - .91). A median split was employed to categorise mothers into high (2) or low (1) levels of sensitivity for the purpose of analysis.

4.2.4.4. Control variables and confounding variables. Details of the control and confounding variable measures included in the current analysis (psychosocial risk stratification variable, sociodemographic deprivation, smoking during pregnancy, Maternal IQ, breastfeeding, gestational age at birth, toddler verbal comprehension ability and maternal postnatal stress) are described in full in sections 3.2.4.3 and 3.2.4.4 of this thesis.

4.2.5. Statistical Methods. Distributions of PNS, EF and confounder/ covariate variables were examined previously for study 2 (PNS prediction of toddler EF) and found to be non-normal. As a median split approach was employed for maternal sensitivity and tactile stimulation data, the proportion of mothers falling above or below the median split was examined. For maternal sensitivity data, 29 % fell below the median and 71 % above; for tactile stimulation data, 57 % fell below the median and 43 % above. Bivariate correlations (Spearman's rho) between predictor, outcome and moderator outcome variables were then examined. See Table 40). Following this, a series of four hierarchical multiple linear regression models were specified to test for moderation of the significant associations between measures of PNS (symptoms of anxiety and stressful life events) and aspects of executive function (working memory and inhibitory control) reported in study 2 (chapter 3) of this thesis by (a) maternal tactile stimulation and (b) maternal sensitivity.

In all models tested, each variable was entered singly in consecutive blocks with forced entry. For each analysis, the models were first specified to include a minimal set of essential control variables (part a). Where the maternal caregiving variables (tactile stimulation/ maternal sensitivity) made a significant contribution to the model, either as a main effect or in the 3-way interaction term (PNS by Sex x maternal caregiving), a second model (part b) was run with a full set of confounders and covariates. An a priori criterion of *p*- values < 0.05 was set to indicate significant findings. SPSS version 22 was used for all analyses.

Following Field (2009), diagnostic statistics were examined to ensure assumptions of multiple linear regression were not violated in the models tested. This involved (a) examination of bi-variate correlations amongst predictor variables,

which were all < .8, indicating that there was no multi-collinearity within the data; (b) visual inspection of scatter plots, which suggested linearity and homoscedasticity (c) use of the Durbin-Watson test statistic, which was consistently < 2, thus confirming independence of residuals, (d) histograms and normal probability plots of residuals which all exhibited approximately normal distributions (e) case wise diagnostics which indicated that the models were not limited by the presence of outliers.

Table 40

Bi-variate correlations (Spearman's rho) amongst predictor, outcome and moderator variables.

	20 wk STAI	32 wk STAI	Postnatal STAI	1st tri SLEs	2nd tri SLEs	3rd tri SLEs	Postnatal SLEs	Tactile stimulation	Maternal sensitivity	Working memory	Inhibitory control
20wk STAI	-	**.53****	**.50****	**.18****	**.19****	**.18****	.13	-.01	-.13	**.14***	.02
32 wk STAI		-	**.52****	**.16***	**.19****	**.21****	.09	-.11	-.09	-.02	-.01
Postnatal STAI			-	.06	.13	**.17***	**.16***	-.10	-.03	.05	.01
1st tri SLEs				-	**.38****	**.32****	**.20****	-.06	-.08	.02	-.00
2nd tri SLEs					-	**.43****	**.31****	-.07	-.03	.11	-.00
3rd tri SLEs						-	**.35****	.01	-.03	.06	.13
Postnatal SLEs							-	.06	-.05	.12	.04
Tactile stimulation								-	.06	.07	-.06
Maternal sensitivity									-	-.05	.04
Working memory										-	**.57****
Inhibitory control											-

** p < .05; ** p< .01*

4.3. Results. –

4.3.1. Bi-variate associations between measures of maternal care-giving.

As can be seen from **Table 40**, maternal tactile stimulation was not significantly correlated with maternal sensitivity. This was unexpected as both measures were considered, a priori, to assess related parenting constructs and is discussed further in section 4.4.1.

4.3.2. Moderation of the Association between PNS and Toddler Executive Function by Maternal Caregiving Behaviours.

A series of four multiple linear regression models were run to test the moderation of the PNS prediction of toddler EF reported in study 2 (chapter 3 of this thesis) by measures of maternal caregiving behaviour (tactile stimulation and maternal sensitivity). The PNS prediction of indices of toddler EF reported in study 2 were (a) anxiety symptoms in the third trimester (STAI score at 32 weeks) in interaction with sex in the prediction of toddler working memory and (b) stressful life events (SLEs) experienced in the first trimester in interaction with sex in the prediction of toddler inhibitory control.

Models 15 and 16 (Table 41 and Table 42) tested for moderation of the effects by tactile stimulation (TS); models 17 and 18 (Table 43 and

Table 44) tested for moderation of the effects by maternal sensitivity. As can be seen from Table 41 and Table 42, the overall models testing moderation of the PNS effects reported in chapter 2 of this thesis by tactile stimulation (models 15a and 16a) were each significant. However, the neither the 3-way interaction term (Sex x PNS by sex) nor the main effect of tactile stimulation made a significant contribution to either of the models tested. As expected, toddler age at time of EF assessment and Verbal comprehension ability made significant contributions to each of the models.

Table 41

Model 15a Regression Model Testing the Moderation of the 3rd Trimester (32 week) STAI score X Sex prediction of Toddler WM by Tactile Stimulation.

			95% CI						
Model 15a	β	*p*-value	lower	upper	Adjusted R^2	ΔR^2	ΔF	df	*p* - value

Risk stratification	-0.05	.46	-0.85	0.39	.00	.00	0.59	1, 205	.44
Toddler age	**0.12**	**.09**	**-0.02**	**0.26**	**.04**	**.05**	**10.92**	**1, 204**	**.00**
VC	**0.30**	**.00**	**0.10**	**0.28**	**.12**	**.08**	**17.70**	**1, 203**	**.00**
Sex	0.05	.80	-1.62	2.10	.12	.01	1.88	1, 202	.17
3rd tri STAI	-0.61	.40	-0.50	0.20	.12	.00	0.16	1, 201	.69
TS	-0.03	.89	-2.14	1.86	.11	.00	0.13	1, 200	.71
Sex x TS	0.07	.79	-1.07	1.40	.11	.00	0.03	1, 199	.87
Sex x 3rd tri STAI	**0.40**	**.58**	**-0.15**	**0.26**	**.13**	**.02**	**4.79**	**1, 198**	**.03**
3rd tri STAI x TS	0.11	.88	-0.21	0.25	.13	.00	1.09	1, 197	.30
Sex x 3rd tri STAI x TS	0.11	.88	-0.12	0.14	.12	.00	0.02	1, 196	.88

F (10, 196) = 3.84, $p < 0.01$

VC = Verbal Comprehension; TS = Tactile Stimulation

Table 42

Model 16a Regression Model Testing the Moderation of the 1st Trimester SLEs x Sex Interaction Prediction of Toddler IC by Tactile Stimulation.

			95% CI						
Model 16a	β	*p*-value	lower	upper	Adjusted R^2	ΔR^2	ΔF	df	*p* - value
Risk stratification	-0.01	.87	-0.27	0.23	.00	.00	0.00	1, 205	1.00
Toddler age	**0.17**	**.02**	**0.01**	**0.12**	**.06**	**.07**	**14.19**	**1, 204**	**.00**
VC	**0.31**	**.00**	**0.04**	**0.12**	**.14**	**.09**	**21.17**	**1, 203**	**.00**
Sex	-0.15	.57	-1.28	0.70	.14	.00	0.99	1, 202	.32
1st tri SLEs	-0.76	.25	-2.56	0.66	.14	.00	0.04	1, 201	.85
TS	-0.15	.58	-1.32	0.74	.13	.00	0.16	1, 200	.69
Sex x TS	0.17	.63	-0.48	0.79	.13	.00	0.02	1, 199	.90
Sex x 1st tri SLEs	0.80	.23	-0.39	1.64	.14	.01	2.77	1, 198	.10
1st tri SLEs x TS	0.44	.50	-0.68	1.40	.13	.00	0.00	1, 197	.99
Sex x 1st tri SLEs x TS	-0.46	.48	-0.90	0.42	.13	.00	0.51	1, 196	.48

F (10, 196) = 4.08, $p < 0.01$

VC = Verbal Comprehension; TS = Tactile Stimulation.

In model 17a (Table 43), the prediction of toddler working memory by the interaction between third trimester (32 week) STAI score and sex of toddler was tested for moderation by maternal sensitivity. The overall model was significant (F (10, 184) = 3.97; $p < 0.01$). As can be seen in Table 43, toddler age at EF assessment and verbal comprehension ability made significant contributions to the model as expected. Neither the main effect of maternal sensitivity nor the 3- way

interaction term (3rd trimester STAI by sex by maternal sensitivity) made a significant contribution to the model.

Table 43

Model 17a Regression model testing the moderation of the 3rd trimester (32 week) STAI score X Sex prediction of toddler WM by maternal sensitivity.

			95% CI						
Model 17a	β	*p*-value	lower	upper	Adjusted R^2	ΔR^2	ΔF	df	*p* - value
Risk stratification	-0.07	.34	-0.94	0.32	.00	.00	0.54	1, 196	.46
Toddler age	**0.12**	**.10**	**-0.02**	**0.26**	**.05**	**.05**	**11.15**	**1, 195**	**.00**
VC	**0.33**	**.00**	**0.12**	**0.30**	**.13**	**.08**	**18.74**	**1, 194**	**.00**
Sex	0.05	.86	-2.21	2.65	.13	.01	1.68	1, 193	.20
3rd tri STAI	-0.29	.75	-0.51	0.37	.13	.00	0.07	1, 192	.80
Sensitivity	-0.13	.55	-2.86	1.53	.13	.01	1.40	1, 191	.24
Sex x sensitivity	0.07	.83	-1.23	1.52	.12	.00	0.00	1, 190	.97
Sex x 3rd tri STAI	**0.18**	**.84**	**-0.22**	**0.27**	**.14**	**.02**	**4.51**	**1, 189**	**.04**
3rd tri STAI x Sensitivity	-0.24	.79	-0.29	0.22	.14	.00	0.21	1, 188	.65
Sex x 3rd tri STAI Sensitivity	0.35	.68	-0.11	0.17	.13	.00	0.17	1, 187	.68

VC = Verbal Comprehension.

In models18a and 18b (

Table 44*)* the prediction of toddler inhibitory control by 1st trimester SLEs in interaction with sex was tested for moderation by maternal sensitivity. The models were each significant, $F (10, 187) = 4.78$, $p < 0.01$; and $F (15, 182) = 3.30$ $p < .01$) respectively. Toddler age at EF assessment and verbal comprehension ability each made a significant contribution to the model as expected. The 3-way interaction term (Sex by 1st trimester SLEs by maternal sensitivity) made a significant contribution to the model, accounting for an additional 3% of the variance in toddler inhibitory control, after accounting for the pre- and postnatal confounders specified. Gestational age at birth also made a significant contribution when entered in the last step.

Table 44

Models 18a and 18b, Regression models testing the moderation of the 1st trimester SLEs x Sex interaction prediction of toddler IC by Maternal sensitivity.

			95% CI						
Model 18a	β	*p*-value	lower	upper	Adjusted R^2	ΔR^2	ΔF	df	*p* - value
Risk stratification	-0.01	.90	-0.26	0.23	-.005	.00	0.01	1, 196	.94
Toddler age	**0.16**	**.02**	0.01	0.12	**.04**	**.05**	**11.26**	**1, 195**	**.00**
VC	**0.31**	**.00**	0.04	0.11	**.14**	**.10**	**22.22**	**1, 194**	**.00**
Sex	-0.72	.04	-2.61	-0.05	.15	.01	3.12	1, 193	.08
1st tri SLEs	-2.11	.01	-4.58	-0.66	.14	.00	0.04	1, 191	.84
Sensitivity	-0.60	.04	-2.38	-0.05	.14	.00	0.03	1, 192	.87
Sex x sensitivity	1.01	.03	0.10	1.53	.14	.00	0.39	1, 190	.53
Sex x 1st tri SLEs	2.35	.01	0.55	3.03	.14	.01	1.68	1, 189	.20
1st tri SLEs x Sensitivity	1.95	.02	0.25	2.47	.14	.00	0.04	1, 188	.85
Sex x1st tri SLEs x Sensitivity	**-2.16**	**.01**	**-1.63**	**-0.22**	**.16**	**.03**	**6.74**	**1, 187**	**.01**
			95% CI						
Model 18b	β	*p*-value	lower	upper	Adjusted R^2	ΔR^2	ΔF	df	*p* - value
Risk stratification	-0.03	.69	-0.30	0.20	-.01	.00	0.01	1, 196	.94

Deprivation	0.01	.90	-0.27	0.30	-.01	.00	0.24	1, 195	.63
Maternal IQ	-0.02	.79	-0.03	0.02	-.01	.00	0.28	1, 194	.60
Smoking	0.00	.96	-0.32	0.34	-.02	.00	0.06	1, 193	.80
Breastfeeding	-0.01	.93	-0.37	0.33	-.02	.00	0.00	1, 192	.97
Toddler age	**0.18**	**.02**	**0.01**	**0.13**	**.03**	**.05**	**10.81**	**1, 191**	**.00**
VC	**0.30**	**.00**	**0.04**	**0.11**	**.12**	**.10**	**21.79**	**1, 190**	**.00**
Postnatal SLEs	0.03	.73	-0.28	0.39	.12	.00	0.22	1, 189	.64
Sex	-0.76	.03	-2.71	-0.12	.13	.01	2.78	1, 188	.10
1st tri SLEs	-0.60	.01	-4.70	-0.75	.12	.00	0.06	1, 187	.81
Sensitivity	-2.20	.04	-2.40	-0.05	.12	.00	0.01	1, 186	.94
Sex x Sensitivity	1.03	.02	0.11	1.55	.12	.00	0.47	1, 185	.49
Sex x 1st tri SLEs	2.41	.00	0.59	3.09	.12	.01	1.61	1, 184	.21
1st tri SLEs x Sensitivity	1.98	.02	0.27	2.51	.11	.00	0.02	1, 183	.90
Sex x 1st tri SLEs x Sensitivity	**-2.20**	**.01**	**-1.65**	**0.23**	**.14**	**.03**	**6.41**	**1, 182**	**.01**
Gestational age at birth	**0.15**	**.03**	**0.00**	**0.03**	**.16**	**.02**	**4.98**	**1, 181**	**.03**

VC = Verbal Comprehension.

Standardised predicted values of inhibitory control from regression model 18b are plotted in Figure 9 and Figure 10 in male and female toddlers separately in order to illustrate the nature of the underlying moderation of the relationship between 1st trimester SLEs and inhibitory control by maternal sensitivity. An opposing pattern of effects was evident in male and female toddlers. In males, increasing exposure to SLEs (1st trimester) was associated with poorer inhibitory control in the context of lower maternal sensitivity at 7 months of age but this was no longer evident in the context of higher maternal sensitivity.

