THE LONG-TERM NEUROPHYSIOLOGICAL EFFECTS OF PRENATAL NICOTINE EXPOSURE ON EXECUTIVE FUNCTIONING: AN fMRI STUDY OF YOUNG ADULTS

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For my mother and father

Thank you for providing me with the most secure of bases from which I was able to follow my dreams

Abstract

Maternal smoking during pregnancy has often been associated with numerous adverse outcomes for the offspring. However, its long-term effects are not well established. Given the high prevalence of maternal smoking during pregnancy, an understanding of these effects is essential. Therefore, the aim of the present dissertation was to shed light on the long-term neurophysiological effects of prenatal nicotine exposure on three different executive functioning processes by assessing participants in young adulthood, using functional magnetic resonance imaging (fMRI). Participants imaged were members of the Ottawa Prenatal Prospective Study, a longitudinal study that collected a unique body of information on participants from infancy to young adulthood, which allowed for the measurement of an unprecedented number of potentially confounding drug exposure variables. The dissertation consists of three separate original manuscripts. In manuscript 1, participants completed a response inhibition task, in manuscript 2 participants completed a verbal working memory task and in manuscript 3 participants completed a visuospatial working memory task. Taken together, results from all three manuscripts showed that prenatal nicotine exposure leads to altered neural functioning during executive functioning processing that continues into young adulthood. These significant results highlight the need for education about the repercussions of women smoking during pregnancy.

Statement of Co-Authorship

The three manuscripts included in this dissertation were prepared in collaboration with my dissertation supervisor. Included as co-authors are Dr. Peter Fried, as his original participants were recruited in the study and Dr. Ian Cameron, who helped with MRI settings. As the primary author on all manuscripts, I was responsible for the conceptualization of the research question and methods, planning and execution of statistical analyses, and preparation of manuscripts. Dr. Smith provided guidance and assistance in all aspects of the project.

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Chapter 1

General Introduction

The Long-Term Neurophysiological Effects of Prenatal Nicotine Exposure on Executive Functioning: An fMRI Study of Young Adults

Prenatal nicotine exposure via maternal smoking is one of the most modifiable developmental neurotoxins. Despite continued public awareness campaigns and education about the effects of maternal smoking on the offspring, a large proportion of women worldwide continue to smoke while pregnant. In the United States, according to data from the 2008 Pregnancy Risk Assessment and Monitoring System, approximately 13% of women reported regularly smoking cigarettes while pregnant (Centre for Disease Control and Prevention, 2008). In Europe, an estimated 6-22% of women report smoking cigarettes during pregnancy (European Perinatal Health Report, 2008). Although cigarette smoke contains thousands of harmful chemicals, nicotine is the leading candidate responsible for causing perinatal damage (Slotkin, Greer, Faust, Cho, & Seidler, 1986; Slotkin, 2008). This is largely because nicotine acts on nicotinic acetylcholine receptors (nAChRs) which are expressed in the brain at a very early stage of development (Hellström-Lindahl, Gorbounova, Seiger, Mousavi, & Nordberg, 1998). These receptors, when activated by acetylcholine play a critical role in many aspects of brain development (for review see Dwyer, McQuown, & Leslie, 2009). As a result, premature stimulation of nAChRs by nicotine is believed to disrupt the timing of the trophic effects of acetylcholine, leading to profound effects on neurodevelopment (for review see Slotkin, 2004).

Nicotinic Acetylcholine Receptors

Prenatal nicotine exposure effects brain development by mimicking the neurotransmitter acetylcholine and binding to nAChRs (for review see Pauly & Slotkin, 2008). nAChRs are ligand-gated ion channels that are found throughout the central nervous system of both humans and rodents with binding sites for acetylcholine and nicotine (for review see Gotti, Moretti,

Gaimarri, Zanardi, Clementi, & Zoli, 2007). nAChRs occupy three main interconvertable states: open, closed and desensitized. When acetylcholine or other ligands bind to nAChRs, it causes the channel to open. It is during the open state of the channel that positively charged ions such as sodium, potassium and calcium flow in. A few milliseconds later, the receptor closes. Continuous exposure to acetylcholine or other ligands stabilizes the desensitized state, which renders the receptor unresponsive to the ligand and will remain closed (for review see Dani & Bertrand, 2007).

nAChRs consist of alpha and beta subunits which come together to form a pentamer around a central ion channel (for review see Gotti & Clementi, 2004). Nine different alpha subunits, designated alpha 2 to alpha 10, and three different beta subunits, designated beta 2 to beta 4, have been identified thus far. These subunits combine in either a homomeric or heteromeric configuration. In the homomeric configuration, the nAChR pentamer is made up of only alpha subunits including alpha 7, alpha 8 or alpha 9. In the heteromeric configuration, the nAChR pentamer is typically made up of three beta subunits with two alpha subunits. The alpha subunits that combine with beta subunits to form heteromeric nAChRs include alpha 2 to 6 with beta 2 to 4. It is the composition of subunits that determine the affinity the receptor has for specific ligands, the ions which gate it and the desensitization characteristics (for review see Gotti et al., 2007).

The two most common nAChR subtypes expressed in the central nervous system are heteromeric alpha 4 beta 2 receptors and homomeric alpha 7 receptors (for review see Jones, Sudweeks, & Yakel, 1999). Alpha 4 beta 2 nAChRs have a high affinity for nicotine and acetylcholine, and are permeable to sodium. In contrast, alpha 7 nAChRs have a low affinity for

nicotine and acetylcholine and are permeable to sodium and calcium (for review sees Dani & Bertrand, 2007).

nAChRs are detected in the brains of humans and rodents early in development (Agulhon et al., 1998; Hellström-Lindahl et al., 1998; Zoli, Le Novère, Hill, & Changeux, 1995). In humans, the alpha 4 beta 2 nAChRs first appear in the brainstem at six weeks of development and in the cortex at eight weeks of conception (Hellström-Lindahl et al., 1998). The alpha 7 nAChRs are first found in the brainstem at five weeks and then in the cortex at nine weeks of development (Falk, Nordberg, Spieger, Kjaeldgaard & Hellström-Lindahl, 2002; Hellström-Lindahl et al., 1998; Hellström-Lindahl & Court, 2000). Within these regions, nAChRs are found in several locations including dendrites, cell bodies, axons, as well as presynaptically and postsynaptically (for review see Dani & Bertrand, 2007).

nAChRs are expressed early in the developing brain because when activated by acetylcholine they play a critical role in brain development (for review see Dwyer, McQuown, & Leslie, 2009). In addition, they are the primary targets for nicotine in the brain, thereby providing a window of vulnerability for prenatal nicotine exposure to disrupt key neurodevelopmental processes.

Function of Acetylcholine in Brain Development

Acetylcholine is a major excitatory neurotransmitter in the central nervous system that is synthesized, stored and released by cholinergic neurons (for reviews see Gotti & Clementi, 2004; Jones, Sudweeks, & Yakel, 1999). Acetylcholine, acts on nAChRs to govern various processes in the brain including learning and memory (Atri et al., 2004), executive functioning (Ellis et al., 2006; Green et al., 2005) and mood (Mineur & Picciotto, 2010). Acetylcholine, like other neurotransmitters, also plays a critical role during brain development influencing early cellular

processes including neural cell proliferation, migration, differentiation, apoptosis and synaptogenesis (for review see Slotkin, 2004). Thus, excessive cholinergic stimulation at inappropriate times by nicotine during gestation is believed to disrupt the timing of the trophic actions of acetylcholine (for review see Slotkin, 2004).

In addition, early in development, functional nAChRS are expressed on other developing neurotransmitter neurons (Azam, Chen, & Leslie, 2007; Leslie, Gallardo, & Park, 2002). When these nAChRs are located presynaptically and are activated by acetylcholine, it induces the release of a number of neurotransmitters including dopamine, norepinephrine, serotonin, acetylcholine and glutamate (for review see Gotti et al., 2007). This neurotransmitter release is crucial for the maturation of several neurotransmitter pathways (for review see Dwyer, McQuown, & Leslie, 2009). For example, dopaminergic neurons first appear in the rat brain in the substantia nigra and the ventral tegmental area around 12 to 15 days of gestation (Specht et al., 1981; Marchard & Poirier, 1983). Shortly thereafter, these neurons express alpha 4 and beta 2 nAChRs subunit mRNAs on their cell bodies, and functional receptors are detectable as early as 17 to 18 days of gestation, where they regulate dopamine release from fetal striatal terminals (Azam, Chen, & Leslie, 2007). This dopamine release is crucial for the maturation of the dopamine pathway, as Jung & Bennett (1996) found that mRNAs for D1 and D2 dopamine receptors, which are abundant in the striatum during early development, are not converted into functional proteins until they are stimulated by dopaminergic midbrain neurons. Other developing neurotransmitter neurons including norepinephrine also express nAChRS on their cell bodies and nerve terminals early in development and these receptors have been shown to help mediate norephinephrine release (O'Leary & Leslie, 2006). In addition, spontaneous cholinergic activity is responsible for terminating GABAergic excitation and promoting

inhibition. This event, which is time sensitive, is critical for the maturation of the GABA system (Liu, Neff, & Berg, 2006).

Thus, premature nAChR-induced release of these neurotransmitters by prenatal nicotine exposure will also disrupt development mediated by other neurotransmitters, resulting in dysfunction of numerous pathways and systems within the central nervous system (Navarro, Seidler, Whitmore, & Slotkin, 1988; Muneoka, Nakatsu, Fuji, Ogawa, & Takigawa, 1999; Ribary & Lichtensteiger, 1989; for review see Slotkin, 2004).

The Effects of Prenatal Nicotine Exposure on the Animal Brain

An overwhelmingly large body of animal research has shown that nicotine is a neuroteratogen. Prenatal nicotine exposure is associated with changes in brain morphology. In particular, Roy, Seidler, & Slotkin (2002) found that rats exposed to nicotine in utero exhibited profound structural changes including decreased cell size within the hippocampal region, the dentate gyrus and the somatosensory cortex. These results are consistent with Muhammad and colleagues (2012) who found that rats prenatally exposed to nicotine exhibited altered dendritic morphology within the prefrontal cortex, the parietal cortex and the nucleus accumbens.

Neurochemical changes are also evident as a result of developmental nicotine exposure. Rats prenatally exposed to nicotine via maternal infusions throughout gestation show persistent elevated levels of ornithine decarboxylase activity postnatally, a biomarker typically associated with cell damage and cell death (Slotkin, Greer, Faust, Cho, & Seidler, 1986). Reduced DNA content in the cerebellum and the cerebral cortex has also been found among rats with gestational nicotine exposure, indicative of a decrease in the total number of brain cells (Slotkin, Orband-Miller, Queen, Whitmore, & Seidler, 1987). In addition, persistent elevations of c-fos gene expression in brain regions of animals prenatally exposed to nicotine have also been found, an

indicator of cell injury and cell death (Slotkin, McCook, & Seidler, 1997; Trauth, Seidler, McCook, & Slotkin, 1999). Taken together, these results suggest that prenatal nicotine exposure leads to programmed apoptosis, altered cellular maturation and perturbed cellular replication and differentiation (Slotkin et al., 1987; Slotkin, McCook, & Seidler, 1997; for review see Slikker, Xu, Levin, & Slotkin, 2005).

Prenatal nicotine exposure has also been shown to alter several neurotransmitter systems. For example, Navarro et al. (1988) examined the effects of gestational exposure to high dose nicotine on the development of the dopamine pathways in the offspring of dams. The results revealed that prenatal nicotine produced a significant overall suppression of maturational increases in dopamine and norepinephrine levels in the cerebral cortex. In addition, reduced neural activity was also found, as evidenced by suppression of markers of dopamine and norephinephrine activity, such as tyrosine hydroxylase. Similarly, Muneoka et al. (1997) examined the effects of high dose nicotine, administered via injections or infusion to pregnant rats, on the development of the dopaminergic system in their offspring during neonatal and juvenile periods. Overall, the researchers found that nicotine exposure during pregnancy resulted in a significant decrease in dopamine turnover in the forebrain, indicating reduced activity in the dopaminergic system of the rat. Other neurotransmitters have also been shown to be affected. Abreu-Villaca, Seidler, Tate, Cousins, & Slotkin (2004) found that the cholinergic system exhibited persistent hypoactivity, as evidenced by reduced hemicholinium-3 binding relative to choline acetyltransferase which continued into adolescence following prenatal nicotine exposure. In addition, serotonin and glutamate has also been shown to be effected by prenatal nicotine exposure (Parameshwaran et al., 2012; Xu, Seidler, Ali, Slikker, & Slotkin, 2001). In particular,

Muneoka et al. (1997) found that rats prenatally exposed to nicotine exhibited reduced serotonin turnover in the midbrain, pons, forebrain and cerebellum.

As a whole, it is clear from animal models that premature stimulation of nAChRs by nicotine in utero leads to profound effects on neurodevelopment. This altered brain development may help better understand the cognitive deficits that have often been found in human offspring with prenatal nicotine exposure.

Cognitive Functioning Among Humans Prenatally Exposed to Maternal Smoking

Over the past several decades, the Ottawa Prenatal Prospective Study (OPPS) has extensively studied the effects of prenatal tobacco exposure on offspring from birth to adolescents (Fried & Makin, 1987; Fried & Watkinson, 1988; Fried, O'Connell, & Watkinson 1992a; Fried, Watkinson, & Gray 1992b; Fried, Watkinson, & Gray, 1998; Fried & Watkinson, 2001; Fried, Watkinson, & Gray, 2003). Results from the OPPS, in conjunction with other research, has consistently shown that prenatal cigarette exposure is associated with a multitude of negative cognitive outcomes for the offspring including lowered intellectual and academic functioning (Fried et al., 1992a; 1998; 2001; Mortensen, Michaelsen, Sanders, & Reinisch, 2005; O'Callaghan, Mamum, O'Callaghan, Alati, Williams, & Najman, 2010) altered auditory processing (Fried et al., 1987; 1988; McCartney, Fried, & Watkinson, 1994) and executive functioning deficits (Brook, Brook, & Whiteman, 2000; Cornelius, Ryan, Day, Goldschmidt, & Wilford, 2001; Cornelius, De Genna, Leech, Wilford, Goldschmidt, & Day, 2011; Fried et al., 1992b; 2001; Julvez, Ribas-Fito, Torrent, Forns, Garcia-Esteban, & Sunyer, 2007; Linnet et al., 2003; Nomura, Marks, & Halperin, 2010). Many of these cognitive effects remain at least until the adolescent period. However, little is known about the effects of this substance beyond this stage of development.

One of the advantages of the OPPS is the prospective nature of the study, often preferred to a retrospective design. Women were followed during their pregnancy and information on drug use was obtained during each trimester. A disadvantage of the study, however, is the lack of control over the dose administered, the pattern of use or the timing of exposure. This is a limitation of human drug studies in general. However, given the proximity of measurement of drug use with actual drug use in prospective studies, they have the potential to determine the extent and timing of drug exposure during pregnancy. Another concern with human drug studies is that prenatal tobacco exposure tends to occur with other risk factors that may confound the relationship between exposure and deficits in cognition. Specifically, offspring exposed to tobacco smoke in utero are more likely to be exposed to other prenatal substances, have lower IQ scores, have greater behaviour problems and are more likely to use tobacco and marijuana themselves (Cornelius et al., 2000; 2012; Erickson & Arbour, 2012; Fried et al., 1984; 1998; Goldschmidt et al., 2012; Nomura et al., 2010; O'Callaghan et al., 2010; Porath & Fried, 2005; Rydell, Cnattingius, Granath, Magnusson, & Galanti, 2012). Therefore, current research needs to address these limitations.

Executive Functions

Prenatal tobacco exposure's effect on executive functioning skills is of particular importance. Executive functions represent a cognitive construct composed of multiple, inter-related, higher-level processes responsible for purposeful, goal-directed behaviour (Welsh & Pennington, 1988). Factor analytic studies have identified five key executive functioning processes including response inhibition, cognitive flexibility, planning, working memory and concept formation (Kelly, 2000; Miyake, Friedman, Emerson, Witzki, Howerter, & Wager, 2000; Welsh, Pennington, & Groisser, 1991). These executive functions are called upon when

routine or automatic responses or behaviours would not be adequate for optimal performance (Baddeley, 1986).

Executive functions have been shown to be critical for appropriate academic functioning. For example, St Clair-Thompson & Gathercole (2006) assessed executive functioning abilities and scholastic achievement among 11 year old healthy children. Overall, the researchers found that better working memory skills were associated with higher achievements in mathematics and English, while better response inhibition skills were associated with higher achievements in English, mathematics and science. Similarly, Bull, Espy & Wiebe (2008) found that strong working memory skills at age 4, predicted proficiency in mathematical achievement at 7 years of age. Moreover, Gathercole, Alloway, Willis & Adams (2006) found that among children 6 to 11 years of age with reading disabilities, severity of reading difficulty was negatively associated with working memory ability.