In contrast in females, increasing 1st trimester SLEs were associated with greater inhibitory control in the context of low maternal sensitivity but this was no longer evident in the context of high maternal sensitivity.

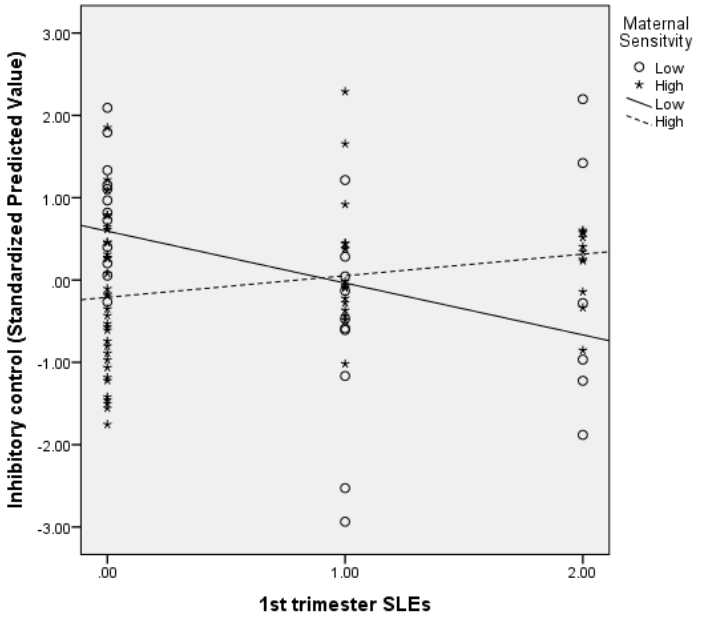


Figure 9

Predicted Values of toddler IC as a Function of Maternal Sensitivity and Maternal Exposure to SLEs in the 1st Trimester. Male Toddlers.

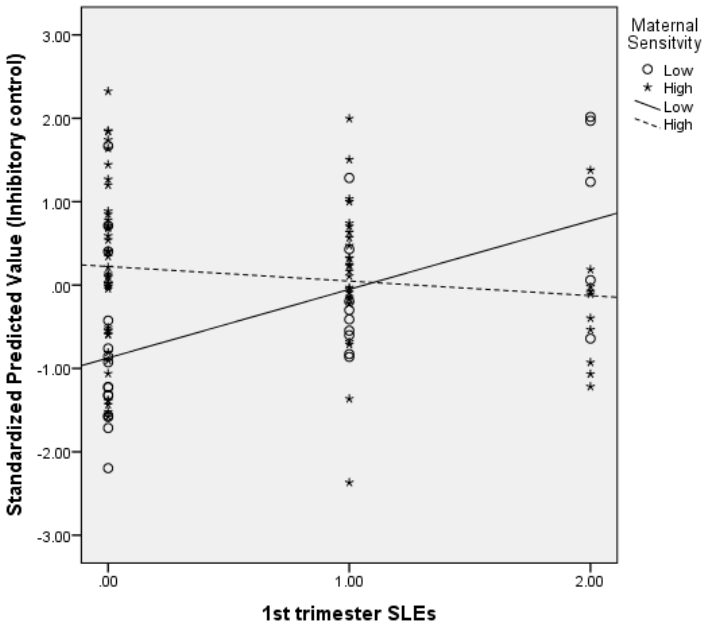


Figure 10

Predicted Values of Toddler IC as a Function of Maternal Sensitivity and Maternal Exposure to SLEs in the 1st Trimester. Female Toddlers

In order to examine the magnitude and statistical significance of the associations observed in Figure 9 and Figure 10, the regression models were next tested in male (models 19a) and female (models 20a and 20b) toddlers separately. The model statistics from these models can be seen in Table 45 and Table 46. In male toddlers (model 19a) the overall model was nearing significance (F (6, 83) = 1.81, p =.11) as was the contribution of the 1st tri SLEs by maternal sensitivity interaction term (β=0.61; p = 0.13). The interaction term accounted for 3% of the variance toddler IC score in males.

In contrast in female toddlers, the overall models were significant, both when specified to include only the minimal essential set of control variables; F (6, 101) = 6.28; p < 0.01; and when specified to include the wider set of control variables and covariates; F (12, 77) = 3.15; p < 0.01). The 1st trimester SLEs x maternal sensitivity interaction term made a significant contribution to the model and accounted for an additional 3 % (β = -0.71) of the variance in inhibitory control scores after adjusting for pre- and postnatal confounders. In males, the interaction term accounted for a similar proportion of the variance (β = 0.61) in inhibitory control outcome but did not achieve statistical significance at the conventional p < .05 level. This may reflect the fact statistical power to detect small to moderate effects was more limited in the analysis of male toddlers due to the slightly reduced sample size.

Table 45

Model 19a Regression model testing the moderation of the 1st trimester SLEs prediction of toddler IC by Maternal Sensitivity in male toddlers only.

			95% CI						
Model 19a	β	*p*-value	lower	upper	Adjusted R^2	ΔR^2	ΔF	df	*p* - value
Risk stratification	0.00	0.99	-0.37	0.36	-0.01	0.00	0.01	1, 88	0.94
Toddler age	0.07	0.55	-0.05	0.10	0.00	0.02	2.14	1, 87	0.15
VC	**0.28**	**0.02**	**0.01**	**0.11**	**0.05**	**0.06**	**5.77**	**1, 86**	**0.02**
1st tri SLEs	-0.65	0.11	-1.60	0.16	0.05	0.00	0.25	1, 85	0.62
Sensitivity	-0.20	0.17	-0.89	0.16	0.04	0.00	0.19	1, 84	0.66
1st tri SLEs x Sensitivity	0.61	0.13	-0.11	0.89	0.05	0.03	2.36	1, 83	0.13

VC = Verbal Comprehension

Table 46

Models 20a and 20b, Regression models testing the moderation of the 1st trimester SLEs prediction of toddler IC by Maternal Sensitivity in female toddlers only.

			95% CI						
Model 20a	β	*p*-value	lower	upper	Adjusted R^2	ΔR^2	ΔF	df	*p* - value
Risk stratification	-0.02	.86	-0.37	0.30	-.01	.00	0.00	1, 106	.98
Toddler age	**0.26**	**.01**	**0.03**	**0.19**	**.09**	**.11**	**12.67**	**1, 105**	**.00**
VC	**0.34**	**.00**	**0.04**	**0.14**	**.21**	**.12**	**16.29**	**1, 104**	**.00**
1st tri SLEs	0.77	.02	0.14	1.91	.21	.01	1.73	1, 103	.19
Sensitivity	0.18	.11	-0.09	0.89	.21	.00	0.16	1, 102	.69
1st tri SLEs x sensitivity	**-0.67**	**.05**	**-1.00**	**-0.01**	**.23**	**.03**	**4.04**	**1, 101**	**.05**

			95% CI						
Model 20b	β	*p*-value	lower	upper	R^2	ΔR^2	ΔF	df	*p* - value

Risk stratification	-0.02	.81	-0.40	0.31	-.01	.00	0.00	1, 106	.98
Deprivation	0.08	.43	-0.23	0.53	-.01	.01	0.66	1, 105	.42
Maternal IQ	0.04	.66	-0.02	0.04	-.01	.01	1.31	1, 104	.26
Smoking	0.00	.97	-0.51	0.49	-.02	.00	0.06	1, 103	.80
Breastfeeding	0.04	.70	-0.40	0.59	-.02	.01	0.73	1, 102	.40
Toddler age	**0.26**	**.01**	**0.03**	**0.20**	**.07**	**.10**	**11.48**	**1, 101**	**.00**
VC	**0.34**	**.00**	**0.04**	**0.15**	**.18**	**.11**	**14.62**	**1, 100**	**.00**
Postnatal SLEs	-0.01	.96	-0.53	0.50	.18	.00	0.30	1, 99	.59
Sensitivity (med split)	0.78	.03	0.11	1.98	.18	.01	1.18	1, 98	.28
1st tri SLEs	0.19	.12	-0.11	0.93	.17	.00	0.17	1, 97	.69
1st tri SLEs x sensitivity	**-0.71**	**.05**	**-1.05**	**-0.01**	**.20**	**.03**	**3.98**	**1. 96**	**.05**
Gestational age at birth	0.07	.42	-0.01	0.03	.19	.00	0.65	1, 95	.42

VC = Verbal Comprehension

4.3.3. Power analysis. Post-hoc power analyses were conducted using the software package G-Power (Faul et al., 2007). Following Cohen (1988), the recommended effect sizes were: small ($f^2 = .02$), $f^2 = .15$ (medium) and $f^2 = .35$ (large). A threshold of > 0.80 was employed to indicate sufficient power.

4.3.3.1. Power analysis for the test of moderation of the PNS x Sex prediction of toddler EF (IC and WM) by maternal tactile stimulation. The pooled sample size of male and female toddlers (n = 207) was used, and a 10 predictor variable equation (reflecting the minimal set of essential confounder and covariate variables) was employed. The post hoc power analyses revealed the statistical power to detect large effects was 0.99; to detect medium effects was 0.98 and to

detect small effects was 0.21. Thus, there was more than sufficient power (i.e. > 0.80) to detect moderate to large effects, but less than adequate statistical power to detect small effects.

4.3.3.2. Power analysis for the test of moderation of the PNS x Sex prediction of toddler EF (WM and IC) by maternal sensitivity.

The pooled sample size of male and female toddlers (n =198) was used. A 12 predictor variable equation (reflecting the comprehensive set of confounder variables and covariates) was employed and the fore- mentioned recommended effect sizes of Cohen (1988) were followed. An alpha level of $p < 0.05$ used was. The power analysis revealed the statistical power to detect large effects was 0.99, to detect medium effects was 0.94 and to detect small effects was 0.16. Thus, there was more than sufficient power (i.e. > 0.80) to detect moderate to large effects, but less than adequate statistical power to detect small effects.

Post- hoc power analysis for the analyses examining moderation of the PNS x sex prediction of IC by maternal sensitivity in male and female toddlers separately was also conducted. A 12 predictor variable equation (reflecting the comprehensive set of confounder variables and covariates) and an alpha level of $p < 0.05$ was employed. In females (n= 108) the power analysis indicated the statistical power to detect large effects was 0.97, to detect medium effects was 0.62 and to detect small effects was 0.01. In males, the power to detect large effects was 0.93, to detect medium effects was 0.51 and to detect small effects was 0.01. Thus, when the sample was split by sex, and included the comprehensive set of confounder variables and covariates, there was still more than adequate power (i.e. > 0.80) to detect large effects but the power to detect small or moderate effects was inadequate.

4.4. Discussion.

Previously identified sex-specific effects of prenatal stress on emerging toddler executive functions were significantly moderated by maternal sensitivity but not tactile stimulation in early life. This held true for toddler inhibitory control but not working memory. A pattern of moderation of PNS by maternal sensitivity on inhibitory control was observed in both sexes and the strength of the associations were similar in magnitude (accounting for 3% of the variance in outcome), however single group analyses achieved statistical significance only for females in this study. Possible explanations, linked to statistical power are considered later in this discussion. However, the whole sample analysis did reveal a significant three-way interaction between infant sex, PNS exposure and maternal sensitivity on inhibitory control, arising from the opposing direction of underlying effects in males and females.

This opposing pattern of effects in males and females warrants particular attention and can be considered in the context of theories of foetal programing and evolutionary theories which raise important points regarding sex-dependent effects in development. Each will be considered in turn. First, the Developmental Origins of Health and Disease (DOHaD), previously referred to as the 'foetal origins' hypothesis (Barker, 1998) and the 'predictive adaptive response' concept (e.g. Godfrey et al., 2007).

The fundamental tenet of these hypotheses is that the foetus adapts to cues in the environment in-utero and the subsequent matching (or non- matching) of the postnatal environment predicts outcomes. The DOHaD or foetal origins hypothesis was first described by Barker to explain observed associations between low birth weight and chronic conditions of adult health, particularly heart disease and type II diabetes (Barker, 1998). The hypothesis proposes that the foetus adapts in utero to the environment it expects to enter once leaving the womb, based on cues from the uterine environment. This can be adaptive when conditions in utero accurately reflect the postnatal environment, or maladaptive when there is a mismatch between the prenatal and the postnatal environment. In the case of heart disease and type-II diabetes for example low birth weight, reflecting low availability of nutrients prenatally, carries an evolved adaptation to thrive in food scarce environments in later life, such that the foetus' metabolic systems are physiologically programmed to expect nutritionally sparse environments. This confers an advantage when the postnatal environment matches, i.e. a lack of nutritional availability, but risk when there is a mismatch such as the high calorie diets of many modern societies. In this case, the metabolic systems programmed for food scarcity are unable to efficiently process the highly available high calorie foods consumed in the postnatal

environment. It is suggested that it is this mismatch between expected nutritional deficits and actual food surplus that results in obesity and eventually type II diabetes and/ or heart disease. Similarly, the predictive adaptive response mechanism is described as a form of developmental plasticity in which cues received in early development in utero can influence the development of particular phenotypes, which are adapted to thrive in an environment matching the environment from which those cues came. When the predicted and actual environments differ, the mismatch can have adverse consequences for adaptive survival.

Evolutionary hypotheses of sex biased preferential maternal investment (e.g. Trivers & Willard, 1973) may also be drawn upon in understanding the differences observed in male and female toddlers in the current study. Specifically, such hypotheses assert that reproductive strategies when maternal conditions during pregnancy are poor, such as the presence of PNS, involve less investment in male foetus' and greater investment in females. According to such hypotheses, adaptations to PNS cues arising from foetal programming / the predictive adaptive response mechanism may be more evident in females; with males therefore exhibiting vulnerability to prenatal adversity irrespective of matching or non-matching of the postnatal environment. Indeed, the hypothesis asserts that exposure to prenatal adversity will always affect male foetus' more than females (Sandman et al., 2013).

Applied to the current findings, a postnatal environment of low maternal sensitivity (characterised by a lack of appropriate and warm responding and the presence of inconsistent, unresponsive and intrusive responding) may be considered a postnatally adverse environment for infants. In females, we observed enhanced

inhibitory control following exposure to higher levels of PNS (chapter 3 of this thesis), which remained in the context of subsequent low levels of maternal sensitivity (postnatal adversity) but was no longer evident in the context of high sensitive caregiving. So, here the relative enhancement in IC or adaptive preparedness for an adverse environment disappears when the predicted environment is not adverse and no longer requires the infant to be 'threat-ready' to ensure survival. An optimal outcome indexed by higher levels of inhibitory control at age 2.5 years was observed when the prenatal environment 'matched' the postnatal so the female infant could be said to have been prepared for adversity in utero by the exposure to PNS. In the context of low levels of PNS (non-adverse) and subsequent low maternal sensitivity (adverse) the female infant could be seen to be unprepared in utero for later adversity. In line with this, visual inspection of Fig 10 suggests that these females exhibit the lowest predicted IC scores in the current study.