Executive functioning skills are also crucial for appropriate social and behavioural competence. Jacobsen, Williford & Pianta (2011) investigated the relationship between children's executive functioning skills, assessed both before and during elementary school, and sixth grade behavioural functioning. Overall, the researchers found that executive functioning skills significantly predicted teacher and parental reports of children's problem behaviour, including poor self-control, emotional reactivity, delinquent behaviour and social problems. In particular, better performance on both preschool and fourth grade measures of response inhibition were associated with decreases in teacher-reported sixth grade behavioural problems, while increases in planning ability were found to be associated with lower parent-rated behavioural problems. Consistent with these findings, adolescent boys with a history of aggressive behaviour performed significantly worse on measures of working memory and

showed a stronger tendency for perseverative responding on a card playing task, compared to non-aggressive youth (Sequin, Phil, Harden, Tremblay & Boulerice, 1995; Sequin, Arseneault, Boulerice, Harden, Tremblay, 2002), while deficits in response inhibition and planning ability have been linked to reactive aggression among 10 year old boys (Ellis, Weiss & Lochman, 2009). In addition, a recent meta-analysis found a robust negative relationship between inhibition and externalizing behaviour problems in young children (Schoemaker, Mulder, Dekovic & Matthys, 2012).

Executive functioning skills have also been shown to be important for emotional functioning. For example, Tonks and colleagues (2011) assessed executive functioning skills and emotional functioning using the Strengths and Difficulties Questionnaire, among 9 to 15 year old children. The researchers found that poor working memory was correlated with increased parental and teacher ratings of hyperactivity, while deficits with cognitive flexibility were correlated with increased self-reported emotional difficulties. Similarly, Carlson & Wang (2007) found that better inhibitory control of prepotent responses was positively correlated with preschool children's ability to regulate their emotions, even after controlling for several confounding variables. In addition, using a longitudinal study design, Leech, Larkby, Day & Day (2006), found that difficulties with attention in early childhood predicted higher levels of depressive and anxious symptoms among children at 10 years of age. Moreover, deficits in response inhibition have often been linked to several psychological disorders including attention-deficit/hyperactivity disorder (ADHD) and obsessive compulsive disorder (OCD) (Dimoska, Johnstone, Barry & Clarke, 2003; Woolley, Heyman, Brammer, Frampton, McGuire & Rubia, 2008).

Taken together, these results suggest that executive functioning skills are important for academic, social, behavioural and psychological functioning and executive dysfunction can lead to impairments in many of these domains.

Executive Functions and Neuroanatomy

Executive functioning skills increase throughout childhood and adolescence. The building blocks of executive functioning skills are evident in the first year of life, increase between the ages of 3 and 6 years, undergo major changes between 7 and 11 and continue to develop during adolescence (for review see Diamond, 2002). The improved performance in executive functioning tasks tends to parallel increases in white matter, suggestive of myelination that has been shown to occur from infancy to adolescence (Pfefferbaum, Mathalon, Sulivan, Rawles, Zipursky & Lim, 1994), particularly within the frontal cortex (Klingberg, Vaidy, Gabrieli, Moseley & Hedehus, 1999; Paus et al., 1999). In fact, studies have shown that white matter growth in frontal regions is correlated with better performance on executive functioning tasks (Mabbott, Noseworthy, Bouffett, Laughlin & Rockel, 2006; Nagy, Westerberg & Klingberg, 2004). Given the changes that take place during the child and adolescent years, in order to elucidate the long-term effects of maternal smoking during pregnancy on executive functioning, it is crucial to study offspring at an age when the prefrontal cortex has matured.

Frontal-cortical areas have often been associated with executive functioning skills, including the dorsolateral prefrontal cortex, which is involved in cognitive flexibility (Stuss, Bisschop, Alexander, Levine, Katz, & Izukawa, 2001) and working memory manipulation (Barbey, Koenigs, Grafman, 2012), the inferior frontal gyrus, which has been linked to response inhibition (Aron, Fletcher, Billmore, Sahakian & Robbins, 2003), the orbital prefrontal cortex, which has been associated with emotion regulation (Golkar et al., 2012) and the ventrolateral

prefrontal cortex, which has been shown to be involved in working memory maintenance (Narayanan, Prabhakaran, Bunge, Christoff, Fine, & Gabrieli, 2005). Patients with lesions in the medial prefrontal cortex have been shown to perform poorly on the Iowa Gambling Task, a measure of decision making (Manes et al., 2002) and imaging studies have shown increased activity in both the dorsolateral and ventrolateral prefrontal cortex during the performance of the Wisconsin Card Sorting Test, a measure of concept formation (Monchi, Petrides, Petre, Worsley, & Dagher, 2001).

More recently, however, a large body of research has shown that executive functions are not solely subserved by the frontal cortex, but also by other cortical and subcortical regions that are strongly connected to the frontal lobes through numerous pathways. For instance, the prefrontal cortex is strongly connected with the striatum (Leh, Ptito, Chakravarty, & Strafella, 2007) and patients with focal lesions in the striatum exhibit attention switching difficulties (Cools, Ivry, & D'Esposito, 2006), while damage to the caudate nucleus often leads to perseverative responding on executive functioning measures (Nys, van Zandvoort, van der Worp, Kappelle, & de Haan, 2006). The prefrontal cortex is also connected to the temporal lobes (Axmacher, Scmitz, Wagner, Elger, & Fell, 2008) and patients with hippocampal damage show impairments on spatial working memory tasks (Abrahams et al., 1999). In addition, cerebellar damage leads to deficits in verbal working memory tasks (Ravizza, McCormick, Schlerf, Justus, Ivry, & Fiez, 2006), while the nucleus accumbens, is important for goal-directed behaviours (Pennartz, Groenewegen, & Lopes da Silva, 1994). Moreover, patients with right parietal lobe damage often exhibit significant impairments with visuospatial working memory tasks (Berryhill & Olson, 2008). Thus, the integrity of the connections of the frontal cortex with the rest of the brain help to regulate executive functioning processes.

Three executive functions that were examined in the present dissertation include response inhibition, verbal working memory and visuospatial working memory. Response inhibition refers to the ability to suppress inappropriate or unwanted responses that can interfere with the attainment of future goals (Mostofsky & Simmonds, 2008). Working memory is defined as a limited-capacity system that involves the active maintenance and manipulation of both verbal and visuospatial information for a brief amount of time, followed by its retrieval (Baddeley, 2003). Lesion and neuroimaging studies have confirmed that these executive functioning processes depend upon several cortical and subcortical structures, in addition to the frontal cortex (Aron et al., 2003; Aron & Poldrack, 2006; Barbey et al., 2012; Garavan, Ross, & Stein, 1999; Narayanan et al., 2005; Ravizza et al., 2006).

Neuroimaging of Prenatally Exposed Offspring

Despite a large body of cognitive research showing that prenatal cigarette exposure leads to deficits in executive functioning, the effects of in utero cigarette exposure on brain function is not well established. One of the most commonly used tools to study brain function or activity is functional magnetic resonance imaging (fMRI). fMRI is a non-invasive neuroimaging technique that relies on the blood oxygen level dependent (BOLD) effect, which is based on changes in blood oxygenation that occur with changes in neuronal activity (for reviews see Arthurs & Bonifice, 2002; Logothetis & Wandell, 2004). Shortly after neural activity increases in a region of the brain, oxygen rich hemoglobin (oxyhemoglobin) is infused into that region. The active neurons metabolize the oxygen, but there is a greater oxygen supply than can be consumed, resulting in an increase of the ratio of oxyhemoglobin to deoxyhemoglobin (low level of oxygen) in the tissue. Oxygenated and deoxygenated hemoglobin have different magnetic properties with oxyhemoglobin being diamagnetic and deoxyhemoglobin being paramagnetic. The diamagnetic

properties of the oxyhemoglobin provide an increased magnetic signal to that of deoxyhemoglobin and this can be quantified with fMRI. Therefore, the BOLD effect can map neural activity or brain function during many cognitive processes, including response inhibition, verbal working memory and visuospatial working memory. This technique offers excellent spatial resolution (2-3 millimeters) and moderate temporal resolution.

fMRI typically employs paradigms called blocked designs. Blocked designs involve the presentation of stimuli sequentially within alternating task conditions, usually an experimental condition and a control condition. The two conditions are similar except that the control condition excludes demands on the process of interest. Neural activity related to the process of interest is found by subtracting neural activity of the control condition from that of the experimental condition.

Three of the most commonly used fMRI paradigms to examine response inhibition, verbal working memory and visuospatial working memory are the Go/No-Go task, the letter 2-back task and the visuospatial 2-back task, respectively. For the Go/No-Go task, participants are required to respond to a specific letter in one condition and refrain from responding to that same letter in another condition. The letter 2-back task involves the presentation of letters, one at a time and participants are required to respond when the same letter that appeared two stimuli prior is presented again. For the visuospatial 2-back task, 9 different spatial locations are presented one at time and participants are required to respond when the same spatial location of an object that appeared two stimuli prior is presented again. Data from lesion studies and imaging studies have validated the use of these tasks as measures of executive functioning (Liddle, Kiehl, Smith, 2001; Menon, Adleman, White, Glover, & Reiss, 2001; Muller, Machado, & Knight, 2002;

Owen, McMillian, Laird, & Bullmore, 2005; Pfefferbaum, Desmond, Galloway, Menon, Glover, & Sillivan, 2001; Tsuchida & Fellows, 2009).

To date, only three studies have investigated brain function in offspring prenatally exposed to cigarette smoke during performance of executive functioning tasks (Bennett et al., 2009, 2012; Jacobsen, Slotkin, Menci, Frost & Pugh, 2007). Given the limited research, a consistent pattern of activation has yet to be determined, and a full understanding of the effects of prenatal tobacco exposure on neural processing during executive functioning tasks has yet to be attained. Moreover, participants in each of the studies were either children or adolescents. Thus, the neuronal effects of maternal smoking during pregnancy on executive functioning beyond the adolescent period have not been examined.

Overview of Dissertation

The aim of the present dissertation was to investigate the long-term neurophysiological effects of prenatal nicotine exposure on executive functioning by assessing participants in young adulthood, an age when the prefrontal cortex which subserves executive functioning processes has matured, using fMRI. Moreover, to address some of the limitations in previous research, participants in the study were recruited from the OPPS, where maternal prenatal drug use was gathered prospectively and, their offspring has continually been followed from infancy to young adulthood, with detailed information gathered on their current and past drug use, cognitive/behavioural performance and over 4000 lifestyle variables. The use of participants from the OPPS allowed for the measurement and statistical control of these potentially confounding variables.

The present dissertation consists of three separate original manuscripts. Participants for all three papers were members of the OPPS. The original sample size consisted of

approximately 150 participants. A total of 36 participants were imaged. However, 11 participants were not included in the analysis due to meeting exclusionary criteria once tested. Specifically, a total of 7 prenatally exposed participants were excluded as 2 tested positive for cocaine, 1 tested positive for amphetamines, 3 reported using marijuana on the day of testing and 1 had an abnormal structural MRI scan. A total of 4 non-exposed controls were excluded as 2 tested positive for amphetamines and 2 reported using marijuana on the day of testing. Thus, the results for each of the manuscripts are based on a total of 25 participants, with 12 participants in the prenatally exposed group and 13 participants in the non-exposed group.

The first original manuscript investigated the long-term neurophysiological effects of prenatal nicotine exposure on response inhibition in young adults. Neural activity during performance of a Go/No-Go task was compared between exposed and non-exposed participants using fMRI. Despite similar task performance, different neural activation patterns were found between the two groups related to response inhibition.

The second original manuscript investigated the long-term neurophysiological effects of prenatal nicotine exposure on verbal working memory in young adults. fMRI data was collected from participants prenatally exposed to nicotine and non-exposed controls during completion of a 2-Back task. Major findings revealed different activation patterns related to increasing amounts of prenatal nicotine exposure.

The third original manuscript investigated the long-term neurophysiological effects of prenatal nicotine exposure on visuospatial working memory in young adults. Neural activity during performance of a visuospatial 2-back task was examined among participants prenatally exposed to nicotine and non-exposed controls. Major findings revealed different activation patterns related to increasing amounts of prenatal nicotine exposure. These results were

compared with the long-term effects of prenatal marijuana on visuospatial working memory to reveal a similar impact of the two drugs.

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Chapter 2

Manuscript I

The Long-Term Effects of Prenatal Nicotine Exposure on Response Inhibition: An fMRI Study of Young Adults

Longo, C.A., Fried, P.A., Cameron, I., Smith AM. (2013). The long-term effects of prenatal nicotine exposure on response inhibition: An fMRI study of young adults. *Neurotoxicology and Teratology. Accepted for Publication.*

Abstract

The long-term effects of prenatal nicotine exposure on response inhibition were investigated in young adults using functional magnetic resonance imaging (fMRI). Participants were members of the Ottawa Prenatal Prospective Study, a longitudinal study that collected a unique body of information on participants from infancy to young adulthood, which allowed for the measurement of an unprecedented number of potentially confounding drug exposure variables including: prenatal marijuana and alcohol exposure and current marijuana, nicotine and alcohol use. Twelve young adults with prenatal nicotine exposure and 13 non-exposed controls performed a Go/No-Go task while fMRI blood oxygen level-dependent responses were examined. Despite similar task performance, participants prenatally exposed to nicotine demonstrated significantly greater activity in several region of the brain that typically subserve response inhibition including the inferior frontal gyrus, the inferior parietal lobe, the thalamus and the basal ganglia. In addition, prenatally exposed participants showed greater activity in relatively large posterior regions of the cerebellum. These results suggest that prenatal nicotine exposure leads to altered neural functioning during response inhibition that continues into adulthood. This alteration is compensated for by recruitment of greater neural resources within regions of the brain that subserve response inhibition and the recruitment of additional brain regions to successfully perform the task. Response inhibition is an important executive functioning skill and impairments can impede functioning in much of everyday life. Thus, awareness of the continued long-term neural physiological effects of prenatal nicotine exposure is critical.

Keywords: Prenatal nicotine exposure; response inhibition; young adults; fMRI

The Long-Term Effects of Prenatal Nicotine Exposure on Response Inhibition: An fMRI Study of Young Adults

Cigarette smoking during pregnancy remains a worldwide public health concern, despite a plethora of research highlighting its deleterious effects to the mother and fetus. Approximately 10% of Canadian women aged 20 to 44 reported regularly smoking cigarettes while pregnant in a 2009 Canadian Tobacco Use Monitoring Survey (Health Canada, 2009). These rates increase in the United Kingdom, where according to the 2010 Infant Feeding Survey, 12% of mothers report smoking throughout their pregnancy (The Health and Social Care Information Centre, 2011).

Cigarette smoke contains thousands of chemicals (U.S. Department of Health and Human Services, 1989), many of which are toxic and can potentially modify the development of the brain. However, of these chemicals, research has consistently implicated nicotine as the main neuroteratogen (Slotkin et al., 1986; Slotkin, Cho, & Whitmore, 1987). This is largely because nicotine can rapidly cross the placental tissue into the fetal brain at a level higher than that of their mothers (Luck et al., 1985). Second, nicotine acts on nicotinic acetylcholine receptors (nAChRs) by mimicking the neurotransmitter acetylcholine (for review see Dwyer, McQuown, Leslie, 2009).

nAChRs are ligand-gated ion channels with binding sites for acetylcholine and nicotine (Gotti et al., 2007). These receptors are detected in various regions of the human brain, including the frontal cortex, within the first and second trimesters (Hellström-Lindahl et al., 1998) and play an important role in many aspects of brain development (for review see Dwyer, McQuown, & Leslie, 2009). Prenatal nicotine exposure is believed to disrupt the timing of the trophic actions of acetylcholine, by binding to nAChRs receptors prematurely, resulting in

dysfunction of numerous pathways and systems within the central nervous system (Navarro et al., 1988; Muneoka et al., 1999; Ribary & Lichtensteiger, 1989; for review see Slotkin, 2008).

The frontal cortex, specifically the prefrontal cortex, plays an important role in higher-level cognitive processes, such as executive functioning skills (for a review see Hanna-Pladdy, 2007; Muller & Knight, 2006). Executive functions involve several types of processes including: decision making, planning, focused attention, concept formation, response inhibition, cognitive flexibility and working memory (Denckla, 1993).

An executive functioning process of particular importance, given its utility to the successful completion of many everyday tasks, is response inhibition. Response inhibition refers to the ability to suppress inappropriate or unwanted responses that can interfere with the attainment of future goals (Mostofsky & Simmonds, 2008). In fact, deficits in response inhibition have often been linked to several psychological disorders including attention-deficit/hyperactivity disorder (ADHD) and obsessive compulsive disorder (OCD) (Dimoska et al., 2003; Woolley et al., 2008).

Lesion and imaging studies have greatly increased our understanding of the neural circuitry involved in response inhibition. Various regions within the frontal cortex have often been implicated in response inhibition. Specifically, the right inferior frontal cortex (Aron et al., 2003) and the dorsolateral prefrontal cortex (Sasaki et al., 1993) have been associated with the successful suppression of inappropriate responses. Patients with damage to the medial prefrontal cortex experience difficulties with response inhibition tasks (Lehmkuhler & Meseulam, 1985). In addition, patients with dysfunction in the orbitofrontal circuits often exhibit personality changes, including behavioural disinhibition (Eslinger & Damasio, 1985). However, response inhibition is not solely subserved by the frontal cortex but by other cortical and subcortical

regions that are richly interconnected to the frontal lobes through numerous pathways, including the anterior cingulate (Kiehl et al., 2000), the basal ganglia (Aron & Poldrack, 2006) and the inferior parietal lobe (Garavan et al., 1999).