In males, the significantly lowered inhibitory control observed following exposure to PNS in Chapter 3 of this thesis remained in the context of lowered maternal sensitivity assessed at 7 months of life. Therefore, matching of the pre- and postnatal environments did not confer an advantage in males, in line with the sex biased preferential maternal investment hypothesis of Trivers & Willard (1973). However, the negative impact of PNS on IC observed in study 2, was no longer evident in the context of high sensitive caregiving; therefore it would appear that the adverse effects of PNS in males were reversed by a good quality caregiving environment postnatally. Taken together, this suggest that in females, it is the matching versus non-matching of pre-and postnatal adversity that predicts outcomes. Males on the other hand appear to be vulnerable to prenatal adversity

even if the postnatal environment matches this. However, the findings indicate that the observed effects of prenatal adversity in males can be reversed by an optimal postnatal care-giving environment.

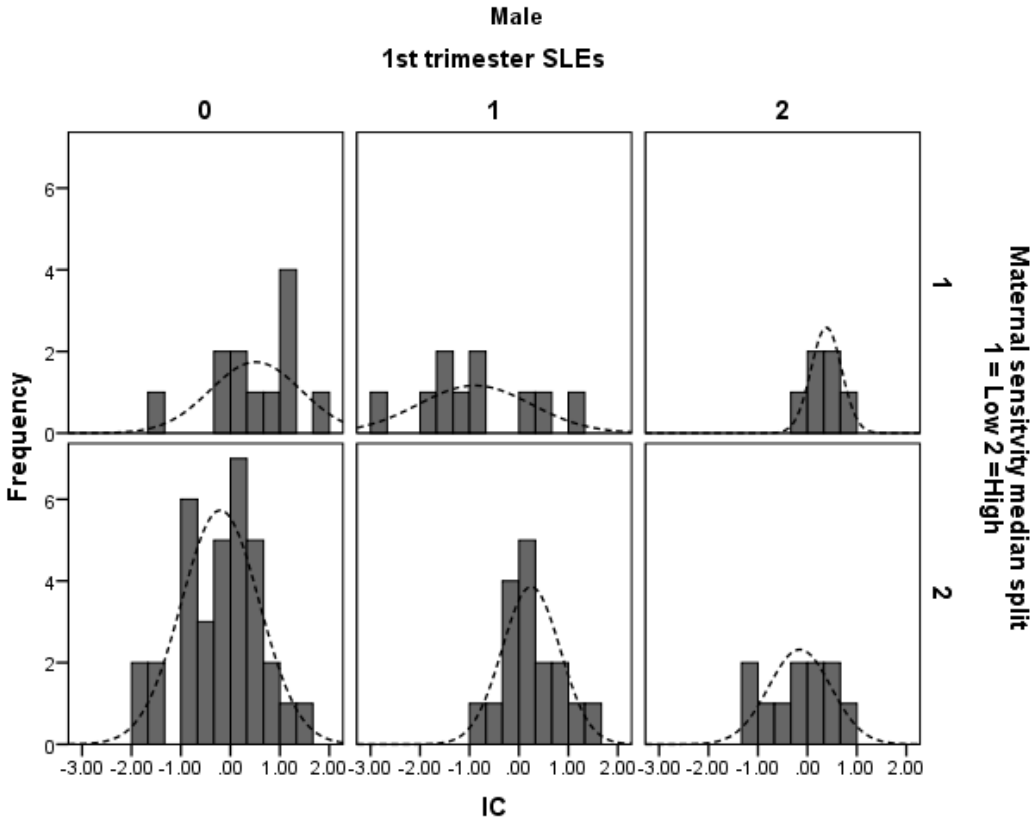


Figure 11

Histograms to show distributions of IC in males for different levels of exposure to SLEs in the 1st trimester and exposure to low or high maternal sensitivity.

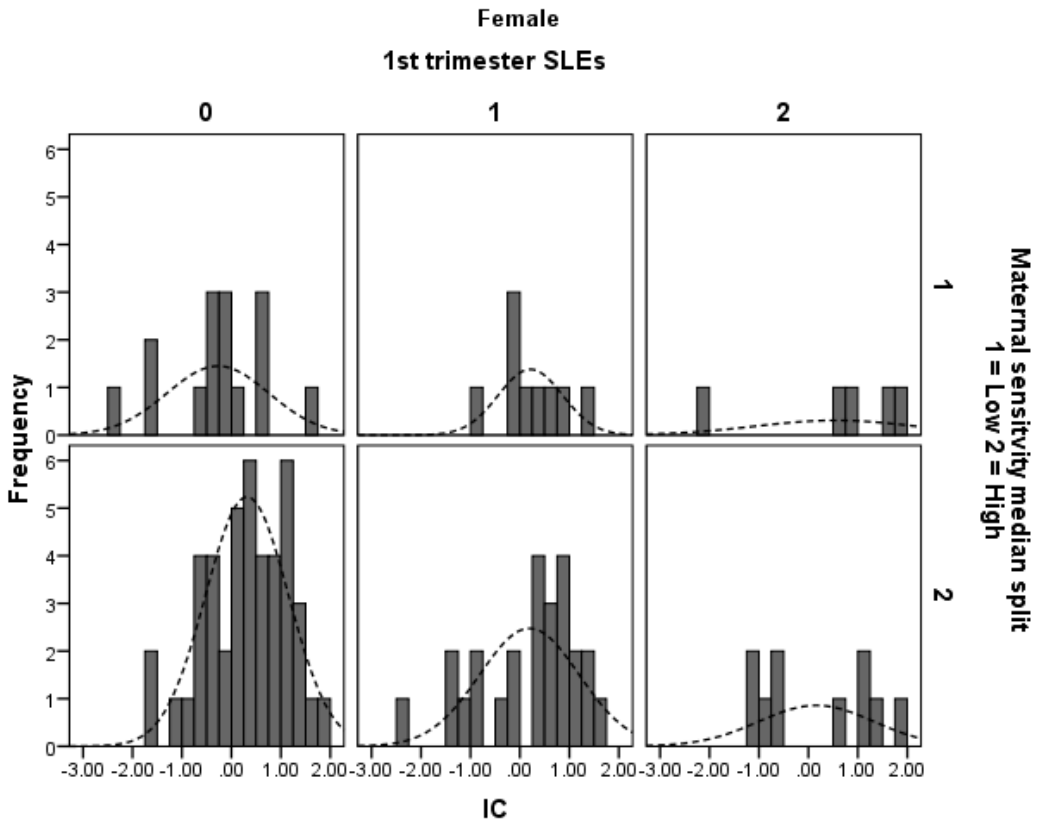


Figure 12

Histograms to show distributions of IC in females for different levels of exposure to SLEs in the 1st trimester and exposure to low or high maternal sensitivity.

4.4.1. Null findings, methodological limitations and directions for future research. Contrary to expectations, we did not find a moderating influence of maternal sensitivity on the prediction of toddler working memory from the 3rd trimester STAI score by sex interaction. This may reflect a lack of moderating influence of maternal sensitivity on some cognitive domains (in this case working memory) as opposed to moderation in the case of others (i.e. inhibitory control). However, since no previous studies have examined moderation of the association between PNS and component executive functions by maternal care-giving behaviour for comparison, this is speculative and therefore requires replication.

Also, we did not observe a moderating influence of maternal tactile stimulation on the PNS predictions of toddler EF examined. We cannot conclude with confidence that this null finding represents a lack of moderating influence of this form of maternal caregiving behaviour or is an artefact of aspects of the current study design. The measure of maternal tactile stimulation employed was the frequency with which mothers stroked their infants and was assessed in the early postnatal period (over the first 12 weeks). Toddler EF outcome, on the other hand, was assessed at 2½ years. Thus, a moderating effect of tactile stimulation on the association between PNS and EF development may have been masked by the influence of other aspects of the environment on EF development over this period. As this is the first investigation of moderation of the association between PNS and EF by tactile stimulation, replication is required to be sure that this null finding is not a result of such artefacts.

However, the unexpected finding of a lack of bivariate association between the two measures of maternal care-giving raises the possibility that they assess somewhat different aspects of the mother-infant caregiving relationship. Such differences may account for the lack of moderating influence of tactile stimulation in the current study. Indeed, maternal sensitivity was also examined much earlier in development (7 months) than toddler EF but did demonstrate a moderating influence on the association between PNS and inhibitory control. This measure is likely to reflect a more persistent feature of maternal caregiving that endures throughout the period between its measurement and the measurement of EF. Studies show maternal sensitivity is associated with maternal scaffolding behaviour (cognitive support, directiveness of instruction, praise and criticism), which in turn has been associated with better EF development (Hammond et al., 2010). Thus

maternal sensitivity may be a better indicator of a prolonged parenting effect that directly fosters EF development, compared with the tactile simulation measure.

It is plausible that these two indices of maternal care-giving behaviour, in assessing somewhat different aspects of the mother- infant care-giving relationship, influence different aspects of infant development. In contrast to maternal sensitivity, tactile stimulation in the early postnatal period may facilitate the development of infant emotional regulation in the context of PNS (as reported by Sharp et al., 2012) but not aspects of cognitive development. This is in contrast to the evidence from the animal literature demonstrating a facilitative effect of maternal tactile stimulation in offspring cognitive development and a moderating effect on the association between PNS and offspring cognitive development. However, inter species differences in the trajectories of neural development in the postnatal period in relation to the timing of tactile stimulation may account for these differences. As this is the first investigation of its kind in the human literature, further studies are warranted to understand the pattern of effects.

A limitation of the current study is the multiple comparisons conducted and associated risk of type-I error. With only one analysis showing a significant moderating effect of maternal caregiving behaviours out four analyses conducted, it may be argued that this was a chance finding. We did not apply an adjustment to *p*-values, as is often advised to guard against type-I errors, as this carries the risk of type -II errors (Rothman, 1990). Furthermore, our aim of detecting 3-way interactions also put the models tested under strain to detect significant but small effects, with our sample size. To our knowledge, this is the first investigation examining moderation of the association between PNS and EF specifically (as opposed to cognitive function more generally) at this early age; therefore we

exercised caution in not adjusting coefficient alpha. However, the results should be interpreted with caution and replication is required.

4.4.2. Study Findings in the Context of Previous Research, methodological strengths and Novel Contributions to the field. To our knowledge, this is the first study examining the moderation of the association between PNS and EF by maternal caregiving behaviours. Overall, our findings are consistent with the only two previous studies in the literature (to our knowledge) that have examined this effect in early development (Bergman et al., 2007& Grant et al., 2010) and build upon them in a number of ways.

First, we examined more specific measures of infant cognitive development, i.e. indices of EF, as opposed to the more global measures (BSID-MDI) employed by both Bergman et al. (2010) and Grant et al. (2010). The predictive value of the BSID-MDI in early development to cognitive function at later ages has been called into question (e.g. Talge, Neal and Glover, 2007). Early EF abilities have been implicated in supporting the development of later academic skills and EF deficits have been implicated in a number of developmental disorders (as outlined in section 2.1.4)

Second, we examined aspects maternal care-giving directly via assessment of maternal sensitivity towards their infants during play, as opposed to inferring the quality of care-giving through attachment classification (as in Bergman et al.'s, 2010 study). Though a widely used and well-validated measure of parent-child relationship quality, the coding of attachment classification during Ainsworth's Strange Situation, as employed by Bergman et al. (2010), is based largely upon the infant's behaviour towards their mother as opposed to the mother's behaviour towards her child, specifically assessing "the extent to which the child uses the

parent as a secure base for exploration" (p. 1208). Though this may indeed reflect overall qualities of the dyadic relationship, it may be argued it is a rather indirect measure of maternal care-giving behaviours per se, as the infant is an active player in the attachment classification received. The influence of maternal sensitivity reported here (and by Grant et al., 2010) however, is more clearly centred on the mothers' responses to the infant and thus has more clear cut potential for interventions aimed at improving outcomes.

Third, though Grant et al. (2010) examined maternal sensitivity directly, the generalizability of their findings may be limited by characteristics of the sample studied in that they were largely middle class and well-educated. The current sample exhibits a diverse range of socioeconomic and educational backgrounds. Furthermore, Grant et al.'s study examined prenatal stress with a dichotomous approach, with women classified as either anxious or non-anxious on the basis of meeting diagnostic criteria for at least one anxiety diagnosis during pregnancy. It is not clear whether these findings are generalisable to women experiencing lower level symptoms of prenatal anxiety within the normal range. The current study findings may be more generalizable due to the use of a continuous measure of symptoms of anxiety in a large community sample experiencing symptoms across the range.

4.4.3. Conclusion. To our knowledge, the current study is the first investigation of a moderating influence of maternal caregiving behaviours on the association between PNS and toddler EF. We have further shown sex-dependent effects in this moderating influence, a finding not previously reported as far as we are aware. The current findings can be explained in terms of evolutionary mechanisms and hypotheses of foetal adaptations to the environment in utero and

subsequent matching of the postnatal environment and the sex biased preferential maternal investment hypothesis of Trivers and Willard, (1973). Our findings, of moderation of the associations between PNS and EF by maternal sensitivity are broadly consistent with a limited number of previous studies but improve upon their methodologies and extend their findings in as number of important ways. Our findings have important implications for interventions aimed at improving outcomes for children at risk of sub optimal EF development due to their mothers' experience of stress during pregnancy, and therefore warrant replication.

5. Overall Conclusions

This thesis examined the latent structure of EF at an age that has been largely neglected by previous research, reporting an integrative structure of two separable but moderately correlated EF components- working memory (WM) and inhibitory control (IC) (Chapter 2). We examined the prediction of these component EFs by two relatively independent measures of prenatal stress at multiple time points during gestation, a comparison that to our knowledge has not previously been reported in pre-schoolers (Chapter 3). Finally, we tested moderation of the PNS prediction of toddler WM and IC reported in chapter 3 by maternal care-giving behaviours, again a previously unreported investigation as far as we aware (Chapter 4).

5.1. Novel contributions to the field, implications of the findings and directions for future research.

First, we have demonstrated that EF exhibits an integrative structure very early in its development as it is just coming 'on-line' during toddlerhood. To our knowledge, only one previous study in the literature (Mulder et al., 2014) has examined the latent structure of EF in children as young as the current sample. Mulder et al. (2014) also reported an integrative structure of EF comprising 2 distinguishable yet moderately correlated factors. The nature of the factors reported by Mulder et al. (2014) differ to those of the current thesis, with these authors focussing on 'hot' and 'cool' EF rather than WM and IC, however this is likely to reflect differences in the specific aims of the two studies and related differences in the tasks administered. Nevertheless the current study provided important replication of an integrative latent structure of EF very early in development, as

these abilities are just emerging and the results are in line with those reported in older samples of children (Huizinga et al., 2006; Lehto et al., 2003; Lonigan et al., 2016; Lerner & Lonigan 2014, Usai et al., 2014 and Miller et al., 2012. Since ours was the first study to focus on the latent structure of affectively neutral aspects of emerging EF in toddlers, future research should aim to replicate the current findings. Outside of a large cohort study design it may be possible to ideally administer a larger number of developmentally appropriate tasks, than was possible in the current study, that are considered a priori to draw on working memory and inhibitory control abilities in toddlers.