Given that nicotine compromises critical neural pathways in the developing brain, it is not surprising that a large body of neurocognitive research, across different developmental levels, has shown that offspring prenatally exposed to nicotine show deficits in response inhibition. Research on infants and toddlers has been small given that response inhibition is more difficult to assess in this age group. Nonetheless, using retrospective reports of mothers' cigarette use during pregnancy, Brook et al. (2000) found that mothers who smoked during pregnancy were significantly more likely to rate their 2-year-old children as impulsive on a structured questionnaire, compared to mothers who did not smoke during pregnancy. In line with this research, a prospective study found that infants exposed to prenatal tobacco smoke were more excitable and had higher scores on a stress scale than infants not exposed to prenatal tobacco smoke (Law et al., 2003).

In contrast to the limited research during the infant and toddler years, several studies have examined the effects of prenatal nicotine exposure on response inhibition during childhood. The Ottawa Prenatal Prospective Study (OPPS), an ongoing, longitudinal investigation of the effects of prenatal drug exposure on offspring, initiated in 1978, has extensively studied response inhibition among children prenatally exposed to cigarette smoke. At 4-7 years of age, exposed and non-exposed OPPS children were given a visual and auditory continuous performance task (CPT) as a measure of sustained attention and response inhibition. Overall, the researchers found that prenatal cigarette exposure was associated with increased errors of commission on both the visual and auditory CPT, suggesting difficulties with impulsivity (Kristjansson, Fried, &

Watkinson, 1989). These results remained despite controlling for several confounding variables related to maternal smoking, including: lower income, lower educational attainment and, maternal use of other substances during pregnancy. Streissguth and colleagues (1984) also found a significant relationship between prenatal tobacco exposure and an increased number of commission errors during a vigilance task among 4 year old children. Using a more thorough executive functioning battery on 6 year old children from the OPPS, discriminant function analysis revealed a dose-response association between prenatal cigarette exposure and impulsive behaviour. In particular, higher amounts of maternal smoking during pregnancy was associated with lower scores on a response inhibition task and increased errors of commission on a sustained vigilance task (Fried, Watkinson, & Gray, 1992). Similarly, in another longitudinal prospective cohort study conducted within the Pittsburgh area, researchers found that at 10 years old, children born to mothers who smoked during pregnancy were significantly more likely to make perseverative responses on a card sorting test (Cornelius et al., 2001) and exhibited more difficulty with the interference component of the Stroop task, both suggesting difficulties with inhibition, compared to non-exposed children (Cornelius et al., 2011). In addition, they were more likely to be rated as active and impulsive by their mothers (Cornelius et al., 2011). Consistent with these findings, numerous studies have found a strong association between maternal smoking during pregnancy and attention deficit hyperactivity disorder (ADHD) (Linnet et al., 2003; Nomura et al., 2010; Piper & Corbett, 2012). This is quite important given that one of the hallmark features of ADHD is difficulties with inhibition (American Psychiatric Association, 2001).

Only a few studies have examined the effects of prenatal cigarette exposure on response inhibition among adolescents, with mixed results. The OPPS found that an impulsivity factor,

which was mainly derived from the CPT, was associated with prenatal tobacco exposure, but only among 13 year old adolescents not 14 to 16 year olds (Fried & Watkinson, 2001). Similarly, in a follow-up study with the same cohort, neither perseverative errors on the WCST or interference on the Stroop was associated with maternal smoking in utero (Fried, Watkinson, & Gray, 2003). In contrast, a retrospective cohort study continued to find a strong association between maternal smoking during pregnancy and attention deficit hyperactivity disorder (ADHD) among adolescent offspring (Milberger et al., 1998). Taken together, results are inconsistent with regards to whether the deficits in response inhibition associated with in utero exposure to tobacco smoke continue into adolescents and beyond. Studying offspring after the adolescent period is important, given that the prefrontal cortex, which subserves executive functioning, grows exponentially during the adolescent years (for review see Diamond, 2002).

Despite a large body of neurocognitive research examining the effects of prenatal nicotine exposure on the offspring, the neuronal effects of in utero nicotine exposure on the human brain is not well established. To date, only one study has used functional magnetic resonance imaging (fMRI) to shed light on the neural mechanisms that underlie response inhibition deficits among offspring prenatally exposed to maternal smoking. Specifically, Bennett et al. (2009) compared neural function among 12-year old adolescents exposed and non-exposed to tobacco in utero during a response inhibition task. The researchers found that tobacco exposed children showed greater activity in a relatively large and diverse set of brain regions including the left frontal, right occipital, and bilateral temporal and parietal regions compared to non-exposed youth. According to the authors, greater activation in multiple brain regions among prenatally exposed youth suggests less efficient processing due to altered neural functioning. However, given the limited research, a consistent pattern of activation has yet to be determined

and thus a full understanding of the effects of prenatal nicotine exposure on neural processing during response inhibition has yet to be attained. In addition, no prospective study has used fMRI to investigate the long-term neural effects of prenatal nicotine exposure on response inhibition in a sample of young adults.

The aim of the present study was to examine the long-term neuronal effects of prenatal nicotine exposure on response inhibition in young adults from the OPPS, using fMRI blood oxygen level dependent (BOLD) response. Using participants from the OPPS allowed for the measurement of several risk factors that tend to co-occur with prenatal tobacco exposure including: exposure to other prenatal substances, low IQ scores, behavioural problems, and current use of tobacco (Cornelius et al., 2000; 2012; Erickson & Arbour, 2012; Fried, Watkinson, & Gray, 1998; Fried, Innes, & Barnes, 1984; Goldschmidt et al., 2012; Nomura et al., 2010; O'Callaghan et al., 2009; Porath & Fried, 2005; Rydell et al., 2012). Response inhibition was assessed using a Go/No-Go task. Evidence from fMRI research conducted on healthy controls has found that the Go/No-Go task depends on prefrontal and parietal cortical integrity, specifically, the dorsolateral prefrontal cortex, inferior frontal cortex, inferior parietal lobule, anterior cingulate, premotor cortex, thalamus and the caudate (Casey et al., 1997; Liddle et al., 2001; Menon et al., 2001; Rubia et al., 1998; Smith et al., 1998 & Stevens et al., 2007). It was hypothesized that prenatal nicotine exposure would impact the efficiency of neurocircuitry supporting response inhibition and that this would result in greater activity in the inferior frontal cortex, the basal ganglia and the anterior cingulate.

Methods

Participants

Participants were recruited from the OPPS. Initial recruitment procedures have been described in detail elsewhere (Fried et al., 1980; 1988). Briefly, pregnant women volunteered to participate in the study after becoming aware of the research either by notices in the media or by signs placed in the offices of their obstetricians. The study was described as investigating prenatal lifestyle habits and their effects on offspring. Upon volunteering to participate, mothers-to-be were interviewed, usually in their homes, once during each trimester. The repeated interviews during pregnancy provided a means of checking the reliability of the self-report and were typically conducted by the same researcher, thereby helping to establish rapport with the participant. The information collected by a trained female interviewer included socioeconomic status, mothers age, education, physical and mental health of both parents, a 24 hour dietary recall and present drug use during pregnancy with particular emphasis upon cigarette, alcohol and marijuana usage. Families were Caucasian, from middle-class, low-risk homes, and no parent of the participant was reported to have an Axis I diagnosis based on the Diagnostic and Statistical Manual of Mental Disorders (DSM) at the time of pregnancy. In addition, mothers were excluded if they used substances other than tobacco, marijuana or alcohol during pregnancy.

Twelve prenatally exposed participants (6 females and 6 males; mean age 21) and 13 non-exposed controls (7 females and 6 males; mean age 21) were included in the analysis. The prenatally exposed nicotine group was defined as participants who were exposed at any point in utero to any amount of nicotine. The control group was defined as participants with no exposure at any point in utero to any amount of nicotine. For prenatal nicotine exposure, a nicotine score

was determined by multiplying the number of tobacco cigarettes smoked on average per day, at the time of pregnancy, by the nicotine content of the brand specified. A package of cigarettes of average strength contains approximately 16 mg of nicotine. The 12 prenatally exposed participants were exposed to an average of 14.13 mg of nicotine in utero daily (range of 0.03-35 mg of nicotine/daily), while the 13 non-exposed participants were never exposed to any amount of nicotine in utero.

For both groups, exposure to marijuana and alcohol in utero was also recorded. Marijuana use at the time of pregnancy was recorded in terms of the number of joints smoked per week. Both the quantity and the pattern of alcohol consumption during pregnancy (including beer, wine and liquor) were recorded and converted to ounces of absolute alcohol (AA) per day. Analysis of variance (ANOVA) revealed no significant differences between groups on prenatal marijuana exposure or prenatal alcohol exposure (see Table 1). However, given that both these variables have been shown to impact brain activity (Smith et al., 2004, 2006; Spadoni et al., 2009), they were included as covariates in the fMRI analysis.

Participants with prenatal nicotine exposure are more likely to smoke cigarettes and use marijuana (Cornelius et al., 2000; 2012; Goldschmidt et al., 2012; O'Callaghan et al., 2009; Porath & Fried, 2005; Rydell et al., 2012). Therefore, to increase external validity, participants who smoked cigarettes and marijuana were included. The regular use of these substances was measured among all participants. Regular use of cigarettes was defined as 1 cigarette/day or more. In order to eliminate the acute withdrawal symptoms that come from cessation of regular tobacco use, which includes disruption of attention and memory (Jacobsen et al., 2005), participants were permitted to smoke during the day, however, no tobacco smoking occurred 2 hours prior to testing (confirmed by presence in the hospital MRI unit). Regular use of

marijuana was defined as 1 joint/week or more. To eliminate the acute effects of marijuana on cognitive functioning (Ramaekers et al., 2009), participants were excluded if they smoked marijuana on the day of testing. Given that marijuana use and tobacco use have been shown to impact brain activity (Jacobsen et al., 2007, Smith et al., 2010), values of marijuana and cotinine in the urine samples were used as covariates in the fMRI analysis. Alcohol use is also prevalent among young adults (Johnson et al., 2003) and thus participants who drank alcohol regularly were not excluded. The regular use of alcohol was measured among all participants and defined as 1 drink/week or more. To eliminate the acute effects of alcohol on cognitive functioning (Leitz et al., 2009) participants were excluded if they drank alcohol on the day of testing. Alcohol use has been shown to impact brain activity (Tapert et al., 2004) and therefore it was also used as a covariate in the fMRI analysis.

Participants previously completed a comprehensive psychological battery including the Wechsler Intelligence Scale for Children-III (Wechsler, 1991) (between the ages of 13 and 16) and the National Institute of Mental Health Computerized Diagnostic Interview Schedule for Children (C-DISC) (Bacon, 1997), which assessed for current psychiatric illnesses based on DSM-IV criteria. Parents also previously completed the Conners' Parent Rating Scale-Revised (Conners, 1997) (between the ages of 13 and 16) and family income and mother's education at the time of pregnancy was measured. An ANOVA did not reveal any significant differences between the prenatally exposed group and the non-exposed group on these scales, however, the WISC-III Full Scale IQ score and family income were included as covariates in the fMRI analysis (see Table 2).

Exclusion criteria for both groups included (a) contraindication to fMRI, specifically, having a pacemaker, claustrophobic, mental or electrical implants, accidents leaving metal in the

eye, recent surgery, metal dental work (aside from fillings), piercings that cannot be removed, eye glasses or insufficient vision for viewing the tasks, (b) abnormalities in their structural MRI scan, (c) meeting DSM criteria for an Axis I disorder using the C-DISC, (d) current use of prescription medication with central nervous system effects including psychotropic medication, as well as reported regular use of illicit drugs (defined as once a month or more) including amphetamines, crack, cocaine, heroin, mushrooms, hashish, lysergic acid, steroids, solvents and tranquilizers, (e) testing positive for cocaine, opiates or amphetamines in their urine, (f) history of seizures, diabetes requiring insulin treatment, heart attack, stroke, blood clots, high blood pressure, cancer, brain injury and chronic pain, (g) first language other than English and (h) left-handedness. In addition, all participants signed informed consent prior to being imaged. The study was approved by the Ottawa Hospital Research Ethics board.

Measures

Go/No-Go Task. The Go/No-Go task was used to measure response inhibition processes while in the scanner. The Go/No-Go paradigm involves presentation of letters in white, one at a time, on a black screen for a period of 75 milliseconds, with an interstimulus interval of 925 milliseconds (see Figure 1). Fifty percent of the stimuli were 'X' and the other 50% were other capital letters randomly selected from the remainder of the alphabet. The task was a block design and included two conditions: a control condition (Press for X) and a response inhibition condition (Press for all letters except X). The control condition began with the instruction "Press for X" on the screen for 3 seconds. In this condition, participants were required to press a button with their right index finger when an "X" was presented on the screen. The response inhibition condition began with the instruction "Press for all letters except X" on the screen for 3 seconds. In this condition participants were required to refrain from pressing for "X" and to press for all

other letters with their right index finger. Each condition was followed by a 24 seconds rest period. During this rest period the word "REST" was presented on the screen and no response was required. Both the control (Press for X) and response inhibition condition (Press for all letters except X) were comprised of 27 stimuli, presented every 1 second, for a total of 27 seconds each. Each condition was presented four times. The total duration of the task was 6 minutes and fifty seconds. The order of blocks was counterbalanced. This presentation allowed for manipulation of response inhibition by changing the instructions while maintaining all other features of the task, including number of stimuli, number and type of response the same. This ensured that following the subtraction of the neural activity during the control condition (Press for X) from that during the response inhibition condition (Press for all letters except X) only the neural activity involved in response inhibition was observed in the statistical parametric maps. The Go/No-Go task has been shown to reliably initiate response inhibition activation in healthy controls (Casey et al., 1997; Liddle et al., 2001; Menon et al., 2001).

The task was presented to the participants on a back projection screen, located at the foot of the participants table, via a mirror attached to the head coil. All lighting was turned off and button press responses were recorded via a MRI-compatible fiber optic device (Light-wave Medical, Vancouver, British Columbia).

Drug History Questionnaire (DHQ). The DHQ was used to measure participants' present usage of both licit and illicit substances (see Appendix A). Substances assessed included the regular use of amphetamines, crack, cocaine, heroin, mushrooms, hashish, lysergic acid, steroids, solvents, tranquilizers, marijuana, nicotine and alcohol (as defined above). Both the amount and the number of years of use were recorded among regular users. This survey was adapted from the Monitoring the Future Survey which was used as a national survey focused on

the lifestyles, attitudes, and preferences of American youth (Johnston et al., 2003). The substance use portion of the survey has been shown to have good psychometric properties (O'Malley, Bachman, & Johnston, 1983). Group differences for the amount of nicotine, marijuana and alcohol consumed among regular users was computed by ANOVA using SPSS v. 20, with a statistical threshold set at $p<0.05$.

Urine Sample. Current drug use was verified through a urine sample which was tested for amphetaminies, opiates, cocaine, cannabis, creatinine and cotinine (metabolite of nicotine). All metabolite concentrations were adjusted for creatinine to control for urine dilution. Group differences for marijuana and cotinine urine values were computed by ANOVA using SPSS v. 20, with a statistical threshold set at $p<0.05$.

Imaging Parameters. All imaging was performed using a 1.5 Tesla Siemens Magnetom Symphony MR scanner. Participants lay supine with their head secured in a standard MRI head holder. A conventional T1-weighted spin echo localizer was acquired and used to align the slice orientation for the fMRI scans such that the anterior commissure-posterior commissure (AC-PC) line in the sagittal view were at right angles to the slice selection gradient. Whole brain fMRI was performed using a $T2*$-weighted echo planar pulse sequence (TR/TE 3000/40ms, flip angle 90^{o}, FOV 24 x 24 cm^{2}, 64 x 64 matrix, slice thickness 5 mm, 27 axial slices, bandwidth 62.5 kHz).

Behavioural Performance Parameters and Analysis. For the Go/No-Go task, reaction time for each response, errors of commission and omission were recorded. Errors of commission included any response following the presentation of a non-target stimulus within 900 milliseconds of stimulus presentation. Omission errors were defined as a failure to respond to a target stimulus within 900 milliseconds. Mean reaction times were calculated for all accurate

responses occurring with 900 milliseconds of stimulus presentation. Errors of omission, errors of commission and mean reaction times for both the control condition (Press for X) and the response inhibition condition (Press for all letters except X) were calculated. Group differences were computed by multivariate analysis of covariance using SPSS v. 20, with a statistical threshold set at $p<0.05$.

Whole Brain Analysis. Statistical Parametric Mapping 8 (SPM8) was used to post-process the fMRI data and to perform the statistical analysis. For post-processing, functional images from the first 9 seconds of the initial rest block were discarded to ensure that longitudinal magnetic relaxation (T1 effects) had stabilized. The remaining functional images were realigned to correct for motion by employing the procedures of Friston et al. (1995). The motion correction did not exceed 1 millimeter for any subject. Images were spatially normalized, due to differences in human brain morphology, to match the echo planar imaging (EPI) template provided in SPM8. Following spatial normalization, images were smoothed to eliminate noise interference in the data, with an 8mm full-width at half-maximum Gaussian filter.

Individual participant fixed effects analyses were performed for the comparison of the response inhibition condition (Press for all letters except X) minus the control condition (Press for X). One contrast image was created per person, and these images were then used for second-level random effects analyses that were corrected for multiple comparisons. Random effects analyses eliminate highly discrepant variances between and within individuals in constructing an appropriate error term for hypothesis testing and generalizability to the population. Two-sample t tests were used to compare neural activity during response inhibition between participants with prenatal nicotine exposure and non-exposed controls, at a statistical threshold set at $p<0.05$, both with and without covariates. Prenatal marijuana exposure, prenatal alcohol exposure, current

alcohol use, current marijuana exposure (e.g. marijuana urine) and current nicotine exposure (e.g. cotinine urine), family income and WISC-III Full Scale IQ scores were all used as continuous variables and were entered as covariates. Each covariate was included in the two sample t-test separately to maintain power and to determine if these variables were contributing to the neurophysiological results. Covariates that were not found to significantly contribute to the differences in brain activity between groups were removed from the final analysis.

Procedure

Upon arrival to the hospital, informed consent was attained and MRI compatibility was confirmed. Then participants provided a urine sample which was immediately sent to the Hospital Laboratory for testing. Prior to commencing imaging, participants were required to view the Go/No-Go task outside the scanner and performed a practice session of 10 trials of each condition. This ensured that all participants were able to perform the task accurately. Participants then entered the scanner and task instructions were repeated including to press the response pad as quickly as possible and, if they made a mistake, to continue without thinking about the mistake. Once imaging was completed, participants filled out a drug history questionnaire. Completing the drug questionnaire after scanning ensured the blindness of the fMRI researcher.

Results

Drug questionnaire and urine sample data

Regular marijuana use was reported by three participants from the prenatally exposed group and two participants from the control group. The average number of years of regular marijuana use for the 3 prenatally exposed participants was 5 years (range 4 to 6 years), while the average number of years of regular marijuana use for the 2 non-exposed participants was 3.5

years (range 2 to 5 years). No participant reported smoking marijuana at least 24 hours prior to testing. There were no significant differences between groups for current marijuana use or urine cannabis (see Table 1). All participants from both groups reported regular alcohol use. However, no participant from either group reported drinking more than 8 alcoholic drinks per week on a regular basis. In addition, no participant from either group reported drinking alcohol at least 24 hours prior to testing. No significant differences between groups emerged on current alcohol use (see Table 1). Regular tobacco use was reported by four participants from the prenatally exposed group and two participants from the control group. The average number of years of regular tobacco use for the 4 prenatally exposed participants was 4.7 years (range 1 to 6 years), while the average number of years of regular tobacco use for the 2 non-exposed participants was 5.5 years (range 5 to 6 years). No significant group difference emerged for current tobacco use or cotinine values (see Table 1). No participant from either group reported using illicit drugs on a regular basis or within the month prior to testing.

Behavioural Performance Data

There were no significant performance differences between participants prenatally exposed to nicotine and non-exposed controls on reaction time, errors of omission and errors of commission. This was analysed while controlling for cotinine values (see Table 3).

Whole Brain Analysis

A first-level fixed effects group analysis for all participants, at a *p* value corrected for cluster level at 0.05, for the comparison of the response inhibition condition (Press for all letters except X) minus the control condition (Press for X), confirmed the task was activating areas of the brain typically observed during response inhibition. These areas included the inferior, middle, superior and medial frontal gyri and the anterior cingulate.

When comparing neural activity between participants with prenatal nicotine exposure and non-exposed controls in the second-level random effects two sample t-test analysis without covariates, significant differences in brain activity were found between the prenatally exposed and non-exposed groups. Prenatal marijuana, prenatal alcohol, current alcohol, current marijuana (marijuana urine), IQ and family income did not contribute significantly to the differences in brain activity between groups. Therefore, these variables were eliminated from the final analysis. In contrast, current nicotine (cotinine values) was found to contribute significantly to the differences in brain activity between groups. As a result, this covariate was retained in the final analysis.

The most robust effect of the study was that young adults with prenatal nicotine exposure demonstrated significantly greater activity than non-exposed young adults, while controlling for cotinine values, during the response inhibition condition (Press for all letters except X) minus the control condition (Press for X), at a *p* value corrected for cluster level at <0.05 in a large cluster of 8,446 voxels, in the following regions: the right inferior frontal gyrus, the left anterior cingulate, the thalamus bilaterally, the inferior parietal lobule bilaterally, the right precuneus, the cerebellar tonsil bilaterally, the right culmen, the left declive and the left lentiform nucleus (see Table 4 & Figure 2). All coordinates are in Montreal Neurological Institute (MNI) space. The non-exposed group did not exhibit greater activity in any region of the brain compared to the exposed group.

Discussion

The purpose of the present study was to examine the long-term neurophysiological effects of prenatal nicotine exposure on response inhibition in young adults from the OPPS, using fMRI. Despite similar task performance, significant group differences emerged in the

BOLD response, with prenatal nicotine exposure participants displaying significantly greater and more extensive activation than non-exposed young adults.

The most significant result was observed in the right inferior frontal gyrus and bilateral inferior parietal lobule in that young adults with prenatal nicotine exposure demonstrated significantly greater activation during response inhibition in these areas than non-exposed participants. These regions of the brain have been typically implicated in response inhibition tasks (Liddle et al., 2001; Menon et al., 2001), with the parietal lobule important for sustained visual attention (Pardo, Fox, & Raichle, 1991) and the right inferior frontal gyrus being particularly crucial for the suppression of inappropriate responses (Aron et al., 2004). In fact, as damage to the inferior frontal gyrus increases, the greater the impairment on response inhibition tasks (Aron et al., 2003). The degree of activation within the lateral frontal cortex has also been shown to be strongly associated with the degree of task difficulty, such that tasks that involve a greater degree of difficulty in suppressing responses leads to greater activation within the lateral frontal cortex (Smith et al., 1998). Our results are consistent with those of Bennett et al. (2009) who also found that during a response inhibition task, children prenatally exposed to tobacco exhibited greater activity in the inferior frontal gyrus compared to non-exposed children. The finding of greater activity within these regions among the prenatally exposed group compared to the non-exposed group, suggests that they likely found the task more difficult and had to compensate for this difficulty by working harder to successfully perform this task, as evidenced by the recruitment of greater neural resources within regions of the brain that subserves response inhibition.

The prenatal nicotine group also exhibited greater activity in the lentiform nucleus (consisting of the putamen and globus pallidus), a region within the basal ganglia, compared to

the non-exposed group. The basal ganglia have consistently been shown to be activated during response inhibition tasks due to its role in motor suppression (Aron & Poldrack, 2006; Li et al., 2008; Menon et al., 2001). It is believed that response inhibition is achieved by the inferior frontal gyrus sending a signal to the basal ganglia via the subthalamic nucleus, which excites the globus pallidus and, in turn, cancels the motor program initiated by the Go stimulus (for reviews see Aron et al., 2007; Chambers et al., 2009). Thus, greater activity in the globus pallidus and the right inferior frontal gyrus suggests that the prenatal exposed group had to work harder to suppress the motor response.

The prenatally exposed group also showed greater activity in more posterior regions of the brain including large sections of the cerebellum, compared to the non-exposed group. The cerebellum has been shown to be important for motor response preparation and inhibition (Mostofsky et al., 2003; Simmonds et al., 2007). However, widespread diffuse activity within cerebellar regions is not typical of tasks involving response inhibition, as inhibitory control is often associated with greater activity within frontal regions of the brain (Liddle et al., 2001; Menon et al., 2001). Interestingly, developmental fMRI studies comparing neural activity between healthy children and adults during response inhibition tasks have often shown linear regressive changes with age in predominately posterior brain regions, with children using more cerebellar regions than adults and adults using more prefrontal regions than children to complete response inhibition tasks (Rubia et al., 2006). These developmental differences have been interpreted as delayed maturational processes in the prefrontal cortex of children, with immature prefrontal networks resulting in greater activation in more posterior areas to successfully complete the task (Rubia et al., 2006). The recruitment of greater posterior regions among the

prenatally exposed group to successfully complete the task likely reflects a compensatory mechanism for dysfunction within the prefrontal cortex.

After childhood, the prefrontal cortex undergoes significant maturational processes including increases in white matter volume, which is suggestive of increased myelination within this region (Pfefferbaum et al., 1994). Myelination is generally thought to progress from posterior to anterior brain regions, with frontal regions myelinating last (for review see Sowell et al., 2004). Myelination is directly involved in accelerating communication throughout the brain, allowing the prefrontal cortex to communicate more effectively with other cortical and subcortical brain regions. Enhanced communication between the prefrontal cortex and distant brain regions, aids in the formation of widely distributed, functionally integrated neural circuitry (for review see Luna & Sweeney, 2004). This neural circuitry, in turn, helps to facilitate higher order cognitive functioning skills such as response inhibition, which are dependent upon rapid synchronized communication between brain regions (for review see Deoni et al., 2012). In fact, studies combining diffusion tensor imaging and fMRI have shown that increases in myelination of frontal regions correspond with greater functional recruitment of these regions during executive functioning tasks (Olesen et al., 2003).

The finding of greater activity within a wide variety of cerebellar regions among the prenatally exposed group during response inhibition, compared to the non-exposed group, suggests that the greater difficulty with the task experienced by the prenatally exposed group may likely be the result of perturbed maturation of white matter within the prefrontal cortex, which alters efficient communication between neural circuitry linking the prefrontal cortex and other cortical and subcortical structures. Thus, prenatally exposed young adults must rely on earlier maturing posterior brain regions to compensate for immature frontal networks. This

perturbed maturation appears to continue even after the prefrontal cortex is fully developed as in adulthood.

Consistent with this assumption, diffusion tensor imaging studies have recently found myelin dysfunction and degradation in fibers within the premotor cortex and supplementary motor areas in adolescents with prenatal nicotine exposure; evidence for impaired white matter maturation (Liu et al., 2011). Similarly, Jacobsen and colleagues (2007) also found disturbed white matter integrity in both left and right frontal regions of adolescents prenatally exposed to nicotine. Using magnetic resonance spectroscopy, lower glial metabolite levels have also been found in the brains of young children with prenatal nicotine exposure, compared to non-exposed children, suggestive of abnormalities in glial development (Chang et al., 2012). Glial cells, such as oligodendrocytes, are important for the production of myelin. Recent evidence has shown that functional nAChRs are expressed on oligodendrocyte precursor cells early in development, providing a mechanism by which prenatal nicotine can alter their development (Rogers et al., 2001). In fact, in vitro studies with animals have shown that excessive nAChR stimulation results in oligodendroglial cell damage (Constantinou & Fern, 2009). Glutamate receptors are also present on oligodendrocyte precursor cells early in development and excessive stimulation of the glutamate receptor has been shown to inhibit oligodendrocyte proliferation (Gallo et al., 1994, 1996). Given that prenatal nicotine alters glutamate receptor function and glutamate release (Parameshwaran et al., 2012), this association may be another mode by which nicotine can exert its deleterious effects on the developing brain.

The present study also showed that young adults with prenatal nicotine exposure demonstrated significantly greater activation during response inhibition in the anterior cingulate, compared to non-exposed adults. The anterior cingulate has consistently been shown to be

engaged during response inhibition tasks, due to its role in the detection of conflict (Botvinick et al., 2004; Braver et al., 2001; Menon et al., 2001; Weissman et al., 2003). Tasks requiring the suppression of prepotent responses, such as the Go/No-Go task, often involve conflict, as two possible responses, the correct response and the one being suppressed compete with one another (for review see Botvinick et al., 2004). The greater the conflict, the greater the activation within the anterior cingulate, suggesting that conflict may serve as an index of task difficulty (Botvinick et al., 2004; Paus et al., 1998). Given that the prenatal nicotine group exhibited greater activity in the anterior cingulate, provides further evidence that they likely found the task of inhibiting a prepotent response more difficult and compensated for this difficulty by recruiting greater neural resources within regions of the brain responsible for response inhibition. These findings are consistent with Bennett et al. (2009) who also found greater activity within the anterior cingulate among prenatally exposed children compared to controls.

Further evidence of prenatal nicotine exposure impacting the response inhibition circuitry is that those subjects exposed prenatally also exhibited greater activity in the thalamus bilaterally, compared to the non-exposed group. It has been suggested that prepotent response inhibition is aided by thalamic modulation of subcortical input to cortical motor areas (Brunia, 1997; Logan & Cowan, 1984). Specifically, subcortical input, such as by the basal ganglia, acts to inhibit thalamic output, which in turn, suppresses cortical motor areas, resulting in the inhibition of a motor response (for reviews see Aron et al., 2007; Chambers et al., 2009). Imaging studies have supported this finding by showing that greater activity in the prefrontal cortex and basal ganglia corresponds to decreased activity in the thalamus and cortical motor regions during response inhibition (Stevens et al., 2007). Greater activity in this region during response inhibition among the prenatally exposed group suggests another form of altered neural

activation that perhaps if challenged with a more rigorous response inhibition task or a more ecologically valid task may reveal significant differences in performance variables, such as more errors of commission. Consistent with these findings and the hypothesis of perturbed brain maturation, developmental studies have often found that children exhibit greater bilateral activity in the thalamus compared to adults, whom exhibit activation only in the right thalamus, during response inhibition (Booth et al., 2003; Rubia et al., 2007). Interestingly, studies with rodents have also confirmed that nicotine exposure alters the development of glutamate synapses within thalamocortical circuitry, particularly those circuits projecting to primary sensory areas, by increasing glutamate release prematurely through the activation of nAChRs expressed by these neurons (Aramakis et al., 2000; Aramakis & Metherate, 1998). Dysfunction within the thalamus and its pathways may help explain the auditory processing deficits that have been found among children exposed to prenatal nicotine exposure (Fried & Makin, 1987; McCartney et al., 1994).

The strength of this study is the use of the participants from the OPPS, where information on prenatal nicotine exposure, as well as exposure to other prenatal substances, was gathered prospectively and offspring were followed from infancy to adulthood. This allowed from the measurement of an unparalleled number of lifestyle variables across the lifespan. The unique methodology strengthens the validity of the results and provides outcomes that are able to shed light on the effects of prenatal nicotine exposure on neural processing, with greater control than previous studies. Despite these strengths, several limitations should be addressed. First, the prenatal exposed group was also exposed to marijuana and alcohol in utero as was the control group. Despite examining their effect on neural activity and finding that these substances did not contribute to differences in neural activity between groups, future studies should endeavor to examine participants with only prenatal nicotine exposure. Second, the results cannot be

generalized to other ethnic or socioeconomic status populations as the OPPS is primarily a Caucasian, middle class population. Third, the present study used a block design rather than an event related design. A block design does not permit the separation of response inhibition from response selection or other cognitive processes. However, the design of the task, including the same motor output and sensory input for both conditions, ensured as much as possible that the only difference between the two cognitive tasks was response inhibition. In addition, a block design does not examine the effects of performance on brain activity. An event-related study may have helped to decipher the response inhibition from other cognitive processes and would allow us to take into consideration performance on brain activity. Fourth, although the sample size is generally in keeping with other imaging studies, it remains small and future studies should replicate the results with a greater number of participants.

In conclusion, this was the first fMRI study of the long-term impact of prenatal nicotine exposure on response inhibition. Smoking during pregnancy continues to remain high and this study demonstrates that the effects to the fetus have long lasting implications. Young adults with prenatal nicotine exposure demonstrated significantly greater activity than controls in several regions of the brain typically associated with response inhibition. Greater activity in these regions suggests that the nicotine exposed group had to work harder to successfully perform the task, likely due to dysfunction within the neural circuitry subserving response inhibition. In addition, prenatally exposed participants showed greater activity than controls in relatively large posterior regions of the cerebellum. Widespread activity in this region is not typically associated with response inhibition tasks and is suggestive of compensation for prefrontal dysfunction. Given a harder task, it is likely that the prenatal nicotine group may not be able to compensate and performance may suffer. This is important as response inhibition is

an executive functioning skill required for successful functioning in much of everyday life. Impairments in response inhibition could impede achievements. Thus, awareness of the continued long-term neural physiological effects of prenatal nicotine exposure is critical.