Greater understanding of emerging executive functions and their latent structure in typical development may help to elucidate the cognitive underpinnings of a number of developmental disorders or child mental health problems which are thought to involve executive dysfunction. This, in turn could aid in the early identification of targets for intervention and thus increase the window of opportunity for intervention aimed at improving emotional and behavioural outcomes for children. Similarly, reported predictive relations between EF abilities and later academic skills mean that research into early EF may assist in the early identification of children at risk of later academic difficulties. This again could aid in the identification of such children, thus increasing the potential for intervention aimed at supporting their academic progress.

Second, we report apparent sex-dependent PNS effects on toddler WM and IC after controlling for a wide range of candidate confounders drawn from the literature including maternal cognitive ability, child general verbal ability and exposure to postnatal maternal stress. No whole sample main effects of PNS on EF were identified at any stage of pregnancy. To our knowledge, this is the first study

to examine the PNS prediction of EF abilities specifically (as opposed to cognitive ability more generally) during toddlerhood, and to examine sex differences in the influence of PNS on these abilities. Replication of the observed associations in larger samples would increase confidence in the current findings through increasing the capacity to detect small effects with functional significance.

We observed a continuing theme of opposing effects in male and female toddlers of the influence of PNS on EF. The pattern of sex-specific results supported the a priori hypothesis that there would be a relative a male vulnerability to PNS in EF development. However, the pattern of increasing EF with increasing exposure to PNS observed in females was not predicted, despite there being some studies reporting enhanced EF following PNS in older samples of children (e.g. Buss et al., 2011), though the enhanced ability was observed in males only for IC and for both males and females for WM in Buss et al.'s sample.

Considered in evolutionary terms, our results are in line with theories which posit a reduction in investment in the development of the male foetus in the presence of poor environmental conditions for the mother (in this case, PNS) with a corresponding adaptive agility for the development of the female foetus (Barker, 1998; Godfrey et al., 2007; Sandman et al., 2013; Trivers & Willard, 1973). According to this position, female foetuses were able to adapt to the environment they expected to enter postnatally, which based on cues in utero was one characterised by stress and therefore perhaps environmental risk. In these conditions females adapted to exhibit enhanced WM and IC. Such abilities may have adaptive significance for survival in adverse environments. Increased WM may confer an advantage through enhancing recall of the sources and locations of threat, as well as strategies and safe havens to escape threat in order to protect the

young. Similarly, enhanced IC may help females to select and deselect effective behavioural responses to the environmental threats to which they expect to be exposed based on cues in utero. In contrast, such theories suggest that the male foetus does not display this adaptive agility and are thus left vulnerable to the influence of PNS on development manifest, in this case, in poorer IC and WM. Reduced inhibitory control would perhaps increase the likelihood of aggressive responses under threat and might ensure their survival to mate again in the short term but may also leave the individual vulnerable to not surviving an aggressive bout. We know that reactive aggression in childhood is associated with lower inhibitory control and other neuropsychological deficits. Poorer IC in particular and WM have been reported in children with conduct problems (e.g. Sonuga-Barke, Dalen, Daley & Remington, 2002; Stevens, Quittner, Zuckerman & Moore, 2002), and conduct problems have been linked to exposure to PNS (O'Connor, Heron, Golding, Beveridge & Glover, 2002).

An important direction for future research would be an examination of the persistence of the prenatal stress prediction of poorer WM and IC to later development, and whether the sex differences observed in the current thesis remain. Executive functions are particularly salient in the transition to formal schooling, when greater demands are placed on children's abilities to pay attention, wait one's turn, remember rules and follow instructions. Thus, prospective investigation of the prenatal stress prediction of indices of EF beyond toddlerhood, across the transition to school and beyond is warranted.

Third, Study 3 revealed evidence for moderation of prenatal stress effects on toddler cognitive development by one form of postnatal caregiving, namely maternal sensitivity, but there was no evidence of moderation by early tactile

stimulation. Interestingly, the strength of the direct associations between PNS and EF for males and females that was found to underlie the significant sex by PNS interaction effects in Study 2 were observed to be somewhat weaker in magnitude in prediction of IC than for WM and these associations were non-significant or marginal in females. On reflection, his may have been due to the presence of the further interaction with postnatal maternal sensitivity that was unmeasured at that stage of analysis (Study 2) but was observed in Study 3 for the IC outcome specifically. Indeed in study 3, there was 3-way interaction between sex of toddler, 1st trimester SLEs and maternal sensitivity. In both males and females, exposure to higher levels of maternal sensitivity in the postnatal period led to an elimination of the previously observed PNS effects, whereas in the context of lower sensitivity the pattern of male vulnerability (lower IC) and female enhancement or adaptation remained and were clearly observed. So the underlying effect of the postnatal caregiving environment observed in Study 3, which was unmeasured in Study 2, may explain why the opposing sex-dependent patterns of PNS on IC appeared comparatively 'dampened down' compared with those for working memory. In line with this interpretation, maternal sensitivity was not found to be a moderator of the observed PNS effects on WM.

Since our findings indicate that male children of mothers that experienced stress during pregnancy may be at risk of sub-optimal EF development postnatally, early identification of pregnant women experiencing increased PNS may therefore offer the opportunity for interventions in pregnancy aimed at reducing such stress, in turn reducing the developmental risk associated with executive dysfunction for their unborn child.

Furthermore, findings from the current thesis may suggest policy changes in antenatal care that could help to improve well-being in pregnant women and the unborn child. Specifically, given the current findings of sub-optimal EF associated with prenatal stress in male toddlers, routine screening of women for symptoms of stress such as anxiety and depression at scheduled antenatal appointments deserves consideration. In today's society, many women choose to begin their maternity leave very close to their due date, to maximise the time they can spend with their child after birth. The association between mothers' experience of symptoms of stress (anxiety) during pregnancy and poorer toddler EF was evident for the experience of anxiety in the third trimester, but not in earlier. Thus, pregnant women may benefit from a policy change which promotes maternity leave beginning earlier within the third trimester. This would allow a period of adjustment and preparation for mothers-to-be and could help to alleviate stress.

Conclusion.

We have shown that EF exhibits an integrative latent structure very early in development and that EF abilities are vulnerable to exposure to PNS. This vulnerability appears particularly true for males; however particular maternal care-giving behaviours may eliminate this negative influence of PNS. Females on the other hand appear to show a greater capacity for adaptation to adversity in the prenatal period, making them better adapted to thrive in adverse environments postnatally. It is also worth emphasising that female vulnerability may arise when infants are unexposed to PNS in pregnancy but subsequently experience adverse postnatal environments. Amongst female toddlers the regression model predicted the lowest levels of inhibitory control when lower PNS levels (non-adverse) were

followed by less sensitive caregiving (adverse) and as such the females could be considered ‘unprepared for postnatal environmental adversity’.

More research is undoubtedly required to test out the multiple novel aspects of the investigations presented in this thesis. If replicated, then interventions aimed at reducing maternal stress in pregnancy and the postnatal period and increasing maternal sensitive care-giving in the early postnatal period may be warranted to improve later EF outcomes for children.

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Appendix 1- Ethical Agreement for the study.

Cheshire North & West Research Ethics Committee
Cheshire West PCT
1829 Building
Countess of Chester Health Park
Liverpool Road
Chester
CH2 1HJ

Telephone: 01244 650 334
Facsimile: 01244 650 333

27 June 2006

Professor Jonathan Hill
Professor of Child and Developmental Psychiatry
University of Liverpool, Alder Hey Hospital
Mulberry House, Alder Hey Hospital
Eaton Road
L12 2AP

Dear Professor Hill

Full title of study: **The Wirral Child Health and Development Study**
REC reference number: **05/Q1506/107**

Thank you for your letter of 19 May 2006, responding to the Committee's request for further information on the above research and submitting revised documentation.

The further information has been considered on behalf of the Committee by the Vice-Chairman.

Confirmation of ethical opinion

On behalf of the Committee, I am pleased to confirm a favourable ethical opinion for the above research on the basis described in the application form, protocol and supporting documentation as revised.

Conditions of approval

The favourable opinion is given provided that you comply with the conditions set out in the attached document. You are advised to study the conditions carefully.

Approved documents

The final list of documents reviewed and approved by the Committee is as follows:

Document	*Version*	*Date*
Application		09 January 2006
Investigator CV		
Protocol	1	09 January 2006
Covering Letter		09 January 2006
Summary/Synopsis	1	09 January 2006
Response to Request for Further Information		19 May 2006
Father Information Sheet, Study 1500 - Phases 1, 3, 5 & 7	2	01 May 2006
Study 300 Parent Information Sheet, one year - Phase 8	2	01 May 2006
Study 300 Parent Information Sheet, 6 months - Phase 6	2	01 May 2006

Study 300 Parent Information Sheet, Antenatal Phases 2 & 4	2	01 May 2006
Mother Information Sheet, Study 1500 - Phases 1, 3, 5, & 7	2	01 May 2006
Letter confirming funding - MRC		09 March 2005
Supporting letter from Mr Doyle, Wirral Hospitals NHS Trust		09 December 2005
Supporting letter from Ms Sheila Hillhouse, Birkenhead & Wallasey PCT		09 December 2005
Phase 8: Study 300 12 month mother and baby postnatal assessments	1	09 January 2006
GP Letter Study 1500	1	01 January 2006
GP Letter Study 300		01 January 2006
Parent Consent, Study 1500 - Phases 1, 3, 5 & 7	1	09 January 2006
Consent to contact a relative - Study 1500	1	09 January 2006
Parent Consent, Fathers, - Study 1500 - Phases 1, 3, 5 & 7	1	09 January 2006
Parent Consent - Study 300 Antenatal, perinatal - (Phases 2 & 4)	1	09 January 2006
Study 300 Parent Information Sheet 6 months (Phase 6)	1	09 January 2006
Parent Consent - Study 300, first birthday (Phase 8)	1	09 January 2006
Parent Consent - Study 300, DNA First Birthday (Phase 8)	1	09 January 2006
Phase 1: Study 1500 mother antenatal screen	1	09 January 2006
Phase 1: Study 1500 father antenatal screen	1	09 January 2006
Phase 2: Study 300 mother antenatal interview	1	09 January 2006
Phase 3: Study 1500 pregnancy/obstetric/birth outcomes	1	09 January 2006
Phase 4: Study 300 perinatal baby assessment	1	09 January 2006
Phase 5; Study 1500 6-8 week questionnaire mother	1	09 January 2006
Phase 6: Study 300 6 month postnatal assessments mother and baby	1	09 January 2006
Phase 7: Study 1500 8 month questionnaire and routine health visitor developmental check (mother)	1	09 January 2006
Phase 7: Study 1500 8 month questionnaire (father)	1	09 January 2006

Research governance approval

The study should not commence at any NHS site until the local Principal Investigator has obtained final research governance approval from the R&D Department for the relevant NHS care organisation.

Statement of compliance

The Committee is constituted in accordance with the Governance Arrangements for Research Ethics Committees (July 2001) and complies fully with the Standard Operating Procedures for Research Ethics Committees in the UK.

L13

NHS
National Research Ethics Service
North West 5 Research Ethics Committee - Haydock Park
North West Centre for Research Ethics Committees
3rd Floor - Barlow House
4 Minshull Street
Manchester
M1 3DZ

Telephone: 0161 625 7819
Facsimile: 0161 237 9427

07 June 2010

Professor J Hill
Professor of Child & Adolescent Psychiatry
Room 4.321 Jean McFarlane Building
The University of Manchester
Oxford Road
MANCHESTER M13 9PL

Dear Professor Hill

Full title of study:	**Social, emotional & biological processes in emergent conduct disorders: The Wirral Child Health and Development Study 1-4 years**
REC reference number:	**10/H1010/4**

Thank you for your letter of 08 May 2010, responding to the Committee's request for further information on the above research and submitting revised documentation.

The further information has been considered on behalf of the Committee by Professor Caroline Carlisle (Professor of Education, Nursing and Midwifery).

Confirmation of ethical opinion

On behalf of the Committee, I am pleased to confirm a favourable ethical opinion for the above research on the basis described in the application form, protocol and supporting documentation as revised, subject to the conditions specified below.

Ethical review of research sites

The favourable opinion applies to all NHS sites taking part in the study, subject to management permission being obtained from the NHS/HSC R&D office prior to the start of the study (see "Conditions of the favourable opinion" below).

The Committee has not yet been notified of the outcome of any site-specific assessment (SSA) for the non-NHS research site(s) taking part in this study. The favourable opinion does not therefore apply to any non-NHS site at present. I will write to you again as soon as one Research Ethics Committee has notified the outcome of a SSA. In the meantime no study procedures should be initiated at non-NHS sites.

Conditions of the favourable opinion

The favourable opinion is subject to the following conditions being met prior to the start of the study.

Management permission or approval must be obtained from each host organisation prior to the start of the study at the site concerned.

For NHS research sites only, management permission for research ("R&D approval") should be obtained from the relevant care organisation(s) in accordance with NHS research governance arrangements. Guidance on applying for NHS permission for research is available in the Integrated Research Application System or at http://www.rdforum.nhs.uk. *Where the only involvement of the NHS organisation is as a Participant Identification Centre, management permission for research is not required but the R&D office should be notified of the study. Guidance should be sought from the R&D office where necessary.*

Sponsors are not required to notify the Committee of approvals from host organisations.

It is the responsibility of the sponsor to ensure that all the conditions are complied with before the start of the study or its initiation at a particular site (as applicable).

Approved documents

The final list of documents reviewed and approved by the Committee is as follows:

Document	*Version*	*Date*
Covering Letter - from Dr Helen Sharp, Chartered Consultant Clinical Psychologist and Lecturer in Clinical Child and Adolescent Psychology, University of Liverpool		22 February 2010
REC application	IRAS Version 2.5	22 February 2010
Protocol	1	22 February 2010
Ethical issues and Safety Protocol	1	22 February 2010
Investigator CV - for Professor Jonathan Hill		22 February 2010
Investigator CV - for Dr Helen Sharp		22 February 2010
Participant Consent Form: Phases 10/12 - Mother	1	February 2010
Participant Consent Form: Phases 10/12 - Partner	1	February 2010
Participant Consent Form: Phases 10/12 - Guardian	1	February 2010
Participant Consent Form: Phase 10 - Mother - DNA analysis	1	February 2010
Participant Consent Form: Phases 9,11,12 - Mother - Intensive	1	February 2010
Participant Consent Form: Phases 9,11,12 - Guardian - Intensive	1	February 2010
Participant Consent Form: Phase 9 - Mother - DNA analysis	1	February 2010
Participant Consent Form: Phases 9,11 - Mother - Infant RNA	1	February 2010
Participant Consent Form: Parent - Study 300 GP tracking (previously approved by Cheshire LREC)	1	May 2007
Participant Consent Form: for future contacts (previously approved by Cheshire LREC)	1	February 2010
Participant Consent Form: to contact a relative - extensive sample	1	
Letter to GP and Health Visitor - Extensive/Intensive Study	1	February 2010
Health Visiting Team contact form	1	22 February 2010
Evidence of insurance or indemnity: Letter from Mohammed Zubair, Faculty Research Practice Co-ordinator, The University of Manchester		22 February 2010
Pan-Manchester R&D Notification Form		22 July 2009

Appendix 2- Participant information sheets.

Phase 1, 3, 5 and 7

MANCHESTER 1824

The University of Manchester

Wirral University Teaching Hospital NHS
NHS Foundation Trust

UNIVERSITY OF LIVERPOOL

Study Base:
The Lauries Centre, 142 Claughton Road,
Birkenhead, Wirral, CH41 6EY
Freephone: 0800 051 7597
(from a mobile) 800 051 7597
Text: 07956 297412

Parent Information Sheet (Mother)– Study 1500

Title of study : The Wirral Child Health and Development Study

Investigators: Jonathan Hill, Helen Sharp, Andrew Pickles, Gill Lancaster
Research Staff: Karen Lunt, Carol Bedwell, Belinda Thompson, Julie Carlisle, Kate Marks, Kate Marshall,Liz Green, Florin Tibu, Jo Roberts, Jenny Lee, Nichaela Broyden, Carol Sadler, Jeanette Appleton

You are being invited to take part in a research study. Before you decide whether you want to take part, it is important for you to understand why the research is being done and what it will involve. Please take time to read the following information carefully and discuss it with others if you wish. Ask us if there is anything that is not clear or if you would like more information. Take time to decide whether you wish to take part. Thank you for reading this.

What is the study about?
We would like to invite you to participate in a new study of children's early development from birth to their first birthdays. This study is based at the Universities of Liverpool and Manchester. It is part of a programme of research into how children learn how to behave with other people, and why some children have difficulties controlling their behaviours. In order to fully understand this we need to measure the early development of children in many different ways. The aim of the study is to find out about the effects of many different forms of stress on parents and babies during the antenatal period and in the first months after birth. We know that for some parents and children the effects are quite long lasting, and others find ways of coping. We want to understand these processes better so that services to support families experiencing stress can be improved.

Who is being invited to take part?
We are approaching all first time mothers and their partners who are booked into the antenatal clinic at Arrowe Park Hospital over a two year period. It is important that we have participants in the study with low, medium and high levels of stress. If you have agreed to take this letter home a research midwife will contact you at your 20 week appointment or slightly after, to tell you more about the study, answer any questions you have and to invite you to take part.

Do I have to take part?
It will be up to you to decide whether or not you would like to take part. If you agree, and change your mind later, you can withdraw from the study. This will not affect the care you receive.

How often will I be contacted?
We will contact you again six weeks after the birth of your baby, and when your baby is 8 months old. We would also like to contact some mothers more often up to the first birthdays of their children, so that we can ask them more about their lives, and understand better their ways of coping, and assess their babies' health and development in more detail. If you decide to take part, the computer will tell us who to invite for the additional contacts after we have entered the information you provide now. If your name does come up we hope very much that you will be able to help us, but at this stage we are only asking you to participate now and at 6 weeks and eight months.

What will I be asked to do at each time point?
During your pregnancy we will interview you and ask you to complete some questionnaires about your current health and relationships, and about your expectations of the baby and being a mother. This can be done here at the antenatal clinic or at another clinic on the Wirral or at the study base in the Lauries Centre. It should take about 25 minutes.

We will also ask you for consent for us to have access to your medical records for the pregnancy, the birth, and your new born infant following the birth.

When your baby is 6 weeks old we will send you some short questionnaires about your health, your relationships, and about your baby by post, and ask you to 'Freepost' them back to us.

When your baby is 8 months old we will send you more questionnaires about your health and about your baby, and ask you to return them 'Freepost' to us or return them to your health visitor when you attend for your baby's routine 8 month developmental check-up. We will also ask your health visitor for the results of their 9-12 month assessment of your baby's development.

If you give written consent to take part in this study and you are selected by the computer to be invited for additional contacts, one of the research team named on the front of this information sheet will contact you at home, using the contact details you give to the research midwife. They will only contact you if you agree to it.

How will this information be used?
All information that we receive from you will be treated as strictly confidential, under the guidelines of the Universities of Liverpool and Manchester, the UK Medical Research Council, and the Data Protection Act. Information that we enter on the computer will be identified only by a number. We will report general findings about parents and children, but you or your child will never be identified. The only reason we might have to share information from the study with other people is if there are concerns about you or a child being at risk of serious harm. If that happens we will talk with you first to decide on the best way forward. Concerns like this would be addressed by seeking appropriate forms of help for you and/or following Trust Child Protection Guidelines.

Who is organising and funding the research study?
The study is being run by Professor Jonathan Hill of the University of Manchester and Dr Helen Sharp of the University of Liverpool. The research is funded by the Medical Research Council.

Are there any benefits in taking part in this study?
There are no benefits to you or your child's health in taking part in this study. However we hope that you will feel you are contributing to medical research in a way that will help children and families in the future.

What if something goes wrong?
If you feel you or your child have been harmed by taking part in this research and that the researchers have been negligent or at fault, then you may be able to make a legal claim for compensation to their employer. You might have to pay the legal costs of doing this. However, if you are harmed and the researchers are not at fault, there is no facility for you to make a claim. If you wish to complain or have any concerns about any aspect of the way you have been approached or treated during the course of this study, normal University or National Health Service complaints procedures should be available to you.

Are there any risks to myself or my child taking part in this study?
No, there are no known or likely risks.

Who has reviewed and approved the study?
A team of international experts on child development has reviewed this study for the Medical Research Council. The study has been reviewed and approved by the Research & Development committees of Wirral Hospitals NHS Trust, Wirral PCT and the Cheshire Local Research Ethics Committee.

Can I ask further questions?
When the research midwife meets you, at or after your 20 week scan appointment, she will be very happy to answer any questions you might have. In the meantime, if you would like any more information, please do not hesitate to contact Professor Jonathan Hill, Dr Helen Sharp, or Liz Green on the freephone number shown on the front page.

Appendix 2 (continued)- Participant information sheets, phase 6.

MANCHESTER 1824

The University of Manchester

Wirral University Teaching Hospital NHS
NHS Foundation Trust

UNIVERSITY OF LIVERPOOL

Study Base:
The Lauries Centre, 142 Claughton Road,
Birkenhead, Wirral, CH41 6EY
Freephone: 0800 051 7597
(from a mobile) 800 051 7597
Text: 07956 297412

Parent Information Sheet – Study 300

Title of study : The Wirral Child Health and Development Study

Investigators: Jonathan Hill, Helen Sharp, Andrew Pickles, Gill Lancaster
Research Staff: Karen Lunt, Carol Bedwell, Belinda Thompson, Julie Carlisle, Kate Marks, Nichaela Broyden, Kate Marshall, Florin Tibu, Carol Sadler, Jo Roberts, Jenny Lee, Liz Green

When you were pregnant, and again just after your baby was born you kindly helped us with a study that we are conducting designed to understand better how stress affects mothers to be, their partners and their babies, and how good experiences and support can make a difference. We are following 1500 women up to the first birthday of their babies mainly using questionnaires. In addition we are asking 300 to take part in interviews and to agree to us filming their babies during the first year of their life. You are one of the 300 that we would like to see again now that your baby is nearly 6 months old. Before you decide whether you want to take part, it is important for you to understand why the research is being done and what it will involve. Please take time to read the following information carefully and discuss it with others if you wish. Ask us if there is anything that is not clear or if you would like more information. Take time to decide whether you wish to take part. Thank you for reading this.

What is the study about?
The aim of the study is to find out about the effects of stress on parents and children during the antenatal period and in the first months after birth. We plan to measure each baby's development and how they interact with their mother in some detail. We believe that for some parents and children the effects are quite long lasting, and others find ways of coping. We want to understand these processes better so that services to support families experiencing stress can be improved. We are focussing on mothers for this detailed part of the study because most babies spend most time with their mother.

Who is being invited to take part?
The computer chooses the names of women who we approach based on the information they have given about how much stress they may be experiencing. Because we particularly want to understand about stress in pregnancy the computer is picking more women who are experiencing stress. Your name has been chosen either because you have indicated that you are dealing with quite a lot of stress or because you have said you are not facing a lot.

Do I have to take part?
It will be up to you to decide whether or not you would like to take part. If you agree, and change your mind later, you can withdraw from the study. This will not affect the care you receive.

How often will I be contacted?
Now that your baby is nearly 6 months old we would like to visit you and your baby at home and to come to our study centre for about 1½ hours, We will ask to see you again close to your baby's first birthday.

What will we have to do?

- We would like to see you and your baby once at home and once at the Study Centre. You will be with your baby at all times.
- We will talk with you about your feelings and experiences since the last visit, and ask you about your baby's usual behaviour. We will audio tape part of this talk.
- We would like to make a short video (about 20 minutes) of your baby playing with you .
- We will also make a video of how your baby responds to everyday events such as watching new things, the researcher talking and playing with them, hearing a loud noise or not being allowed to play with a toy for a short time.
- We will put three patches on your baby's back or chest to record your baby's heart while we are watching your baby.
- We will gather two saliva samples from your baby by wiping a cotton swab in his/her mouth at the start of the visit to the Study Centre and once again at the end. This is completely safe and will be used to measure your baby's stress hormones.

Will my expenses be paid?
We will be pleased to organise transport to the interview, or to pay for your transport. We are able to pay up to £30 to compensate you for time lost from home or work or any other expenses incurred from taking part in the study.

How will this information be used?

- We would like to make a video recording of your baby and you so that we go over what has happened in detail afterwards. The recording will be identified only by a number, so that information on it cannot be traced to you. The recording will be kept secure at the university base for up to ten years.
- All information that we receive from you will be treated as strictly confidential, under the guidelines of the Universities of Liverpool and Manchester, the UK Medical Research Council, and the Data Protection Act.
- Information on audio and video recordings, on paper records, and that we enter on to the computer will be identified only by a number. A list of names and addresses of participants and their case numbers will be kept separately and securely in the university base.
- We will report general findings about parents and children, and you or your child will never be identified. Reports will only be based on the ratings that we make from the interview and none of what you say will be reported.
- The only reason we might have to share information from the study with other people is if there are concerns about you or a child being at risk of serious harm. If that happens we will talk with you first to decide on the best way forward. Concerns like this would be addressed by seeking appropriate forms of help for you and following Trust Child Protection Guidelines.

Who is organising and funding the research study?
The study is being run by Professor Jonathan Hill of the University of Manchester and Dr Helen Sharp of the University of Liverpool. The research is funded by the Medical Research Council.

Are there any benefits in taking part in this study?
There are no benefits to your or your child's health in taking part in this study. However we hope that you will feel you are contributing to medical research in a way that will help children and families in the future.

What if something goes wrong?
If you feel you or your child have been harmed by taking part in this research and that the researchers have been negligent or at fault, then you may be able to make a legal claim for compensation to their employer. You might have to pay the legal costs of doing this. However, if you are harmed and the researchers are not at fault, there is no facility for you to make a claim. If you wish to complain or have any concerns about any aspect of the way you have been approached or treated during the course of this study, normal University or National Health Service complaints procedures should be available to you.

Are there any risks to myself or my child taking part in this study?
No, there are no known or likely risks.

Who has reviewed and approved the study?
A team of international experts on child development has reviewed this study for the Medical Research Council. The study has been reviewed and approved by the Research & Development committees of Wirral Hospitals NHS Trust, Wirral Primary Care Trust and the Cheshire Local Research Ethics Committee.

Can I ask further questions?
When the researcher meets you they will be very happy to answer any questions you might have. In the meantime, if you would like any more information, please do not hesitate to contact Professor Jonathan Hill, Dr Helen Sharp, or Liz Green on the freephone number shown on the front page.

Appendix 2 (continued)- Participant information sheets, phase 9 and 10

The University of Manchester

Study Base:
The Lauries Centre,
142 Claughton Road
Birkenhead, Wirral,
CH41 6EY
Freephone: 0800 051 7597
(from a mobile) 800 051 7597
Text: 07956 297412

Participant Information Sheet

Title of study : The Wirral Child Health and Development Study 1-4 years

Investigators: Jonathan Hill, Helen Sharp, Andrew Pickles, John Quinn, Vivette Glover. Research Staff: Liz Green, Niki Sandman, Kate Marshall, Helen Jones, Louise Fisher, Stuart Kehl, Fay Huntley, Nicky Wright.

When you were pregnant, just after your baby was born and when your baby was 12 months old you kindly helped us with this research study. We are now inviting you to take part in this study until your first child is just over 4 years old.

Before you decide whether you want to take part in the next stages of the study, it is important for you to understand why the research is continuing and what it will involve for you and your child. Please take time to read the following information carefully and discuss it with others if you wish. Ask us if there is anything that is not clear or if you would like more information. Take time to decide whether you wish to take part. Thank you for reading this.

What is the purpose of the study?
This study aims to find out how children learn how to behave with other people, and why some children have difficulties controlling their behaviours. To do this we need to measure many aspects of their early development, their experiences, and the ways parents take care of them. We are interested to find out more about the ways that early life stress influences later development as we know that for some parents and children the effects are quite long lasting, and others find ways of coping. Every child is a unique individual and that is partly due to the genes that have been passed on from each parent and partly due to individual life experiences. Genes are like maps inside our bodies that hold information. We now also know that health and behaviour are influenced by genes. This information in our genes is stored in 'DNA', which can be found in our skin cells and saliva. We now also know that genes only influence development when they are switched on. We can tell whether genes are switched on or off at a particular time point from something called 'RNA' which can also be found in our skin cells or saliva. In this study we want to find out more about how genes and different life experiences influence parent's and children's behaviours and development so that NHS services that support families can be improved with this knowledge.

Why have I been invited to take part?
At the time when you were expecting your first child, we approached all women who were booked into the antenatal clinic at Arrowe Park Hospital for their antenatal care over a two year period. During this time we recruited 1286 mothers who were experiencing low, medium or high levels of stress in pregnancy. We asked all mothers reporting higher levels of stress and a sub-group of mothers chosen by the computer at random who were reporting lower levels of stress, to take part in an 'intensive' part of the study. You were one of these mothers and we would like now to follow your child's development up to four years of age if you are happy for us to do so.

NHS Western Cheshire

NHS Wirral

Do I have to take part?
It will be up to you to decide whether or not you would like to take part. If you agree, and change your mind later, you can withdraw from the study. This will not affect the care you receive. If you choose to take part and you find you do not wish to complete a particular assessment or answer a particular question, you will be free to miss that part out but carry on with the rest of the study if you wish or you can just choose to stop that assessment completely.