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Table 1: Prenatal and Current Drug Exposure for Prenatally Exposed and Non-Exposed Participants

Drug Exposure	**Prenatal Nicotine Exposure Group (n=12) M (SD)**	**Non-Exposed Control Group (n=13) M (SD)**	**ANOVA**
Prenatal Marijuana (joints/week)	6.39 (10.80)	3.13 (5.59)	F(1, 23)=0.92 (p=0.35)
Prenatal Alcohol (AA/day)	0.10 (0.08)	0.21 (0.22)	F(1, 23)=3.03 (p=0.11)
Current Nicotine (cigarettes/day)	2.08 (3.52)	1.35 (3.33)	F(1, 23)=0.30 (p=0.60)
Cotinine Urine Values (ug/L)	327.47 (623.02)	224.52 (595.03)	F(1, 23)=0.18 (p=0.69)
Current Marijuana (joints/week)	1.79 (3.82)	0.23 (0.60)	F(1, 23)=2.12 (p=0.16)
Marijuana Urine Values (ug/L)	63.41 (134.72)	18.23 (49.16)	F(1, 23)=1.28 (p=0.27)
Current Alcohol (drinks/week)	2.39 (2.28)	2.24 (2.88)	F(1, 23)=0.02 (p=0.88)

Table 2: Demographic, IQ and Externalizing and Internalizing Characteristics for Prenatally Exposed and Non-Exposed Participants

Measures	**Prenatal Nicotine Exposure Group (n=12) M (SD)**	**Non-Exposed Control Group (n=13) M (SD)**	**ANOVA**
WISC Full Scale IQ	110.46 (10.44)	115.70 (9.57)	F(1, 23)=1.51 (p=0.23)
Conner's (Learning Problems)	0.12 (1.13)	0.05 (0.76)	F(1, 23)=0.19 (p=0.67)
Conner's (Psychosomatic Problems)	0.56 (1.22)	0.44 (1.50)	F(1, 23)=0.05 (p=0.83)
Conner's (Conduct Problems)	0.23 (0.97)	0.12 (0.81)	F(1, 23)=0.50 (*p*=0.36)
Conner's (Anxiety)	0.44 (1.12)	0.25 (1.01)	F(1, 23)=0.19 (p=0.67)
Conner's (Hyperactivity)	-0.16 (0.80)	-0.20 (0.88)	F(1, 23)=0.01 (p=0.91)
Family Income	29,400 (15,223)	37,040 (17,279)	F(1, 23)=1.25 (*p*=0.28)
Mother`s Education (Total Years)	14.545 (0.72)	15.25 (0.69)	F(1, 23)=0.50 (*p*=0.49)

Table 3: Performance data for the two conditions of the Go/No-Go Task for Prenatally Exposed and Non-Exposed Participants, while controlling for cotinine values

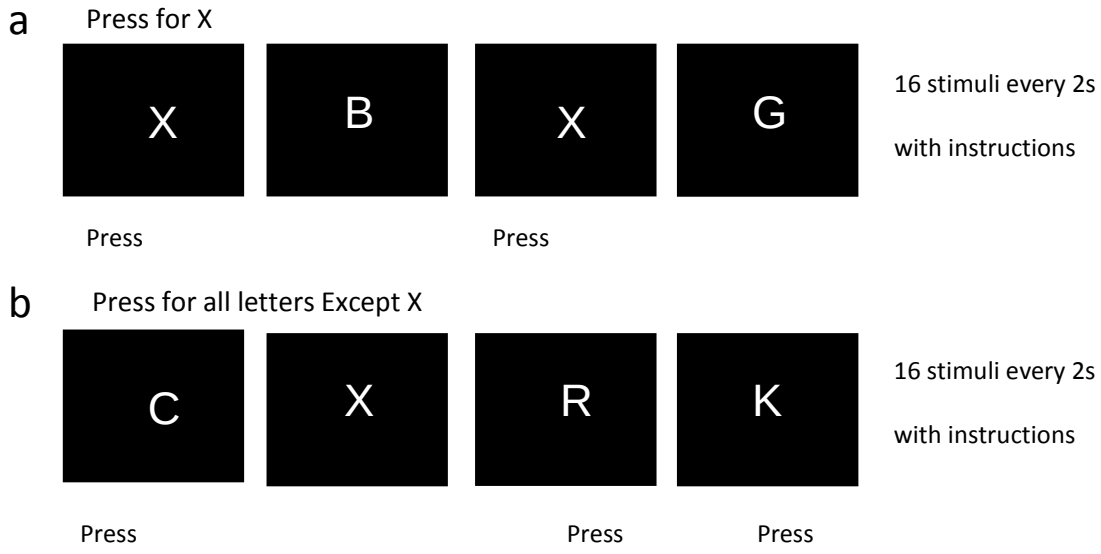
Performance Measure	**Prenatal Nicotine Exposure Group (n=12) M (SD)**	**Non-Exposed Control Group (n=13) M (SD)**	**MANCOVA**
Errors of Omission (Press for X)	0.08 (0.29)	0.15 (0.55)	F(2, 22)=0.14 (p=0.71)
Errors of Omission (Press for all letters except X)	0.15 (0.39)	0.46 (0.97)	F(2, 22)=1.25 (p=0.28)
Errors of Commission (Press for X)	0.44 (0.67)	0.52 (0.78)	F(2, 22)=0.09 (p=0.77)
Errors of Commission (Press for all letters except X)	4.50 (4.81)	4.00 (3.45)	F(2, 22)=0.18 (p=0.66)
Reaction Time (s, Press for X)	0.39 (0.04)	0.39 (0.04)	F(2, 22)=0.08 (p=0.78)
Reaction Time (s, Press for all letters except X)	0.39 (0.04)	0.42 (0.05)	F(2, 22)=1.32 (p=0.26)

Table 4: Significant t-test results comparing neural activity between the prenatally exposed participants and non-exposed participants for the response inhibition condition (Press for all letters accept X) minus the control condition (Press for X), while controlling for cotinine values.

Exposed > Non-Exposed

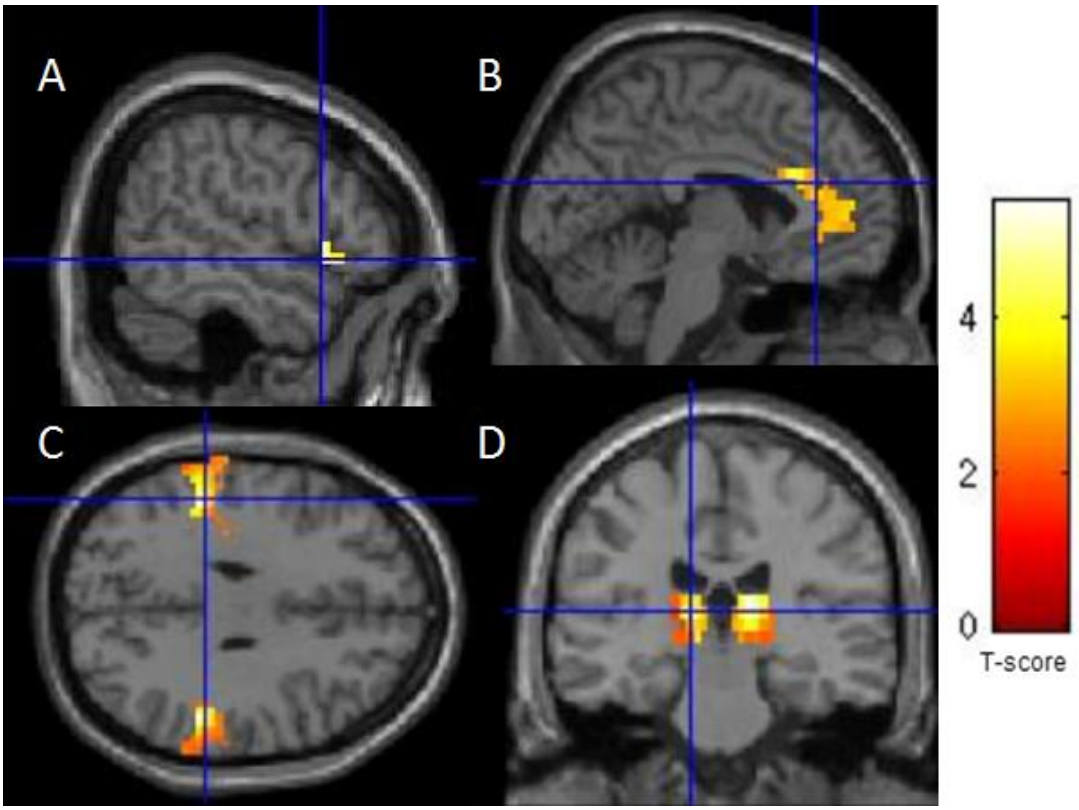
Region	**Coordinates**	**Z value**
R inferior frontal gyrus	48 9 -10	3.34
L anterior cingulate gyrus	-12 21 30	4.10
R thalamus	15 -12 15 9 -21 5	3.80 3.25
L thalamus	-12 -24 10	3.44
R inferior parietal lobule	51 -36 30	3.85
L inferior parietal lobule	-48 -39 30	3.66
R precuneus	18 -60 35	4.32
R cerebellar tonsil	39 -48 40 21 -39 -45	4.05 4.03
L cerebellar tonsil	-33 -45 -40	3.48
R culmen	33 -51 -35 21 -45 -25 12 -45 -25	4.03 3.38 3.31
L declive	-45 -63 -30 -39 -66 -30	3.48 3.45
L lentiform nucleus	-27 0 5	3.30

Figure 1: Go/No-Go Task



a) An example of 4 stimulus presentations for the Press for X condition with 'Press' indicating where an appropriate response should occur. b) An example of 4 stimulus presentations for the Press for all letters except X condition with 'Press' indicating where an appropriate response should occur.

Figure 2: Blue Cross hairs indicate where prenatal nicotine exposure participants demonstrated significantly greater activation than non-exposed participants during the response inhibition condition (Press for all letters accept X) minus the control condition (Press for X), while controlling for cotinine values.



A) Right Inferior Frontal Gyrus (x,y,z= 48, 9, -10) B) Left Anterior Cingulate (x,y,z=-12, 21, 30) C) Parietal Lobule Bilaterally (x,y,z=51, -36, 30 & x,y,z=-48,-39, 30) D) Thalamus Bilaterally (x,y,z=-12, -24, 10; x,y,z= 15, -12, 15 & x,y,z=9, -21, 5).

Chapter 3

Manuscript II

The Long-Term Effects of Prenatal Nicotine Exposure on Verbal Working Memory: An fMRI Study of Young Adults

Longo, C.A., Fried, P.A., Cameron, I., Smith AM. The long-term effects of prenatal nicotine exposure on verbal working memory: An fMRI study of young adults. *Submitted to Drug and Alcohol Dependence.*

Abstract

Using functional magnetic resonance imaging (fMRI), the long-term effects of prenatal nicotine exposure on verbal working memory were investigated in young adults. Participants were members of the Ottawa Prenatal Prospective Study, a longitudinal study that collected a unique body of information on participants from infancy to young adulthood. This allowed for the measurement of an unprecedented number of potentially confounding drug exposure variables including: prenatal marijuana and alcohol exposure and current marijuana, nicotine and alcohol use. Twelve young adults with prenatal nicotine exposure and 13 non-exposed controls performed a 2-Back working memory task while fMRI blood oxygen level-dependent responses were examined. Despite similar task performance, participants with more prenatal nicotine exposure demonstrated significantly greater activity in several regions of the brain that typically subserve verbal working memory including the middle frontal gyrus, precentral gyrus, the inferior parietal lobe and the cingulate gyrus. These results suggest that prenatal nicotine exposure contributes to altered neural functioning during verbal working memory that continues into adulthood. This alteration is compensated for by recruitment of greater neural resources within regions of the brain that subserve verbal working memory to successfully perform the task. Working memory is critical for a wide range of cognitive skills such as language comprehension, learning and reasoning. Thus, these findings highlight the need for continued educational programs and public awareness campaigns to reduce tobacco use among pregnant women.

Keywords: Prenatal nicotine exposure; verbal working memory; young adults; fMRI

The Long-Term Effects of Prenatal Nicotine Exposure on Verbal Working Memory: An fMRI Study of Young Adults

Exposure to cigarette smoke in utero is a leading cause of morbidity and mortality for the developing child. Offspring of women who smoke during pregnancy are at an increased risk of stillbirth (Salihu et al., 2008), chronic respiratory diseases including asthma (Gilliland et al., 2001), low birth weight (Bernstein et al., 2005) and sudden infant death syndrome (Anderson et al., 2005). In addition, prenatal nicotine exposure is associated with several impairments in executive functioning skills (Fried et al., 1998; Julvez et al., 2007). Despite these adverse effects, a relatively large proportion of Canadian women continue to smoke while pregnant (Health Canada, 2009).

Executive functions represent a cognitive construct that refers to the ability to maintain an appropriate problem solving set for the attainment of future goals (Welsh & Pennington, 1988). Executive functions involve several types of processes including: abstract thinking, planning and initiation, focused attention, response inhibition, cognitive flexibility and working memory (Denckla, 1993).

A particularly important executive functioning skill is verbal working memory, which is typically defined as a limited-capacity system that involves the active maintenance and manipulation of verbal information for a brief amount of time (Baddeley, 2003). According to Baddeley`s (1992; 2003) model, verbal working memory is comprised of both a central executive and a phonological loop. The phonological loop, which is specialized for processing verbal information, consists of two components: a phonological store and rehearsal mechanisms that refreshes the contents of this store. The central executive, on the other hand, is an attentional control system important for the regulation and coordination of the various processes

involved in the phonological loop. The central executive is important for manipulation, which involves the reordering and updating of information maintained in working memory (for review see Fletcher & Hansen, 2001). In contrast, the phonological loop is important for maintenance, which involves keeping information in mind in the absence of an external stimulus, either through rehearsal mechanisms or storage (for review see Fletcher & Hansen, 2001). Working memory is critical for a wide range of cognitive skills such as language comprehension, learning and reasoning (Baddeley, 1992) and individuals with working memory deficits are at an increased risk for reading difficulties (Gathercole et al., 2006; Kibby & Cohen, 2008).

An understanding of the neural basis of verbal working memory and support for Baddeley`s model comes from a large body of research using both lesion and neuroimaging studies. For example, the dorsolateral prefrontal cortex, which has been shown to be critical for the manipulation of information within working memory, in addition to maintenance (Collette et al., 1999; Jolles et al., 2011; Narayanan et al., 2005; Rottschy et al., 2012), is strongly affiliated with the central executive (for reviews see Baddeley 2003; Collette & Van der Linden, 2002). The left ventrolateral prefrontal cortex is important for the maintenance of verbal information within working memory (Narayanan et al., 2005; Veltman et al., 2003) specifically, the rehearsal component of the phonological loop (Awh et al., 1996; Geva et al., 2011; Jonides et al., 1997; Li et al., 2012). In addition, the phonological store depends largely on the left inferior parietal cortex (Chen & Desmond, 2005; Jonides et al., 1998; Kirshen et al., 2005; Marvel & Desmond, 2010). However, several other cortical and subcortical structures of the brain have also been shown to be involved in verbal working memory including the cerebellum (Ravizza et al., 2006), the frontal pole (Owen et al., 2005) and the anterior cingulate (Narayanan et al., 2005).

A large body of neurocognitive research, across different developmental levels, has shown that offspring prenatally exposed to cigarette smoke show deficits in working memory. Although not able to measure working memory skills among infants and toddler, tobacco exposed infants have been found to exhibit considerably poorer attention skills after birth, with the magnitude of the difference between exposed and non-exposed group attenuating across the neonatal period (Espy et al., 2011). Consistent with these findings, a large scale prospective study found that among 4 year old children, maternal smoking during pregnancy was associated with decreased working memory performance, as assessed by the McCarthy Scales of Children's Abilities (Julvez et al., 2007). Similarly, Streissguth and colleagues (1984) found a significant relationship between prenatal tobacco exposure and an increased number of omission errors during a vigilance task, suggesting difficulties with sustained attention, among 4 year old children.

Findings in older children continue to support a negative influence of prenatal tobacco exposure on working memory. Using a Dutch birth cohort, Batstra and colleagues (2003) found that children aged 5 to 11 years old whose mothers smoked during pregnancy were significantly more likely to exhibit attention difficulties compared to non-exposed children. Similarly, a longitudinal prospective cohort study conducted within the Pittsburgh area, found that among 6 year old children, second and third trimester prenatal tobacco exposure was associated with increased omission errors on a Continuous Performance Task, suggesting difficulties with sustained attention (Leech et al., 1999). The Ottawa Prenatal Prospective Study (OPPS), an ongoing, longitudinal investigation of the effects of prenatal drug exposure on offspring, initiated in 1978, has extensively studied cognitive functioning among children prenatally exposed to cigarette smoke. At 9 to 12 years of age, children were given a large battery of executive

functioning tasks. Overall, the researchers found a negative dose-response relationship between maternal smoking during pregnancy and performance on executive functioning tasks, after controlling for several prenatal covariates. In particular, higher amounts of maternal smoking during pregnancy was associated with lower scores on the Freedom from Distractibility Index and digit span subtest of the Wechsler Intelligence Scale for Children-3rd edition; measures of sustained attention and working memory (Fried, Watkinson & Gray, 1998). Consistent with these findings, numerous studies have found a strong association between maternal smoking during pregnancy and attention deficit hyperactivity disorder (ADHD) (Linnet et al., 2003; Nomura et al., 2010; Piper & Corbett, 2012). This is interesting in light of the fact that some of the hallmark features of ADHD are difficulties with inattention and working memory (American Psychiatric Association, 2001).

Only a small number of studies have examined the effects of prenatal cigarette exposure on working memory among adolescents. The OPPS sample found, that among 13 to 16 year old adolescents, a working memory factor was negatively associated with maternal cigarette smoking, such that heavily exposed adolescents performed worse on working memory tasks compared to lightly exposed and non-exposed adolescents. These results remained even after controlling for several confounding factors (Fried & Watkinson, 2001). However, using a more extensive executive functioning battery with the same cohort, at the same age, performance on other working memory measures were no longer associated with maternal smoking in utero (Fried, Watkinson, & Gray, 2003). In contrast, in a follow-up study of the Pittsburgh sample, Cornelius et al. (2012a) found that at 14 years of age, children born to mothers who smoked during pregnancy were significantly more likely to be rated by their mothers with attention problems and distractibility, compared to nonexposed youth. Taken together, results are

inconsistent with regards to whether the deficits in working memory associated with in utero exposure to tobacco smoke continue into adolescents and beyond. Studying offspring after the adolescent period is important, given that the prefrontal cortex, which subserves executive functioning, develops exponentially during the adolescent years (for review see Diamond, 2002).

Despite a large body of neurocognitive research examining the effects of prenatal nicotine exposure on the offspring, very few studies have examined the neurophysiological effects of prenatal tobacco exposure in humans during executive functioning tasks. Using functional magnetic resonance imaging (fMRI), Bennett and colleagues (2012) compared neural function among 12-year old children exposed and non-exposed to tobacco in utero during a working memory task. The researchers found that after controlling for several confounding factors, tobacco exposed children showed greater activity in inferior parietal regions, while unexposed children primarily showed greater activity in inferior, middle and superior frontal regions. These differences were observed in the context of correct responses and, according to the authors, suggest that exposed and unexposed children use different brain regions to succeed in working memory tasks. Using an auditory attention task, Jacobsen and colleagues (2007) examined brain activity among adolescent smokers and non-smokers with and without prenatal nicotine exposure. The results revealed that adolescents with prenatal or adolescent exposure to tobacco smoke showed greater activity in the superior temporal gyrus, an area supporting auditory attention, relative to participants with neither prenatal nor adolescent exposure. In addition, adolescents exposed prenatally to tobacco smoke only showed greater activity in the lingual gyrus compared to participants with neither prenatal exposure nor adolescent exposure. According to the authors, greater activity represents loss of efficiency in cortical areas that support auditory attention. However, given the limited research, a consistent pattern of

activation has yet to be determined and thus a full understanding of the effects of prenatal nicotine exposure on neural processing during working memory has yet to be attained. In addition, no prospective study has used fMRI to investigate the long-term neural effects of prenatal nicotine exposure on verbal working memory in a sample of young adults.

The main substance largely believed to be responsible for the cognitive deficits observed in offspring with in utero exposure to cigarette smoke is nicotine (Slotkin et al., 1986; Slotkin, Cho, & Whitmore, 1987). Nicotine has the ability to bind to nicotinic acetylcholine receptors (nAChRs), which are expressed in the human brain early in development (Hellström-Lindahl et al., 1998) and trigger neurodevelopmental events that are normally ascribed to acetylcholine (for review see Dwyer, McQuown, & Leslie, 2009). Acetylcholine, like other neurotransmitters, act to influence early cellular processes including neural cell proliferation, migration, differentiation, apoptosis and synaptogenesis (for review see Pauly & Slotkin, 2008). These processes help to ensure the proper formation of several neuronal circuits and networks within the developing nervous system. Prenatal nicotine exposure is believed to alter the timing of these neurodevelopmental events, by binding to nAChRs receptors prematurely, resulting in dysfunction of numerous pathways within the central nervous system (Navarro et al. 1988; Munenoka et al., 1999; Ribary & Lichtensteiger, 1989; Slotkin, 2008).

The aim of the present study was to examine the long-term neuronal effects of prenatal tobacco exposure on verbal working memory in young adults from the OPPS, using fMRI blood oxygen level dependent (BOLD) response. Using participants from the OPPS allowed for the measurement of several risk factors that tend to co-occur with prenatal tobacco exposure including: exposure to other prenatal substances, low IQ scores, behavioural problems, and current use of tobacco and marijuana (Cornelius et al., 2000; 2012b; Erickson & Arbour, 2012;

Fried et al., 1984; 1998; Goldschmidt et al., 2012; Nomura et al., 2010; O'Callaghan et al., 2009; Porath & Fried, 2005; Rydell et al., 2012). Verbal working memory was assessed using a 2-Back task. Evidence from fMRI research conducted on healthy controls has found that the 2-Back task depends on prefrontal and parietal cortical integrity, specifically, the dorsolateral and ventrolateral prefrontal cortex, frontal poles, medial and lateral posterior parietal cortex, cingulate gyrus and lateral and medial premotor cortex (Casey et al., 1995; Cohen et al., 1994; Dumas et al., 2012; Jonides et al., 1997; Owen et al., 2005; Paskavitz et al., 2010; Rivizza et al., 2004; Veltman et al., 2003). It was hypothesized that prenatal nicotine exposure would impact the efficiency of neurocircuitry supporting verbal working memory and this would result in a greater activity in the dorsolateral prefrontal cortex, ventrolateral prefrontal cortex, inferior parietal lobe and the anterior cingulate.

Methods

Participants

Participants were recruited from the OPPS. Initial recruitment procedures have been described in detail elsewhere (Fried et al. 1980; 1988). Briefly, pregnant women volunteered to participate in the study after becoming aware of the research either by notices in the media or by signs placed in the offices of their obstetricians. The study was described as investigating prenatal lifestyle habits and their effects on offspring. Upon volunteering to participate, mothers-to-be were interviewed, usually in their homes, once during each trimester. The repeated interviews during pregnancy provided a means of checking the reliability of the self-report and were typically conducted by the same researcher, thereby helping to establish rapport with the participant. The information collected by a trained female interviewer included socioeconomic status, mothers age, education, physical and mental health of both parents, a 24 hour dietary

recall and present drug use during pregnancy with particular emphasis upon cigarette, alcohol and marijuana usage. Families were Caucasian, from middle-class, low-risk homes, and no parent of the participant was reported to have an Axis I diagnosis based on the Diagnostic and Statistical Manual of Mental Disorders (DSM) at the time of pregnancy. In addition, mothers were excluded if they used substances other than tobacco, marijuana or alcohol during pregnancy.

Twelve prenatally exposed participants (6 females and 6 males; mean age 21) and 13 non-exposed controls (7 females and 6 males; mean age 21) were included in the analysis. The prenatally exposed group was defined as participants who were exposed at any point in utero to any amount of nicotine. The control group was defined as participants with no exposure at any point in utero to any amount of nicotine. For prenatal nicotine exposure, a nicotine score was determined by multiplying the number of tobacco cigarettes smoked on average per day, at the time of pregnancy, by the nicotine content of the brand specified. A package of cigarettes of average strength contains approximately 16 mg of nicotine. The 12 prenatally exposed participants were exposed to an average of 14.13 mg of nicotine in utero daily (range of 0.03-35 mg of nicotine/daily), while the 13 non-exposed participants were never exposed to any amount of nicotine in utero.

For both groups, exposure to marijuana and alcohol in utero was also recorded. Marijuana use at the time of pregnancy was recorded in terms of the number of joints smoked per week. Both the quantity and the pattern of alcohol consumption during pregnancy (including beer, wine and liquor) were recorded and converted to ounces of absolute alcohol (AA) per day. Analysis of variance (ANOVA) revealed no significant differences between groups on prenatal marijuana exposure or prenatal alcohol exposure (see Table 1). However, given that both these

variables have been shown to impact brain activity (Smith et al., 2004, 2006; Spadoni et al., 2009), they were included as covariates in the fMRI analysis.

Participants with prenatal nicotine exposure are more likely to smoke cigarettes and use marijuana (Cornelius et al., 2000; 2012b; Goldschmidt et al., 2012; O'Callaghan et al., 2009; Porath & Fried, 2005; Rydell et al., 2012). Therefore, to increase external validity, participants who smoked cigarettes and marijuana were included. The regular use of these substances was measured among all participants. Regular use of cigarettes was defined as 1 cigarette/day or more. In order to eliminate the acute withdrawal symptoms that come from cessation of regular tobacco use, which includes disruption of attention and memory (Jacobsen et al., 2005), participants were permitted to smoke during the day, however, no tobacco smoking occurred 2 hours prior to testing (confirmed by presence in the hospital MRI unit). Regular use of marijuana was defined as 1 joint/week or more. To eliminate the acute effects of marijuana on cognitive functioning (Ramaekers et al., 2009), participants were excluded if they smoked marijuana on the day of testing. Given that marijuana use and tobacco use have been shown to impact brain activity (Jacobsen et al., 2007; Smith et al., 2010), values of marijuana and cotinine in the urine samples were used as covariates in the fMRI analysis. Alcohol use is also prevalent among young adults (Johnston et al., 2003) and thus participants who drank alcohol regularly were not excluded. The regular use of alcohol was measured among all participants and defined as 1 drink/week or more. To eliminate the acute effects of alcohol on cognitive functioning (Leitz et al., 2009) participants were excluded if they drank alcohol on the day of testing. Alcohol use has been shown to impact brain activity (Tapert et al., 2004) and therefore it was also used as a covariate in the fMRI analysis.

Participants previously completed a comprehensive psychological battery including the Wechsler Intelligence Scale for Children-III (Wechsler, 1991) (between the ages of 13 and 16) and the National Institute of Mental Health Computerized Diagnostic Interview Schedule for Children (C-DISC) (Bacon, 1997), which assessed for current psychiatric illnesses based on DSM-IV criteria. Parents also previously completed the Conners' Parent Rating Scale-Revised (Conners, 1997) (between the ages of 13 and 16) and family income and mother's education at the time of pregnancy was measured. An ANOVA did not reveal any significant differences between the prenatally exposed group and the non-exposed group on these scales, however, the WISC-III Full Scale IQ score and family income were included as covariates in the in the fMRI analysis (see Table 2).

Exclusion criteria for both groups included (a) contraindication to fMRI, specifically, having a pacemaker, claustrophobic, mental or electrical implants, accidents leaving metal in the eye, recent surgery, metal dental work (aside from fillings), piercings that cannot be removed, eye glasses or insufficient vision for viewing the tasks, (b) abnormalities in their structural MRI scan, (c) meeting DSM criteria for an Axis I disorder using the C-DISC, (d) current use of prescription medication with central nervous system effects including psychotropic medication, as well as reported regular use of illicit drugs (defined as once a month or more) including amphetamines, crack, cocaine, heroin, mushrooms, hashish, lysergic acid, steroids, solvents and tranquilizers, (e) testing positive for cocaine, opiates or amphetamines in their urine, (f) history of seizures, diabetes requiring insulin treatment, heart attack, stroke, blood clots, high blood pressure, cancer, brain injury and chronic pain, (g) first language other than English and (h) left-handedness. In addition, all participants signed informed consent prior to being imaged. The study was approved by the Ottawa Hospital Research Ethics board.

2-Back Task. A 2-back task was used to measure verbal working memory processes while in the scanner. The 2-back paradigm involves presentation of letters in white, one at a time, on a black screen for a period of 1500 milliseconds, with an interstimulus interval of 500 milliseconds (see Figure 1). The task was a block design and included two conditions: a control condition (Press for X) and a working memory condition (2-back). The control condition began with the instruction "Press for X" on the screen for 3 seconds. In this condition, participants were required to press a button with their right index finger when an "X" was presented on the screen. The working memory condition began with the instruction "Press for 2-Back" on the screen for 3 seconds. In this condition participants were required to press a button with their right index finger when the same letter reappeared two letters later. No X`s were presented during this condition. There was also a rest period of 21 seconds following each of the two task conditions. During this rest period the word "REST" was presented on the screen and no response was required. Both the control (Press for X) and working memory condition (2-Back) were comprised of 16 stimuli, presented every 2 seconds, for a total duration of 32 seconds. Each condition was presented six times. The total duration of the task was 7 minutes and thirty seconds. The order of blocks was counterbalanced. This presentation allowed for the manipulation of working memory load by changing the instructions while maintaining all other features of the task the same, including number of stimuli, number and type of response. This ensured that following the subtraction of the neural activity during the control condition (Press for X) from that during the working memory condition (2-Back) only the neural activity involved in working memory processing was observed in the statistical parametric maps. The 2-Back task has been shown to reliability initiate verbal working memory activation in healthy controls (Casey et al., 1995; Cohen et al., 1997; Jonides et al., 1997)

The task was presented to the participants on a back projection screen, located at the foot of the MRI table, via a mirror attached to the head coil. All lighting was turned off and button responses were recorded via a MRI-compatible fiber optic device (Light-wave Medical, Vancouver, British Columbia).

Drug History Questionnaire (DHQ). The DHQ was used to measure participants' present usage of both licit and illicit substances (see Appendix A). Substances assessed included the regular use of amphetamines, crack, cocaine, heroin, mushrooms, hashish, lysergic acid, steroids, solvents, tranquilizers, marijuana, nicotine and alcohol (as defined above). Both the amount and the number of years of use were recorded among regular users. This survey was adapted from the Monitoring the Future Survey which was used as a national survey focused on the lifestyles, attitudes, and preferences of American youth (Johnston et al., 2003). The substance use portion of the survey has been shown to have good psychometric properties (O'Malley, Bachman, & Johnston, 1983). Group differences for the amount of nicotine, marijuana and alcohol consumed among regular users was computed by ANOVA using SPSS v. 20, with a statistical threshold set at $p<0.05$.

Urine Sample. Current drug use was verified through a urine sample which was tested for amphetaminies, opiates, cocaine, cannabis, creatinine and cotinine (metabolite of nicotine). All metabolite concentrations were adjusted for creatinine to control for urine dilution. Group differences for marijuana and cotinine urine values were computed by ANOVA using SPSS v. 20, with a statistical threshold set at $p<0.05$.

Imaging Parameters. All imaging was performed using a 1.5 Tesla Siemens Magnetom Symphony MR scanner. Participants lay supine with their head secured in a standard MRI head holder. A conventional T1-weighted spin echo localizer was acquired and used to align the slice

orientation for the fMRI scans such that the anterior commissure-posterior commissure (AC-PC) line in the sagittal view were at right angles to the slice selection gradient. Whole brain fMRI was performed using a T2*-weighted echo planar pulse sequence (TR/TE 3000/40ms, flip angle 90^{o}, FOV 24 x 24 cm^{2}, 64 x 64 matrix, slice thickness 5 mm, 27 axial slices, bandwidth 62.5 kHz).

Behavioural Performance Parameters and Analysis. For the 2-back task, reaction time for each response, errors of commission and omission were recorded. Errors of commission included any response following the presentation of a non-target stimulus within 900 milliseconds of stimulus presentation. Omission errors were defined as a failure to respond to a target stimulus within 900 milliseconds. Mean reaction times were calculated for all accurate responses occurring within 900 milliseconds of stimulus presentation. Errors of omission, errors of commission and mean reaction times for both the control condition (Press for X) and the verbal working memory condition (2-back task) were calculated. Group differences were computed by multivariate analysis of covariance using SPSS v. 20, with a statistical threshold set at $p<0.05$.

Whole Brain Analysis. Statistical Parametric Mapping (SPM8) was used to post-process the fMRI data and to perform the statistical analysis. For post-processing, functional images from the first 9 seconds of the initial rest block were discarded to ensure that longitudinal magnetic relaxation (T1 effects) had stabilized. The remaining functional images were realigned to correct for motion by employing the procedures of Friston et al. (1995). The motion correction did not exceed 1 millimeter for any subject. Images were spatially normalized, due to differences in human brain morphology, to match the echo planar imaging (EPI) template provided in SPM8. Following spatial normalization, images were smoothed to eliminate noise

interference in the data, with an 8mm full-width at half-maximum Gaussian filter.

Individual participant first-level fixed effects analyses were performed for the comparison of the working memory condition (2-back) minus the control condition (Press for X). One contrast image was created per person, and these images were then used for second-level random effects analyses that were corrected for multiple comparisons. Random effects analyses eliminate highly discrepant variances between and within individuals in constructing an appropriate error term for hypothesis testing and generalizability to the population. Multiple regression was used to assess the relationship between the amount of prenatal nicotine exposure and neural activity during verbal working memory, at a statistical threshold set at $p<0.05$, both with and without covariates. Prenatal nicotine exposure was entered as a continuous variable. Prenatal marijuana exposure, prenatal alcohol exposure, current alcohol use, current marijuana exposure (e.g. marijuana urine), current nicotine exposure (e.g. cotinine urine), family income and WISC-III Full Scale IQ scores were all used as continuous variables and were entered as covariates. Each covariate was included in the regression separately to maintain power and to determine if these variables were contributing to the neurophysiological results. Covariates that were not found to be significantly associated with brain activity were removed from the final analysis.

Procedure

Upon arrival to the hospital, informed consent was attained and MRI compatibility was confirmed. Then, participants provided a urine sample which was immediately sent to the Hospital Laboratory for testing. Prior to commencing imaging, participants were required to view the 2-back task outside the scanner and performed a practice session of 10 trials of each condition. This ensured that all participants were able to perform the task accurately.

Participants then entered the scanner and task instructions were repeated including to press the response pad as quickly as possible and, if they made a mistake, to continue without thinking about the mistake. Once imaging was completed, participants filled out a drug history questionnaire. Completing the drug questionnaire after scanning ensured the blindness of the fMRI researcher.

Results

Drug questionnaire and urine sample data

Regular marijuana use was reported by three participants from the prenatally exposed group and two participants from the control group. The average number of years of regular marijuana use for the 3 prenatally exposed participants was 5 years (range 4 to 6 years), while the average number of years of regular marijuana use for the 2 non-exposed participants was 3.5 years (range 2 to 5 years). No participant reported smoking marijuana at least 24 hours prior to testing. There were no significant differences between groups for current marijuana use or urine cannabis (see Table 1). All participants from both groups reported regular alcohol use. However, no participant from either group reported drinking more than 8 alcoholic drinks per week on a regular basis. In addition, no participant from either group reported drinking alcohol at least 24 hours prior to testing. No significant differences between groups emerged on current alcohol use (see Table 1). Regular tobacco use was reported by four participants from the prenatally exposed group and two participants from the control group. The average number of years of regular tobacco use for the 4 prenatally exposed participants was 4.7 years (range 1 to 6 years), while the average number of years of regular tobacco use for the 2 non-exposed participants was 5.5 years (range 5 to 6 years). No significant group difference emerged for

current tobacco use or cotinine values (see Table 1). No participant from either group reported using illicit drugs on a regular basis or within the month prior to testing.

Behavioural Performance Data

There were no significant performance differences between participants prenatally exposed to nicotine and non-exposed controls on reaction time, errors of omission and errors of commission while controlling for cotinine values (see Table 3).