What will happen to me if I take part ?
We would like to meet with you and your baby when he or she is two, three and four years of age to complete a range of adult interviews, mother-child assessments in the study base, questionnaire packs and a home visit. If you decide to take part we will write to your GP and your child's health visitor to inform them you have agreed to do so.

What will we have to do?

When your child is 2 and 3 years old

Adult interview

- We will talk in detail with you about your feelings, relationships, supports and life experiences since the last visit and audio record our conversation. These interviews will be similar to the ones you have completed on previous occasions.
- Now your child is a toddler we will ask in detail about their development and health including illnesses, accidents, injuries and treatments received since birth. We will ask about your parenting practices including childcare arrangements and daily activities with your child.
- This interview will be done at The Lauries or at home, whichever you prefer.

Mother and child visit to The Lauries

- We would like to see you and your child at the Lauries Centre for half a day.
- We will ask you about your child's behaviours and emotions. For example we will ask what makes him/her anxious, or angry, or happy, and what he/she likes to do with you. We will audio record this conversation also.
- We would like to make a short video (about 15 minutes) of your infant playing with you with some toys and tidying them away at the end. We will be looking at your infant's behaviours during this play time together and the different parenting skills you use.
- We would like to make a DVD of how your baby responds to everyday events such as playing with various toys, exploring a new room with several odd-looking toys or not being allowed to play with a toy for a short time. You will be given a copy of the DVD to keep.
- We would like to find out about your child's development by giving him/her some puzzles to solve.
- We will put three patches on your infant's back (just as we did when your infant was younger) to record your baby's heart during these games and puzzles and a conversation with you. We will make a DVD recording of this too.
- We are also going to see whether some infants are more likely to produce the kinds of hormones that help them to deal with challenging situations. To do this, we will collect some of his/her saliva wiping a set of very small soft sponges in their mouth for 90 seconds at a time. We will blow bubbles while this is done to keep them happy. These soft sponges are smaller than the cotton dental roll we used last time when your baby was one year old. This is a new safe system for infants, and will not produce any allergic reactions. Once we have collected a sample of your infant's saliva it can then be analysed to measure the hormones. We would like to do this four times, once before, and once after exploring the new room with odd looking toys in it, and once before and once after a conversation task with you.
- We would also like to collect skin cells with saliva from your baby's mouth to assess gene activity (RNA analysis) at each time point by briefly rubbing small cotton buds on the inside of your infants cheeks.
- We wish to collect a sample of your own skin cells with saliva from the inside of your mouth for DNA analysis by briefly rubbing small cotton buds on the inside of your cheeks. This is so we can investigate possible links between maternal genes and parenting behaviours.
- We will weigh your child and measure their height, upper arm and head size.

At 3 years only

- We will show your child some photographs of faces (like we did with you when you were pregnant) and ask him/her to say which emotion it is.
- We would also like to visit you about an hour both to see how your child learns and gets along with you at home.

At 4 years only

- We will talk in detail with you about your feelings, relationships, supports and life experiences since the last visit and audio record our conversation. These interviews will be similar to the ones you have completed on previous occasions.
- Now your child will soon start school we will ask in detail about their development and health including illnesses, accidents, injuries and treatments received over the past year. We will ask about your parenting practices including childcare arrangements and daily activities with your child.
- We will ask you in detail about your child's emotions and behaviours

We wish to follow the families in the study for a long time as their children grow up and so if we get funding to do this we may ask you later to consider being in the study for longer. But for now, we are asking you to take part in this study over a three year period from when your child is about 2 years old until they are about 4 ½ years old. Now your child is a toddler we are hoping to study parenting and child behaviours in greater detail than was possible in the earlier stages of your baby's life. At each stage of the study, specially trained research assistants will complete the study interviews or assessments with you and your baby.

Expenses and payments

We are able to give you £40 in high street shopping vouchers each time you complete a yearly assessment. This is to compensate you for time lost from home or work and any other expenses incurred from taking part in the study.

Will my taking part in the study be kept confidential?

- Information on DVD recordings, on audio recordings of interviews with you, and on paper questionnaire records and any information we enter on computers about you will be identified only by a case number. A computer database and paper copies of participant names and addresses and contact details and their case numbers will be kept separately and securely in the university study base so no-one outside of the research team can access this or identify you or your child. All the information you give us is therefore 'pseudoanonymised' which means that it is identified ONLY by a case number and ONLY the research team will be able to link your case number to who you are and the other contact information you give us.
- We would like to make DVD recordings of your baby and you so that we go over what happened in detail afterwards. The recordings will be identified only by a case number, so that information on it cannot be traced to you by anyone outside of the research team. A copy of the recording will be kept securely at each university base for up to thirty years.
- The genetic samples will be analysed pseudo-anonymously too. This means that no records will be generated that directly link your name, your partner's name, or your child's name to the genetic samples. Instead, they will be linked only by the case number. So only the research team will know who the samples belong to. We will analyse the samples for genes that affect infants' health, emotions and behaviour, and not for any other purpose. They will not be kept as part of your medical record. All samples will be destroyed after 20 years. The pseudo-anonymous samples will be analysed by a laboratory technician who is not affiliated with the study, and will have no access to your name, your partner's name, or your child's name.
- All information that we receive from you will be treated as strictly confidential, under the guidelines of the Universities of Liverpool and Manchester, the UK Medical Research Council, and the Data Protection Act. This means that your information will only used by members of the research team and scientific research collaborators from other academic institutions approved by us.
- We will report general research findings about parents and children, and you or your child will never be identified. Reports will be based on the ratings that we make from the interviews, questionnaires or DVD recordings and on occasions when examples of individual responses are reported these will be pseudo-anonymised.
- The only reason we might have to share information from the study with other people is if there are concerns about you or a child being at risk of serious harm. If that happens we will talk with you first to decide on the best way forward. Concerns like this would be addressed by seeking appropriate forms of help for you and by following Trust Child Protection Guidelines.

What will happen to the results of the research study?
We will publish the results of this study in academic journals, at international and national conferences and we will inform study participants of key findings in a study newsletter sent to your home. We also plan to develop a study website where results will be displayed.

What will happen when the research study stops?
When this part of the research comes to an end we hope to secure further funding to continue studying all the families and the children as they grow up through the school years. We would of course ask your permission to do this at a later date.

What are the possible benefits to taking part?
There are no benefits to you or your child's health in taking part in this study. However we hope that you will feel you are contributing to medical research in a way that will help children and families in the future.

What are the possible disadvantages and risks to myself or my child taking part in this study?
No, there are no known or likely risks. It is possible that you may become upset when recalling difficult experiences in your life. If this occurs the interviewer will ask you if you wish to take a break from interviewing or continue. You may also choose to stop the interview completely at any time.

Who is organising and funding the research ?
The study is being led jointly by Professor Jonathan Hill of the University of Manchester and Dr Helen Sharp of the University of Liverpool. The research is funded by the Medical Research Council.

Who has reviewed and approved the study?
A team of international experts on child development has reviewed this study for the Medical Research Council. The study has been reviewed and approved by the Research & Development committees of Wirral Primary Care Trust and Western Cheshire Primary Care Trust and the Northwest 5 Haydock Research Ethics Committee.

What if there is a problem?
Complaints
If you have a concern about any aspect of this study, you should ask to speak to the researchers who will do their best to answer your questions. If they are unable to resolve your concern or you wish to make a complaint regarding the study, please contact a University Research Practice and Governance Co-ordinator on 0161 2757583 or 0161 2758093 or by email to research-governance@manchester.ac.uk.

Harm
In the event that something does go wrong and you or your child are harmed during the research you may have grounds for a legal action for compensation against The University of Manchester and The University of Liverpool but you may have to pay your legal costs. The normal National Health Service complaints mechanisms will still be available to you.

The University of Manchester has cover for no fault compensation for bodily injury, mental injury or death where the injury resulted from a trial or procedure you received as part of the trial. This would be subject to policy terms and conditions. Any payment would be without legal commitment. (Please ask if you wish more information on this). The University would not be bound to pay this compensation where the injury resulted from a drug or procedure outside the trial protocol or the protocol was not followed.

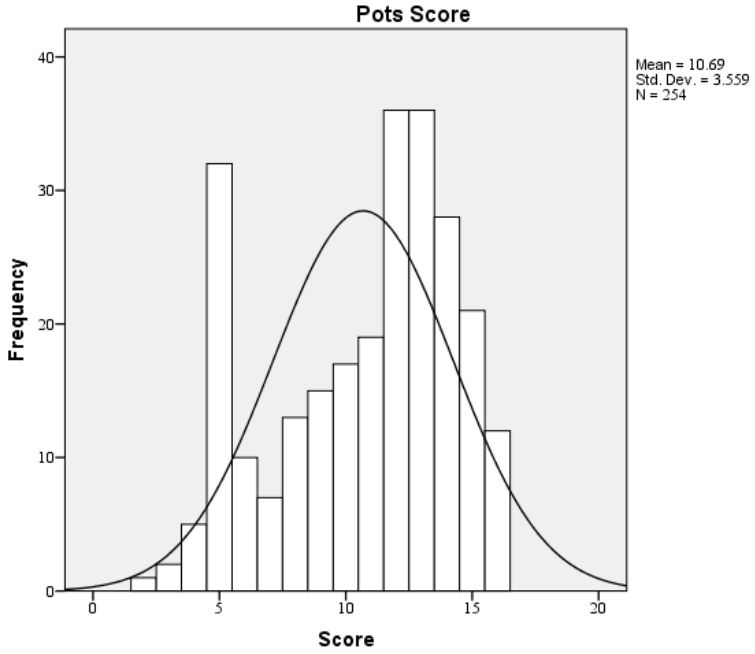
Further information and contact details
When the researcher meets you they will be very happy to answer any questions you might have. In the meantime, if you would like any more information, please do not hesitate to contact Professor Jonathan Hill, Dr Helen Sharp or Liz Green / Niki Sandman (study administrators) on the freephone number shown on the front page.

Appendix 3 – Skewness and Kurtosis for distributions of EF indicator scores.

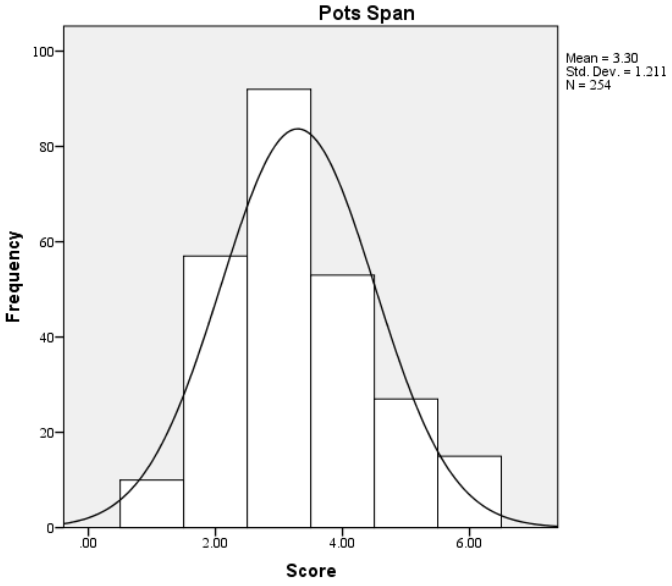
EF Indicator	Skewness	Standard Error	Kurtosis	Standard Error
Pots Score	-0.50	.15	-0.91	.30
Pots Span	0.45	.15	-0.24	.30
Pots Perseverative Errors	-3.30	.15	16.21	.30
Stroop 1 to 4	0.71	.16	-0.91	.32
Stroop 5 to 8	0.44	.20	-0.64	.40
Stroop 9 to 12	0.41	.20	-0.60	.40
Snack Delay Average 1	-1.46	.16	1.51	.32
Snack Delay Average 2	-1.05	.16	0.21	.31
Snack Delay Average 3	-0.79	.16	-0.35	.33
Whisper Average score	0.11	.16	-1.63	.31
Detour Knob score	-0.14	.16	-1.87	.31
Detour Switch score	0.61	.16	-0.77	.32
Working memory factor score	-0.70	.17	0.95	.33
Inhibitory control factor score	-0.52	.17	0.02	.33

Appendix 4 – Histograms displaying distributions of scores for EF indicator scores

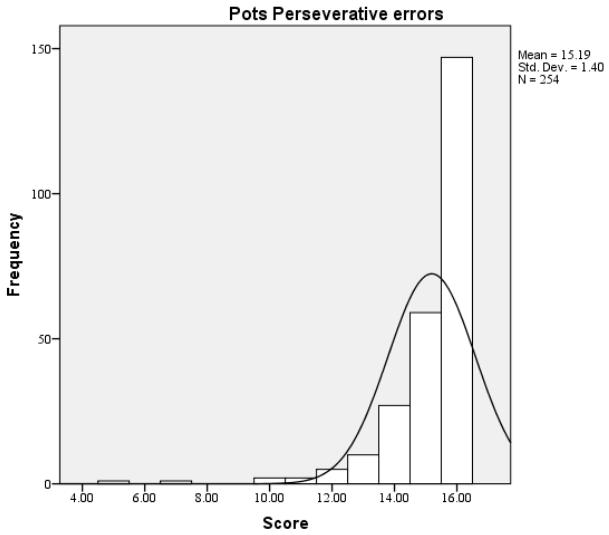
Pots Score.



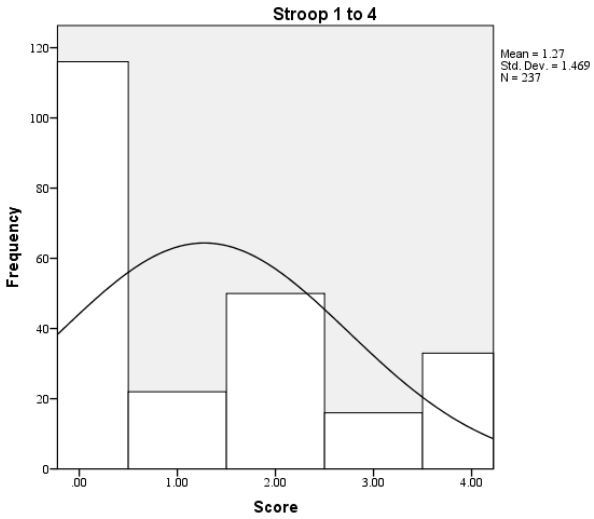
Pots Span.