Whole Brain Analysis

A first-level fixed effects group analysis for all participants, at a *p* value corrected for cluster level at 0.001, for the comparison of the verbal working memory condition (2-back) minus the control condition (Press for X), confirmed the task was activating areas of the brain typically observed during verbal working memory. These areas included the middle, inferior and superior frontal gyri, the inferior and superior parietal lobe, the precuneus, the cerebellum and the thalamus.

When examining the relationship between the amount of prenatal nicotine exposure and neural activity during the verbal working memory in the second-level random effects regression analysis without covariates, there was a significant positive relationship between prenatal nicotine exposure and brain activity. Current nicotine (cotinine values) was the only variable to contribute to this relationship when added as a covariate and thus was included in the final analysis. Prenatal marijuana, prenatal alcohol, current alcohol, current marijuana, IQ and family income did not impact the results and were not included.

The most robust effect of the study was a significant positive relationship between the amount of prenatal nicotine exposure and neural activity, while controlling for cotinine values, during the verbal working memory condition (2-Back) minus the control condition (Press for X),

at a *p* value corrected for cluster level <0.05 in a large cluster of 1875 voxels, in the following regions: the left middle frontal gyrus, the left precentral gyrus, the left inferior parietal lobule and the left cingulate gyrus (see Table 4 & Figure 2). All coordinates are in Montreal Neurological Institute (MNI) space. No significant negative relationship between prenatal nicotine exposure and brain activity was observed.

Discussion

The purpose of the present study was to examine the long-term neurophysiological effects of prenatal nicotine exposure on verbal working memory in young adults from the OPPS, using fMRI. Despite similar task performance, greater prenatal nicotine exposure was associated with significantly greater BOLD response in a relatively large set of brain regions.

The most significant result was observed in the left middle frontal gyrus, corresponding to the dorsolateral and ventrolateral prefrontal cortex. Greater prenatal nicotine exposure was associated with significantly more activity in these areas during verbal working memory. These regions of the brain have consistently been implicated in working memory processes (Jolles et al., 2011; Jonides et al., 1997; Kirschen et al., 2005; Narayanan et al., 2005; Owen et al., 2005; Paskavitz et al., 2010), with verbal working memory showing a predominance for left hemispheric frontal regions (Jennings et al., 2006; Smith et al. 1996). The left ventrolateral prefrontal cortex has been shown to be important for the maintenance of verbal information in working memory (Narayanan et al., 2005; Veltman et al., 2003) and is most closely associated with the phonological loop (Li et al., 2012). In contrast, the left dorsolateral prefrontal cortex is critical for manipulation processes, in addition to maintenance (Barbey et al., 2012; Collette et al., 1999; D' Esposito et al., 1999; Narayanan et al., 2005) and is believed to mediate central executive functions (for reviews see Baddeley, 2003; Collette & Van der Linden, 2002). Studies

examining the relationship between working memory difficulty and brain activation have often demonstrated that increasing working memory load produces increasing brain activity in prefrontal regions (Narayanan et al., 2005; O'Hare et al., 2008; Rypma et al., 1999; Wolf et al., 2010). Thus, the greater activity in frontal regions exhibited by participants with greater prenatal nicotine exposure, suggesting that they likely found the task more difficult and had to work harder to successfully perform the task, as evidenced by the recruitment of greater neural resources in regions of the brain that subserve the central executive and the phonological loop. In line with this assumption, imaging studies conducted on healthy participants have found that working memory task performance negatively predicts fMRI BOLD response; such that individuals with better working memory abilities require fewer neural resources in regions of the brain that subserve working memory to achieve sufficient working memory performance (Nagel et al., 2005). Similarly, studies examining the impact of working memory training on neural activity have often found practice-related decreases in working memory-related brain areas, which are accompanied by improved behavioural performance (Schneiders et al., 2011). According to the authors, these results suggest increased neural efficiency as a result of working memory training.

Our results are consistent with Jacobsen et al. (2007) who found that adolescents with prenatal tobacco exposure exhibited greater activity in the neural circuitry supporting auditory attention, compared to adolescents with neither prenatal nor adolescent exposure to tobacco smoke. In contrast, Bennett et al. (2012) found that children with prenatal tobacco exposure exhibited greater activity in more posterior brain regions than frontal regions during working memory, compared to non-exposed participants. The different findings, however, are likely

attributed to age difference between participants in Bennett's study and those in our study and Jacobsen's study.

The results of the present study are reflective of dysfunction in the neural circuitry subserving working memory. Similarly, Toro and colleagues (2008) measured cortical thickness using MRI in adolescents with and without exposure to maternal smoking in utero and found that the middle frontal and orbitofrontal cortex of the exposed group were thinner compared to the unexposed group. According to the authors, thinning in the prefrontal cortex likely reflects nicotine's dysregulation of various neurotransmitters systems which subsequently decreases neuronal growth.

Consistent with the above hypothesis, developing dopamine neurons within the substantia nigra and the ventral tegmental area express functional nAChRs early in the prenatal period, providing a mechanism by which in utero nicotine exposure can alter their development (Azam et al., 2007). In fact, a large body of animal research has shown that prenatal nicotine exposure consistently produces long-term alterations in the dopaminergic system within the forebrain of the rat (Ribary & Lichtensteigner, 1989). In particular, gestational nicotine exposure leads to a significant overall reduction in dopamine levels and reduced neural activity, as evidenced by the suppression of markers of dopamine activity (Muneoka et al., 1997; Navarro et al., 1988), with the mesocortical dopaminergic system being particularly vulnerable to nicotine exposure during pregnancy (Muneoka et al., 1999). This finding is specifically important given that the mesocortical dopaminergic system transmits dopamine from the ventral tegmental area to the frontal cortex (for review see Janhunen & Ahtee, 2007) and dopamine within the frontal cortex has been shown to play a critical role in executive functioning tasks, with increased dopamine release resulting in faster and more stable working memory performance (Aalto et al., 2005;

Luciana et al., 1992). Alterations within the dopaminergic system may help better understand the link between prenatal nicotine exposure and ADHD (Nomura et al., 2010; Piper & Corbett, 2012), as ADHD is believed to result from dopamine dysfunction (Jucaite et al., 2005).

Greater amounts of prenatal nicotine exposure were also associated with more activity in the left premotor cortex and left supplementary motor areas. These areas have consistently been shown to be activated during verbal working memory tasks (Chen & Desmond, 2005; Jonides et al., 1997; Owen et al., 2005) due to their involvement in preparing and executing a planned motor response (Duque et al., 2012). In fact, activation in the left premotor cortex corresponds with participants being right-handed and using their right hand for responses during the fMRI task. The left precentral gyrus, along with left ventrolateral prefrontal cortex, however, has also been associated with the phonological loop, in particular mediating the rehearsal of verbal information held in mind at a subvocal level (i.e. inner speech) (Awh et al., 1996; Chen & Desmond, 2005; Li et al., 2012; Ravizza et al., 2004), which is an important verbal working memory strategy. Interestingly, recent research using diffusion tensor imaging has found myelin dysfunction and degradation in fibers within the premotor cortex and supplementary motor areas in adolescents with prenatal nicotine exposure; evidence for impaired white matter (Liu et al., 2011). Increased myelination has been associated with better working memory performance (Nagy et al., 2004). Thus, impaired white matter within this region could impede information flow, resulting in participants with greater prenatal nicotine exposure having to work harder at verbal rehearsal, a strategy used in working memory tasks to successfully perform the task.

More prenatal nicotine exposure was also associated with greater activity in the left inferior parietal lobe. This area has been shown to be consistently involved in verbal working memory due to its role in the short-term storage of verbal information in working memory (Chen

& Desmond, 2005; Jonides et al., 1998; Kirshen et al., 2005; Paulesu et al., 1993; Marvel & Desmond, 2010; Ravizza et al., 2004). In addition, neuroimaging studies examining load effects have often found that activity in the parietal lobe increases with increasing working memory load (Kirschen et al., 2005; Veltman et al., 2003), suggesting that harder tasks require more neural resources to adequately maintain information in working memory. Our findings are consistent with Bennett et al. (2012) who also found that children with prenatal tobacco exposure exhibited greater activity in the inferior parietal lobe during working memory, compared to non-exposed children.

Finally, greater activity in the left cingulate gyrus was also associated with increasing amounts of prenatal nicotine exposure. The cingulate gyrus has consistently been shown to be involved in verbal working memory tasks (Chen & Desmond, 2005; Jolles et al., 2011; Jonides et al., 1997; Narayanan et al., 2005). This region of the brain is believed to serve as an index of task difficulty and increased effort (for review see Botvinick et al., 2004), with tasks involving greater effort and increasing difficulty leading to greater activity within this region (Barch et al., 1997; Paus et al., 1998). These findings provide further evidence that participants with more prenatal nicotine exposure likely found the task more difficult and compensated for this difficulty by recruiting greater neural resources within regions of the brain responsible for working memory. If challenged with a harder task, young adults with greater amounts of prenatal nicotine exposure may not be able to compensate and performance may suffer. Recent research has shown that glutamate-glutamine metabolite levels are altered in the anterior cingulate cortex of young children prenatally exposed to nicotine, with higher levels associated with greater impairments on cognitive tasks (Chang et al., 2012). The anterior cingulate is connected to the frontal cortex through numerous pathways and these pathways depend upon

intact glutamatergic systems for information processing. Thus, dysfunction in the glutamate system within the anterior cingulate may be another possible mechanism contributing to the greater difficulty experienced by the prenatally exposed group in the working memory task. Further investigation into the impact of prenatal nicotine exposure on different neurotransmitter systems is warranted.

The strength of this study is the use of the participants from the OPPS, where information on prenatal nicotine exposure, as well as exposure to other prenatal substances, was gathered prospectively and offspring were followed from infancy to adulthood. This allowed for the measurement of an unparalleled number of lifestyle variables across the lifespan. The unique methodology strengthens the validity of the results and provides outcomes that can shed light on the effects of prenatal nicotine exposure on neural processing, with greater control than previous studies. Despite these strengths, several limitations should be addressed. First, the prenatal exposed group was also exposed to marijuana and alcohol in utero as was the control group. Despite examining their effect on neural activity and finding that these substances did not contribute to differences in neural activity between groups, future studies should endeavor to examine participants with only prenatal nicotine exposure. Second, the results cannot be generalized to other ethnic or socioeconomic status populations as the OPPS is primarily a Caucasian, middle class population. Third, a block design was used. A block design does not permit the separation of working memory from other cognitive processes. However, the design of the task, including the same motor output and sensory input for both conditions, ensured as much as possible that the only difference between the two cognitive tasks was working memory. In addition, a block design does not examine the effects of performance on brain activity. An event-related study may have helped to decipher working memory from other cognitive

processes and would have allowed us to take into consideration performance on brain activity. Third, although the sample size is generally in keeping with other imaging studies, it remains small and future studies should replicate the results with a greater number of participants.

In conclusion, this was the first fMRI study of the long- term impact of prenatal nicotine exposure on verbal working memory. Smoking during pregnancy continues to remain a worldwide public health concern and this study demonstrates that prenatal nicotine exposure produces long lasting alterations in neural processing which continues into young adulthood. Greater prenatal nicotine exposure was associated with increased activity in several left hemispheric regions including the middle frontal gyrus, the precentral gyrus, the inferior parietal lobe and the cingulate gyrus. These regions are typically associated with verbal working memory. More activity in these regions suggests that individuals with greater prenatal nicotine exposure likely found the task more difficult and had to compensate for this difficulty by working harder to successfully perform the task. The recruitment of enhanced neural resources to complete the task, suggests dysfunction within the neural circuitry subserving verbal working memory. Working memory is critical for a wide range of cognitive skills such as language comprehension, learning and reasoning. Thus, these findings highlight the need for continued educational programs and public awareness campaigns to reduce tobacco use among pregnant women.

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Table 1: Prenatal and Current Drug Exposure for Prenatally Exposed and Non-Exposed Participants

Drug Exposure	**Prenatal Nicotine Exposure Group (n=12) M (SD)**	**Non Exposed Control Group (n=13) M (SD)**	**ANOVA**
Prenatal Marijuana (joints/week)	6.39 (10.80)	3.13 (5.59)	F(1, 23)=0.92 (p=0.35)
Prenatal Alcohol (AA/day)	0.10 (0.08)	0.21 (0.22)	F(1, 23)=3.03 (p=0.11)
Current Nicotine (cigarettes/day)	2.08 (3.52)	1.35 (3.33)	F(1, 23)=0.30 (p=0.60)
Cotinine Urine Values (ug/L)	327.47 (623.02)	224.52 (595.03)	F(1, 23)=0.18 (p=0.69)
Current Marijuana (joints/week)	1.79 (3.82)	0.23 (0.60)	F(1, 23)=2.12 (p=0.16)
Marijuana Urine Values (ug/L)	63.41 (134.72)	18.23 (49.16)	F(1, 23)=1.28 (p=0.27)
Current Alcohol (drinks/week)	2.39 (2.28)	2.24 (2.88)	F(1, 23)=0.02 (p=0.88)

Table 2: Demographic, IQ and Externalizing and Internalizing Characteristics for Prenatally Exposed and Non-Exposed Participants

Measures	**Prenatal Nicotine Exposure Group (n=12) M (SD)**	**Non-Exposed Control Group (n=13) M (SD)**	**ANOVA**
WISC Full Scale IQ	110.46 (10.44)	115.70 (9.57)	F(1, 23)=1.51 (*p*=0.23)
Conner's (Learning Problems)	0.12 (1.13)	0.05 (0.76)	F(1, 23)=0.19 (*p*=0.67)
Conner's (Psychosomatic Problems)	0.56 (1.22)	0.44 (1.50)	F(1, 23)=0.05 (*p*=0.83)
Conner's (Conduct Problems)	0.23 (0.97)	0.12 (0.81)	F(1, 23)=0.50 (*p*=0.36)
Conner's (Anxiety)	0.44 (1.12)	0.25 (1.01)	F(1, 23)=0.19 (*p*=0.67)
Conner's (Hyperactivity)	-0.16 (0.80)	-0.20 (0.88)	F(1, 23)=0.01 (*p*=0.91)
Family Income	29,400 (15,223)	37,040 (17,279)	F(1, 23)=1.25 (*p*=0.28)
Mother`s Education (Total Years)	14.545 (0.72)	15.25 (0.69)	F(1, 23)=0.50 (*p*=0.49)

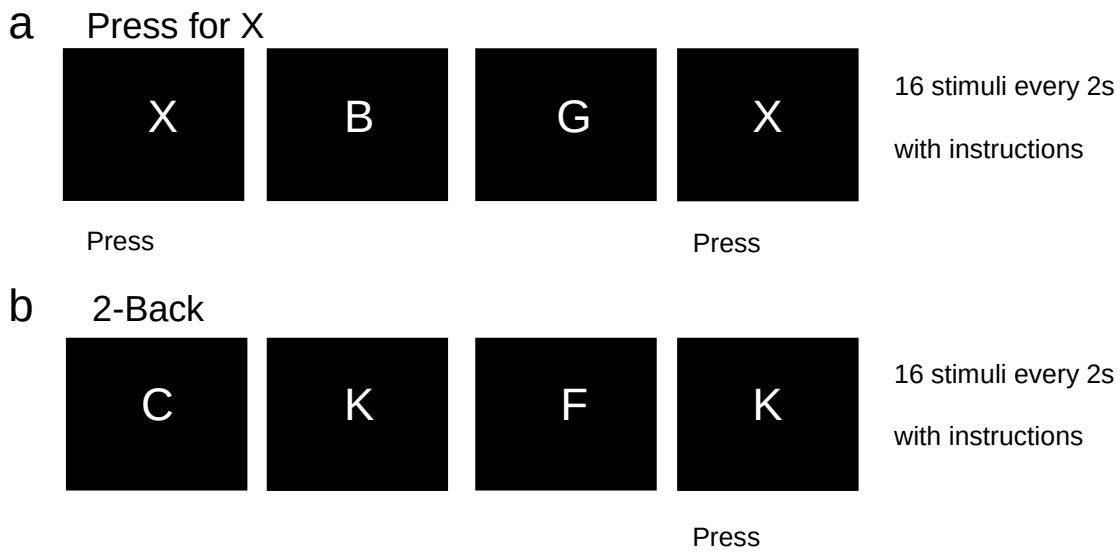
Table 3: Performance data for the two conditions of the 2-Back Task for Prenatally Exposed and Non-Exposed Participants, while controlling for cotinine values.

Performance Measure	**Prenatal Nicotine Exposure Group (n=12) M (SD)**	**Non-Exposed Control Group (n=13) M (SD)**	**MANCOVA**
Errors of Omission (Press for X)	0.09 (0.29)	0.15 (0.55)	F(2, 22)=0.11 (p=0.74)
Errors of Omission (Press for 2-Back)	2.44 (5.08)	2.13 (2.34)	F(2, 22)=0.44 (p=0.84)
Errors of Commission (Press for X)	0.18 (0.39)	0.63 (1.52)	F(2, 22)=2.05 (p=0.17)
Errors of Commission (Press for 2-Back)	0.60 (1.50)	0.90 (1.87)	F(2, 22)=0.31 (p=0.58)
Reaction Time (s, Press for X)	0.42 (0.04)	0.43 (0.07)	F(2, 22)=0.22 (p=0.65)
Reaction Time (s, Press for 2-Back)	0.50 (0.10)	0.52 (0.13)	F(2, 22)=0.22 (p=0.65)

Table 4: Significant positive relationship results between the amount of prenatal nicotine exposure and neural activity, during the verbal working memory condition (2-Back) minus the control condition (Press for X), while controlling for cotinine values.