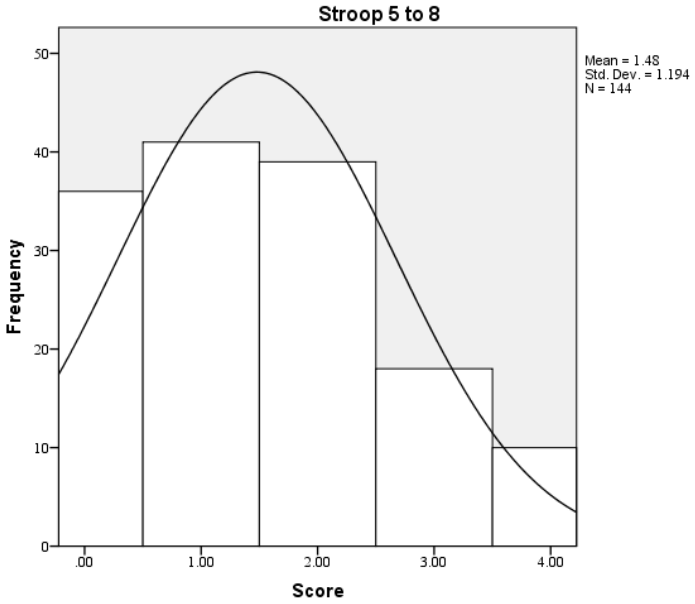
Pots Perseverative Errors.



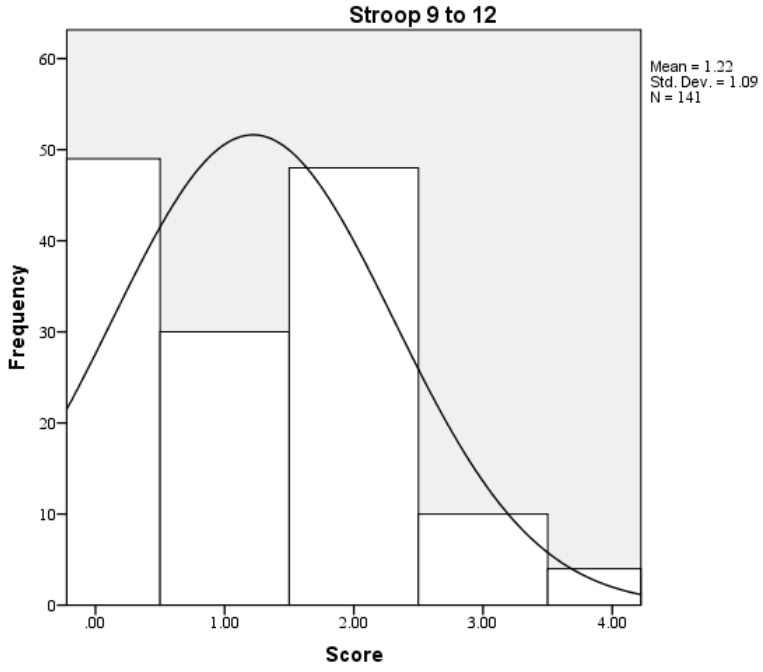
Stroop 1 to 4



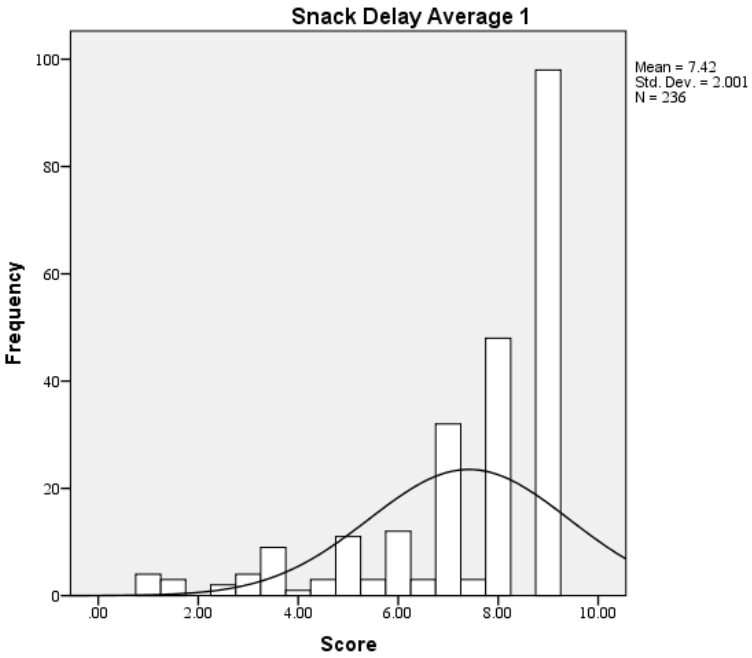
Stroop 5 to 8



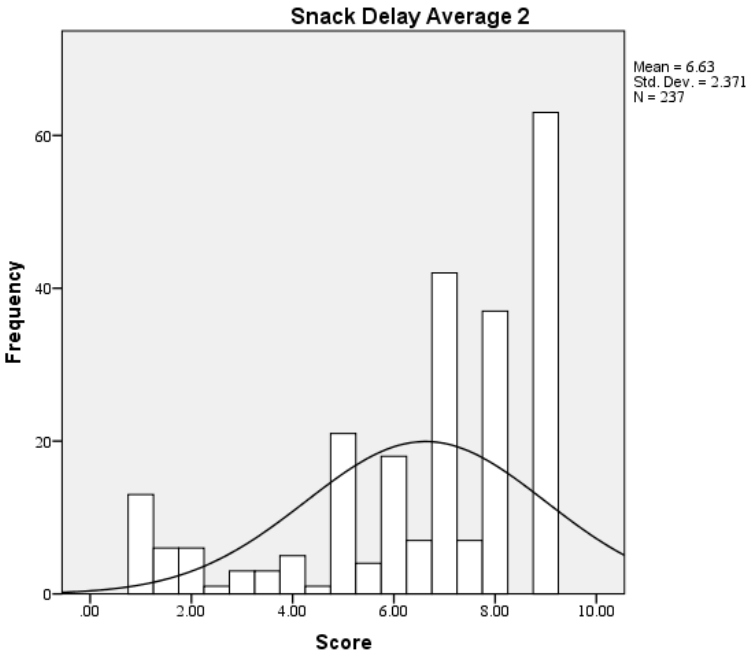
Stroop 9 to 12



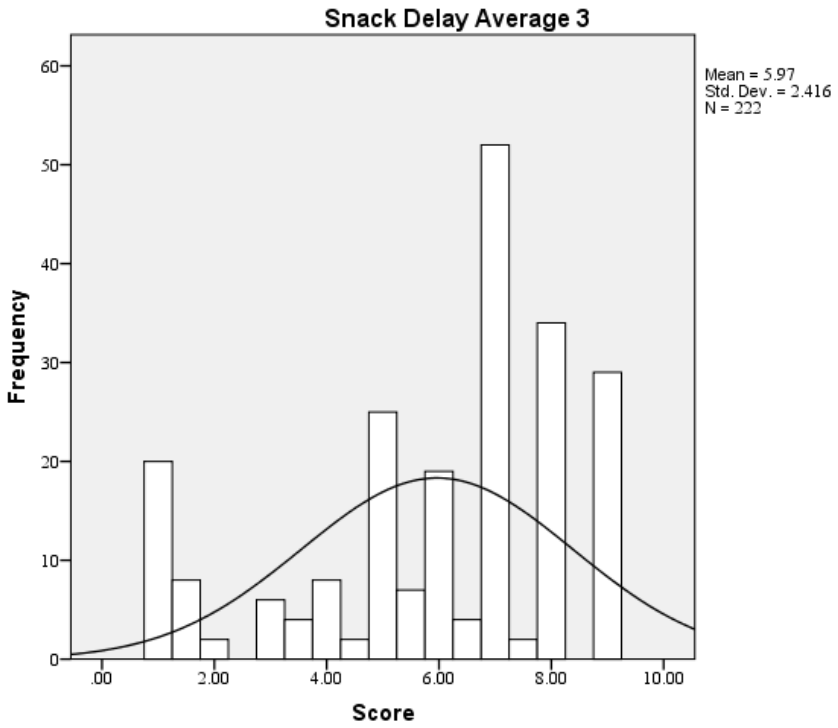
Snack Delay Average 1



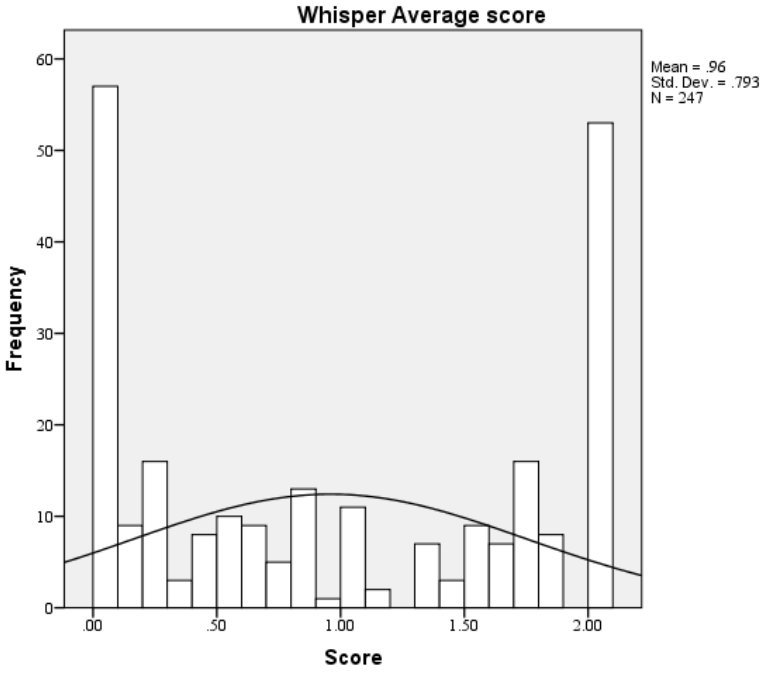
Snack Delay Average 2



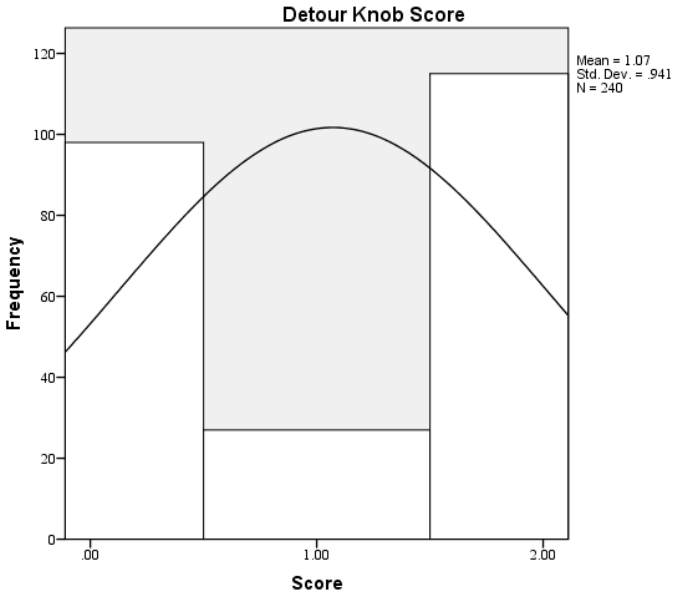
Snack Delay Average 3



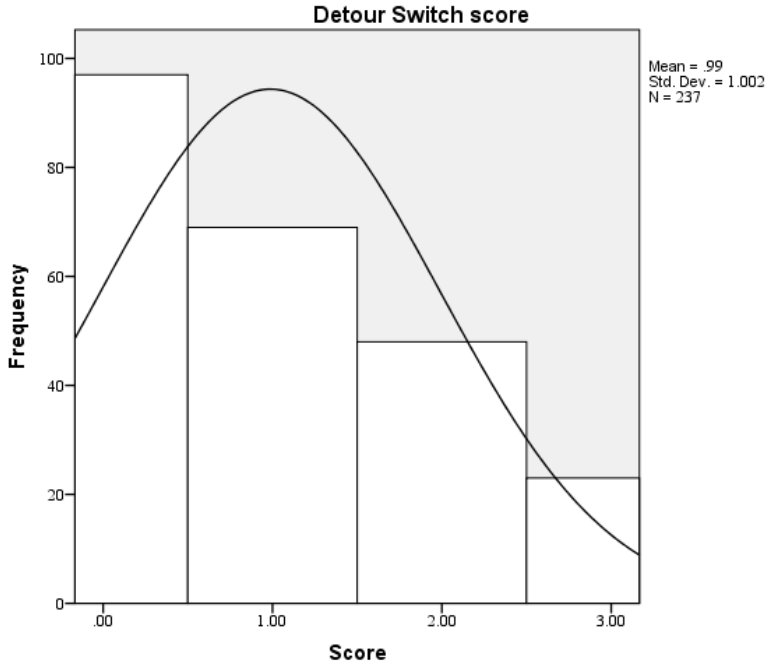
Whisper Average score



Detour Knob score



Detour Switch score



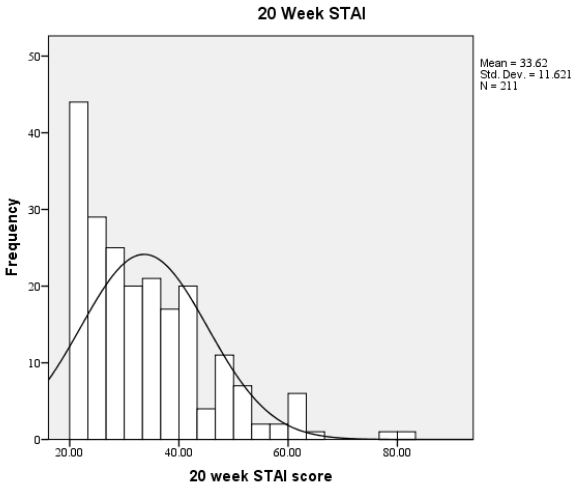
Appendix 4 - Descriptive statistics and Frequency distributions of variables of interest in the regression models predicting toddler EF from PNS variables.

Prenatal stress Predictor variables:

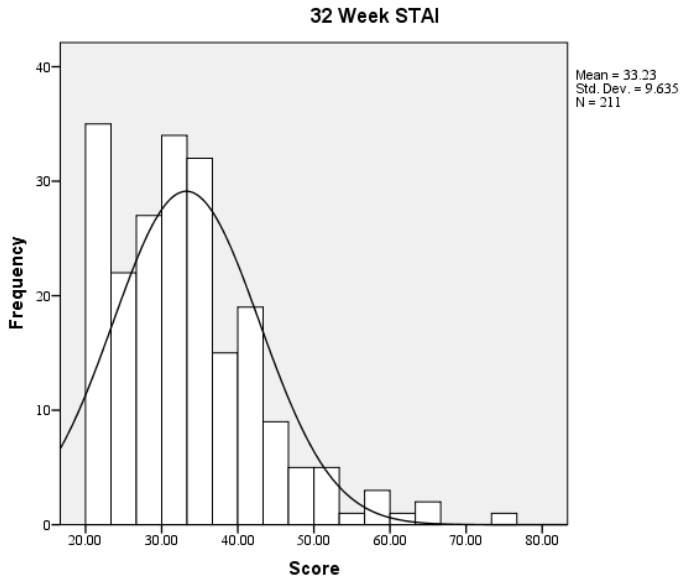
Descriptive statistics for 20 week and 32 week STAI scores.

		20 week STAI	32 Week STAI
N		211	211
Mean		33.62	33.23
SD		11.62	9.63
Variance		135.05	92.82
Range		60.00	56.00
Minimum		20.00	20.00
Maximum		80.00	76.00
Skewness		1.16	1.18
	Std. Error	.17	.17
Kurtosis		1.38	2.11
	Std. Error	.33	.33

Histogram displaying distribution of 20 week STAI scores



Histogram displaying distribution of 32 week STAI scores

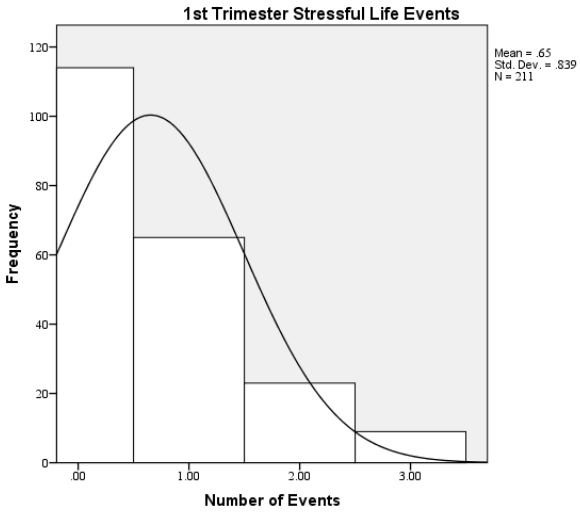


Frequency distributions: Stressful Life Events: 1st trimester, 2nd trimester, 3rd trimester.

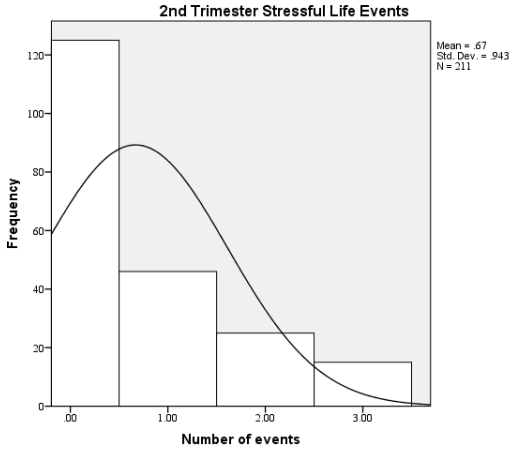
N Stressful	1st trimester		2nd trimester		3rd trimester	
Life events	Frequency	Percent	Frequency	Percent	Frequency	Percent
0	114	54.	125	59	123	58
1	65	31	46	22	61	29
2	23	11	25	12	15	7
3 or more	9	4	15	7	12	6
Total	211	100.0	211	100.0	211	100.0
Skewness	1.17	-	1.22	-	1.43	-
S. E.$_{skew}$	.17	-	.17	-	.17	-
Kurtosis	.62	-	.35	-	1.34	-
S.E. $_{kurtosis}$	.33	-	.33	-	.333	-

Histograms displaying frequency distributions of Stressful Life Events: 1st trimester, 2nd trimester, 3rd trimester.

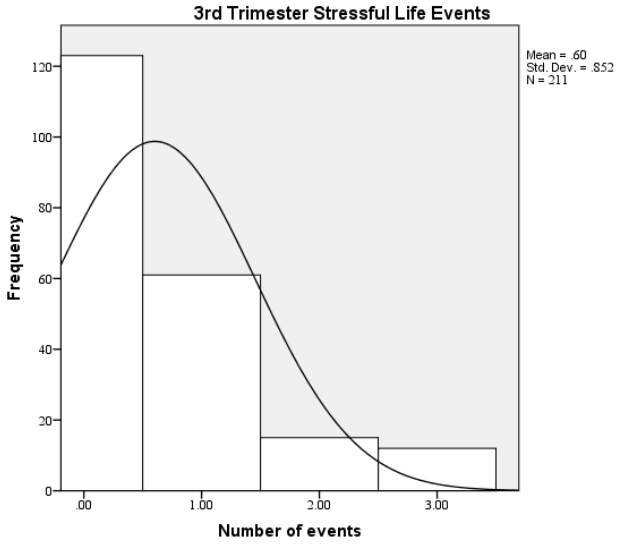
1st trimester Stressful Life Events.



2nd trimester Stressful Life Events.



3rd trimester Stressful Life Events.



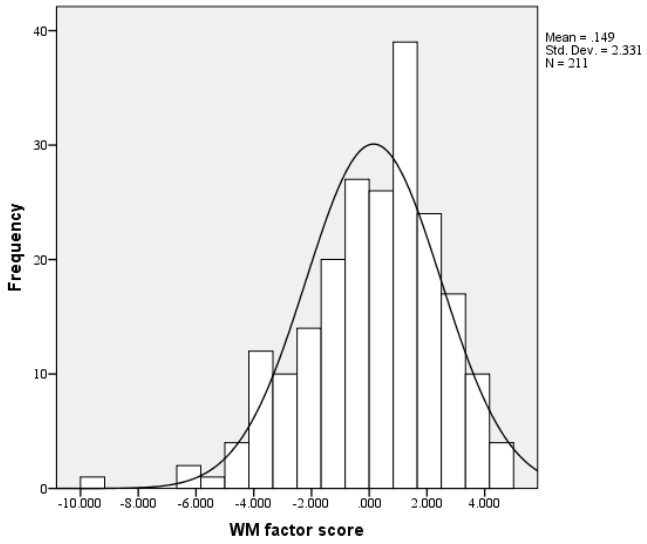
Executive Function Outcome measures: Working memory (WM) factor score and Inhibitory control (IC) factor score.

Descriptive statistics

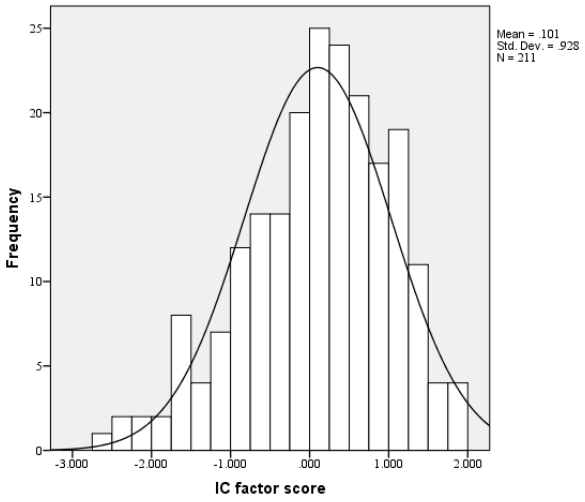
		WM factor score	IC factor score
N		211	211
Mean		.15	.10
SD		2.33	.93
Variance		5.4	.87
Range		14.12	4.67
Minimum		-9.80	-2.68
Maximum		4.39	2.00
Skewness		-.70	-.52
	Std. Error of	.17	.17
Kurtosis		.95	.02
	Std. Error	.33	.33

Histograms displaying frequency distributions of EF outcome measures: Working memory and inhibitory control factor scores.

Working memory factor scores



Inhibitory control factor scores

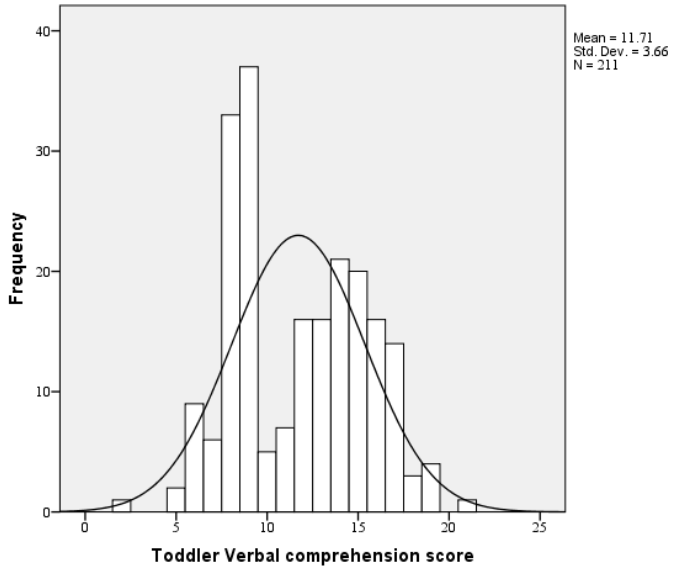


Covariates and confounders:

Toddler Verbal comprehension score: Descriptive statistics.

	Verbal comprehension score
N	211
Mean	11.71
Std. Deviation	3.66
Variance	13.40
Range	19
Minimum	2
Maximum	21
Skewness	.11
Std. Error	.17
Kurtosis	-.95
Std. Error	.33

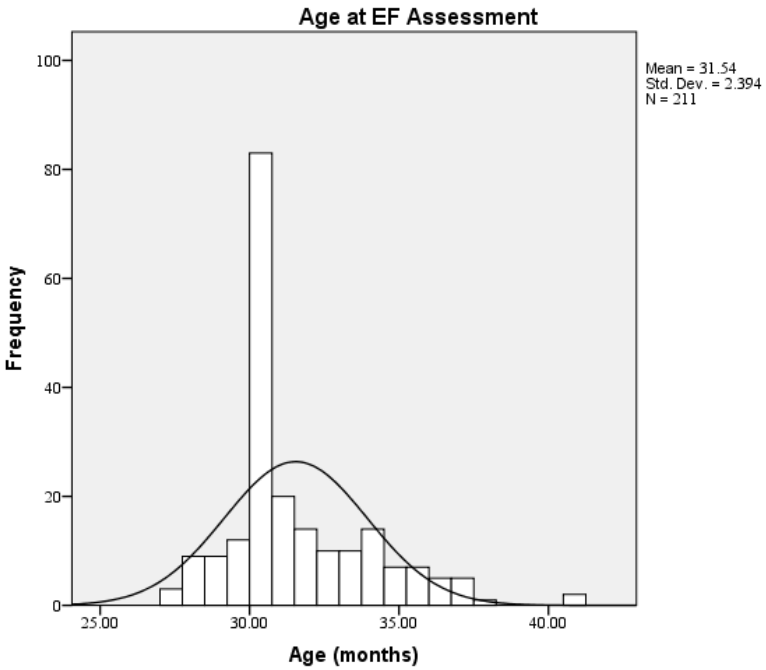
Histogram displaying the frequebcy distribution of toddler verbal comprehension scores



Toddler age at time of EF assessment (months): Descriptive statistics.

	Age EF assessment
N	211
Mean	31.54
Std. Deviation	2.39
Variance	5.73
Range	13.96
Minimum	27.27
Maximum	41.23
Skewness	1.22
Std. Error	.17
Kurtosis	1.71
Std. Error	.33

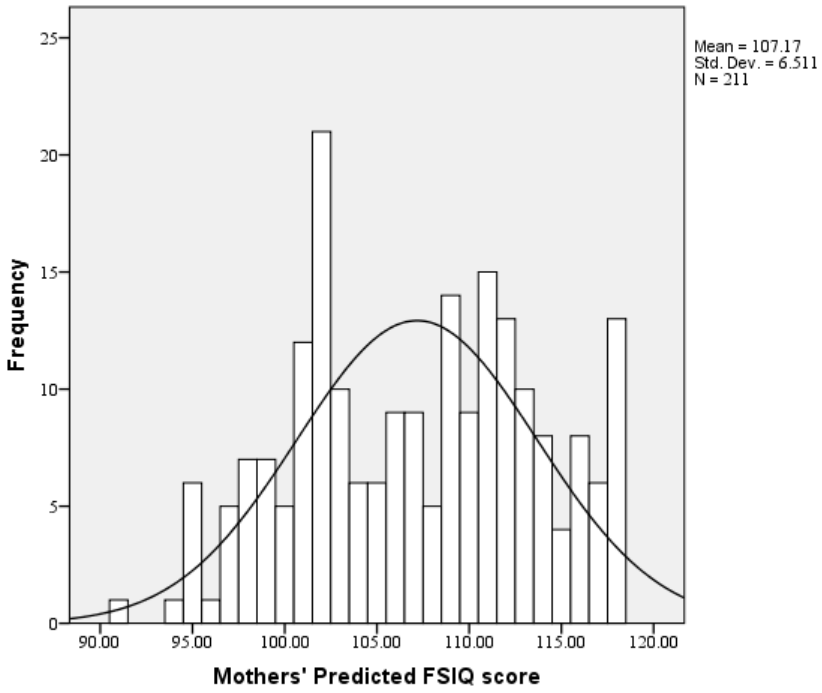
Histogram displaying frequency distributions of toddler age at time of EF assessment



Maternal IQ: WTAR Predicted FSIQ: Descriptive statistics

	Mothers' predicted FSIQ score
N	211
Mean	107.17
SD	6.51
Variance	42.40
Range	27.00
Minimum	91.00
Maximum	118.00
Skewness	-.09
Std. Error	.17
Kurtosis	-.95
Std. Error	.33

Histogram displaying frequency distribution of Mothers' predicted FSIQ scores



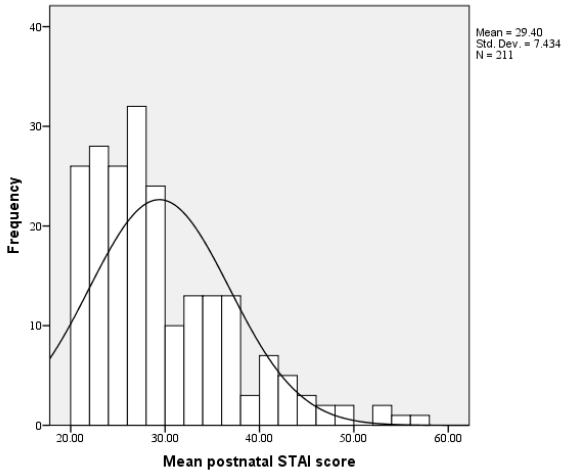
Postnatal stress.

Postnatal STAI score (Mean score across administrations at child age 4 - 8 weeks, 6 months, 12 months old and 2 ½ years) and Postnatal Stressful Life Events (Mean number of events per 12-week period in the postnatal period)

Descriptive statistics.

	Postnatal STAI	Postnatal SLEs
N	211	211
Range	36.48	2.03
Minimum	20.00	0
Maximum	56.48	2.03
Mean	29.40	0.5
Std. Deviation	7.43	0.40
Variance	55.26	0.16
Skewness	1.19	1.31
Std. Error	.17	.17
Kurtosis	1.25	2.01
Std. Error	.33	.33

Histogram displaying frequency distribution of Mean Postnatal STAI scores



Histogram displaying Frequency distribution of Postnatal Stressful Life Events (Mean number of events per 12-week period in the postnatal period).