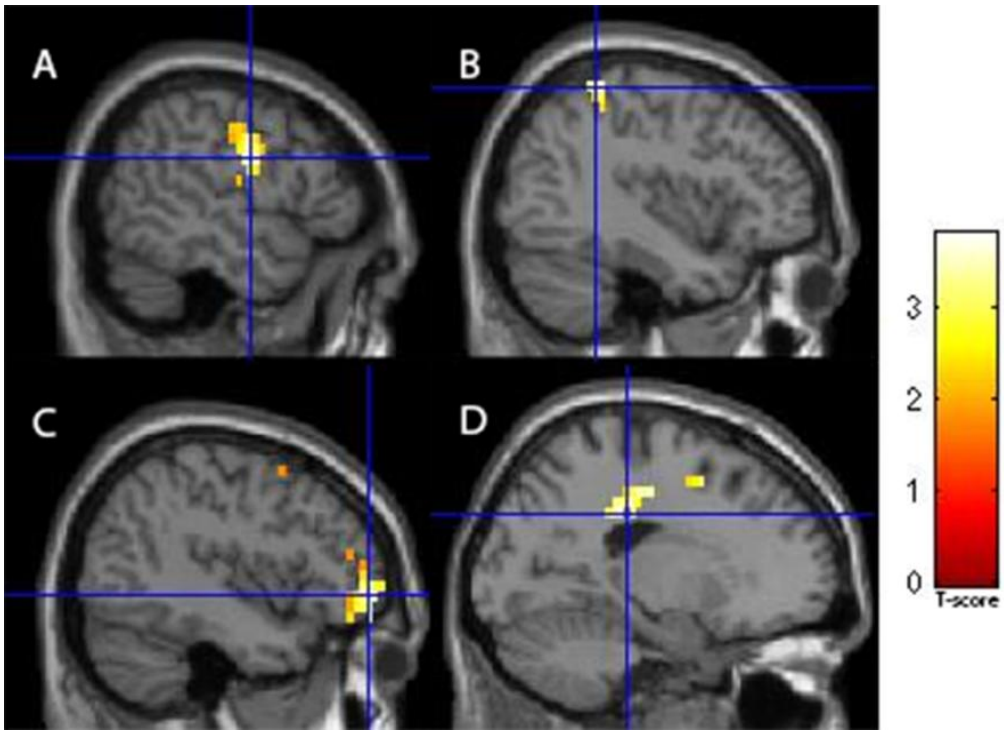
Region	Coordinates	z value
L middle frontal gyrus	-39 48 -15 -42 51 -5 -39 48 0	3.20 3.09 3.05
L precentral gyrus	-51, -5, 25, (Brodmann area 6)	3.06
L inferior parietal lobe	-33 -48 55 -39, -45, 55, (Brodmann 40)	3.00 3.00
L cingulate gyrus	-18 -30 35	2.80

Figure 1: 2-Back Task



a) An example of 4 stimulus presentations for the Press for X condition with 'Press' indicating where an appropriate response should occur. b) An example of 4 stimulus presentations for the 2-Back condition with 'Press' indicating where an appropriate response should occur.

Figure 2: Blue Cross hairs indicate where increasing amounts of prenatal nicotine exposure was associated with greater activity during the verbal working memory condition (2 Back) minus the control condition (Press for X), while controlling for cotinine values.



A) Left Precentral gyrus (x,y,z= -51, -5, 25) B) Left Inferior Parietal Lobule (x,y,z= -39, -45, 55)
C) Left Middle Frontal Gyrus (x,y,z= -39, 48, -15) D) Left Cingulate Gyrus (x,y,z= -18, -30, 35)

Chapter 4

Manuscript III

The Long-Term Effects of Prenatal Nicotine Exposure on Visuospatial Working Memory: An fMRI Study of Young Adults

Longo, C.A., Fried, P.A., Cameron, I., Smith AM. The long-term effects of prenatal nicotine exposure on visuospatial working memory: An fMRI study of young adults. *Submitted to Current Clinical Pharmacology.*

Abstract

Using functional magnetic resonance imaging (fMRI), the long term effects of prenatal nicotine exposure on visuospatial working memory were investigated in young adults. Participants were members of the Ottawa Prenatal Prospective Study, a longitudinal study that collected a unique body of information on participants from infancy to young adulthood. This allowed for the measurement of an unprecedented number of potentially confounding drug exposure variables including: prenatal marijuana and alcohol exposure and current marijuana, nicotine and alcohol use. Twelve young adults with prenatal nicotine exposure and 13 non-exposed controls performed a visuospatial 2-Back working memory task while fMRI blood oxygen level-dependent responses were examined. Despite similar task performance, participants with more prenatal nicotine exposure demonstrated significantly less activity in a relatively large set of right hemispheric prefrontal regions including the middle frontal gyrus, the superior frontal gyrus and the inferior frontal gyrus. There was also less activity in posterior regions including the precuneus and the occipital gyrus. These regions of the brain are typically associated with visuospatial working memory and less activity suggests that participants with more prenatal nicotine exposure likely reached working memory capacity. In addition, more prenatal nicotine exposure was also associated with significantly greater activity in a relatively large set of left prefrontal regions including the middle frontal gyrus, the inferior frontal gyrus, the medial frontal gyrus and the superior frontal gyrus. Widespread activity in these regions is not typically associated with visuospatial working memory tasks and is suggestive of compensation for right hemispheric prefrontal dysfunction. In conclusion, these results suggest that prenatal nicotine exposure contributes to altered neural functioning during visuospatial working memory that continues into young adulthood. Working memory is an important

executive functioning skill required for much of everyday living. Thus, awareness of the continued long-term neurophysiological effects of prenatal nicotine exposure is critical.

Keywords: Prenatal nicotine exposure; visuospatial working memory; young adults; fMRI

The Long-Term Effects of Prenatal Nicotine Exposure on Visuospatial Working Memory: An fMRI Study of Young Adults

The Ottawa Prenatal Prospective Study (OPPS), initiated in 1978, is an ongoing, longitudinal study that has extensively studied the effects of prenatal drug exposure on offspring. Two drugs that have received particular attention in the OPPS have been cigarette smoke and marijuana. Their consequences on neurocognitive outcomes, across different developmental levels, have been investigated, with findings implicating markedly different effects.

During the neonatal period, a neurological status examination of offspring in the OPPS found that prenatal exposure to cigarette smoke was associated with increased tremors and poor auditory habituation, while prenatal exposure to marijuana was associated with increased tremors, startles, and poor habituation to visual stimuli (Fried et al., 1987). When the same cohort was assessed again at 12 to 24 months of age, results for prenatal cigarette exposure paralleled those found during the neonatal period, in particular, reduced responsiveness on auditory-related items. In addition, lower overall mental development scores on the Bayley Scales of Infant Development were also noted at 12 months of age only. In contrast, marijuana use during pregnancy was not associated with mental, motor or language outcome variables at 24 months of age, after controlling for several confounding variables (Fried et al., 1988). Results remained consistent at 5 to 6 years of age, when prenatal exposure to cigarette smoke was significantly associated with lower cognitive scores on the McCarthy Scales of Children Abilities and lower receptive language scores. Prenatal marijuana exposure was again, not related to any cognitive items (Fried et al., 1992a). At 6 years of age, when facets of executive functioning were investigated in both OPPS groups, results found that prenatal cigarette exposure was significantly associated with impulsive behaviour. In particular, higher amounts of maternal

smoking during pregnancy were associated with lower scores on a response inhibition task and increased errors of commission on a vigilance task. In contrast, prenatal marijuana exposure was associated with increased omission errors on the vigilance task, suggesting difficulties with sustained attention (Fried et al., 1992b).

Further differences between the nicotine smoking and marijuana groups were revealed at 9 to 12 years of age when OPPS participants were administered a large battery of cognitive measures. Consistent with previous findings, greater prenatal cigarette exposure was associated with lower overall intellectual functioning, lower verbal scores and lower scores on measures of working memory. Prenatal marijuana exposure, on the other hand, was associated with lower scores on measures involving perceptual reasoning and visual-spatial constructional abilities (Fried et al., 1998). In a follow-up study with the same cohort, at the same age, prenatal cigarette smoke was associated with significantly lower scores on measures of basic visuoperceptual processing, which negatively impacted more complex visuoperceptual tasks. In contrast, prenatal marijuana had no effect on basic visuoperceptual skills but was associated with significantly lower scores on higher order visuoperceptual abilities requiring integration, construction and synthesis (Fried et al., 2000)

During the adolescent period, results continued to support a unique effect of both drugs on different facets of attention. At 13 and 16 years of age, prenatal cigarette exposure was more strongly associated with the working memory component of attention, such that heavily exposed adolescents performed worse on working memory tasks, compared to lightly exposed and non-exposed adolescents. On the other hand, prenatal marijuana exposure was more strongly associated with the stability component of attention, such that heavily exposed

adolescents performed worse on tasks measuring sustained attentional effort over time, compared to lightly exposed and non-exposed adolescents (Fried et al., 2001).

As a whole, these results suggest that prenatal cigarette smoke and prenatal marijuana exposure each have unique effects on cognition. In particular, prenatal cigarette smoke appears to effect global intellectual functioning, verbal skills, auditory and basic visuoperceptual processing and working memory. In contrast, prenatal marijuana exposure has no effect on global intellectual functioning, but appears to impact higher order visuoperceptual processing and sustained attention. The effects of both these drugs continue into at least adolescents but little is known about whether the adverse outcomes continue into young adulthood.

Given the above results, a particularly important executive functioning task that would appear to be greatly impacted by in utero exposure to cigarette smoke is visuospatial working memory. Visuospatial working memory is typically defined as a limited-capacity system that involves the active maintenance and manipulation of visuospatial information for a brief amount of time (Baddeley, 2003). According to Baddeley`s (1992; 2003) model, visuospatial working memory is comprised of both a central executive and a visuospatial sketchpad. The visuospatial sketchpad is specialized for processing visual and spatial information, while the central executive is an attentional control system important for the regulation and coordination of the various processes involved in the visuospatial sketchpad. The central executive is important for manipulation, which involves the reordering and updating of information maintained in working memory (for review see Fletcher & Hansen, 2001). In contrast, the visuospatial sketchpad is important for maintenance, which involves keeping information in mind in the absence of an external stimulus (for review see Fletcher & Hansen, 2001).

An understanding of the neural basis of visuospatial working memory and support for Baddeley`s model comes from a large body of research using both lesion and neuroimaging studies. For example, the dorsolateral prefrontal cortex, which has been shown to be critical for the manipulation of information within working memory, in addition to maintenance (Barbey et al., 2012; Glahn et al., 2002) is strongly affiliated with the central executive (for reviews see Baddeley 2003; Collette & Van der Linden, 2002). In contrast, predominately right parietal and occipital regions, which have been shown to be involved in the maintenance of visuospatial information within working memory (Manoach et al., 2004; Sala et al., 2003; Umla-Runge et al., 2011), are believed to subserve the visuospatial sketchpad (for review see Baddeley 2003). In fact, patients with right parietal lobe damage often exhibit significant impairments with visuospatial working memory tasks (Berryhill & Olson, 2008). In addition, the superior frontal sulcus has also been implicated in the short-term storage of spatial information (Nystrom et al., 2000). However, several other cortical and subcortical structures of the brain have also been shown to be involved in visuospatial working memory including the precuneus (Ventre-Dominey et al. 2005), the frontal eye fields (D'Esposito et al., 1998) and the premotor cortex (Owen et al., 2005).

Smith et al. (2006) used the OPPS sample to investigate the long term impact of prenatal marijuana on visuospatial working memory using fMRI but effects of prenatal nicotine have not yet been reported. The aim of the present study was to examine the long-term neuronal effects of prenatal nicotine exposure on visuospatial working memory in the young adults from the OPPS, using fMRI blood oxygen level dependent (BOLD) response. In addition, a comparison of these results with the findings from Smith et al. (2006) was performed to assess the similarities and differences between the two drugs. Visuospatial working memory was assessed using a

visuospatial 2-Back task. Evidence from fMRI research conducted on healthy controls has found that the visuospatial 2-Back task depends on prefrontal and parietal cortical integrity, specifically, the dorsolateral prefrontal cortex, the superior frontal gyrus, the inferior and superior parietal lobule, the precuneus, the cingulate cortex and occipital gyrus (Carlson et al., 1998; Pfefferbaum et al., 2001; Postle et al., 2000). It was hypothesized that prenatal nicotine exposure would impact the efficiency of neurocircuitry supporting visuospatial working memory and that this would result in greater activity in the dorsolateral prefrontal cortex, the parietal cortex, and the anterior cingulate.

Methods

Participants

Participants were recruited from the OPPS. Initial recruitment procedures have been described in detail elsewhere (Fried et al., 1980; 1988). Briefly, pregnant women volunteered to participate in the study after becoming aware of the research either by notices in the media or by signs placed in the offices of their obstetricians. The study was described as investigating prenatal lifestyle habits and their effects on offspring. Upon volunteering to participate, mothers-to-be were interviewed, usually in their homes, once during each trimester. The repeated interviews during pregnancy provided a means of checking the reliability of the self-report and were typically conducted by the same researcher, thereby establishing rapport with the participant. The information collected by a trained female interviewer included socioeconomic status, mothers age, education, physical and mental health of both parents, a 24 hour dietary recall and present drug use during pregnancy with particular emphasis upon cigarette, alcohol and marijuana usage. Families were Caucasian, from middle-class, low-risk homes, and no parent of the participant was reported to have an Axis I diagnosis based on the Diagnostic and

Statistical Manual of Mental Disorders (DSM) at the time of pregnancy. In addition, mothers were excluded if they used substances other than tobacco, marijuana or alcohol during pregnancy.

Twelve prenatally exposed participants (6 females and 6 males; mean age 21) and 13 non-exposed controls (7 females and 6 males; mean age 21) were included in the analysis. The prenatally exposed group was defined as participants who were exposed at any point in utero to any amount of nicotine. The control group was defined as participants with no exposure at any point in utero to any amount of nicotine. For prenatal nicotine exposure, a nicotine score was determined by multiplying the number of tobacco cigarettes smoked on average per day, at the time of pregnancy, by the nicotine content of the brand specified. A package of cigarettes of average strength contains approximately 16 mg of nicotine. The 12 prenatally exposed participants were exposed to an average of 14.13 mg of nicotine in utero daily (range of 0.03-35 mg of nicotine/daily), while the 13 non-exposed participants were never exposed to any amount of nicotine in utero.

For both groups, exposure to marijuana and alcohol in utero was also recorded. Marijuana use at the time of pregnancy was recorded in terms of the number of joints smoked per week. Both the quantity and the pattern of alcohol consumption during pregnancy (including beer, wine and liquor) were recorded and converted to ounces of absolute alcohol (AA) per day. Analysis of variance (ANOVA) revealed no significant differences between groups on prenatal marijuana exposure or prenatal alcohol exposure (see Table 1). However, given that both of these variables have been shown to impact brain activity (Smith et al., 2004, 2006; Spadoni et al., 2009), they were included as covariates in the fMRI analysis.

Participants with prenatal nicotine exposure are more likely to smoke cigarettes and use marijuana (Cornelius et al., 2000; 2012; Goldschmidt et al., 2012; O'Callaghan et al., 2009; Porath & Fried, 2005; Rydell et al., 2012). Therefore, to increase external validity, participants who smoked cigarettes and marijuana were included. The regular use of these substances was measured among all participants. Regular use of cigarettes was defined as 1 cigarette/day or more. In order to eliminate the acute withdrawal symptoms that come from cessation of regular tobacco use, which includes disruption of attention and memory (Jacobsen et al., 2005), participants were permitted to smoke during the day, however, no tobacco smoking occurred 2 hours prior to testing (confirmed by presence in the hospital MRI unit). Regular use of marijuana was defined as 1 joint/week or more. To eliminate the acute effects of marijuana on cognitive functioning (Ramaekers et al., 2009), participants were excluded if they smoked marijuana on the day of testing. Given that marijuana use and tobacco use have been shown to impact brain activity (Jacobsen et al., 2007a; Smith et al., 2010), values of marijuana and cotinine in the urine samples were used as covariates n the fMRI analysis. Alcohol use is also prevalent among young adults (Johnson et al., 2003) and thus participants who drank alcohol regularly were not excluded. The regular use of alcohol was measured among all participants and defined as 1 drink/week or more. To eliminate the acute effects of alcohol on cognitive functioning (Leitz et al., 2009) participants were excluded if they drank alcohol on the day of testing. Alcohol use has been shown to impact brain activity (Tapert et al., 2004) and therefore it was also used as a covariate in the fMRI analysis.

Participants previously completed a comprehensive psychological battery including the Wechsler Intelligence Scale for Children-III (WISC-III; Wechsler, 1991) (between the ages of 13 and 16) and the National Institute of Mental Health Computerized Diagnostic Interview Schedule

for Children (C-DISC) (Bacon, 1997), which assessed for current psychiatric illnesses based on DSM-IV criteria. Parents also previously completed the Conners' Parent Rating Scale-Revised (Conners, 1997) (between the ages of 13 and 16) and family income and mother's education at the time of pregnancy was measured. An ANOVA did not reveal any significant differences between the prenatally exposed group and the non-exposed group on these scales, however, the WISC-III Full-Scale IQ score and family income were included as covariates in the fMRI analysis (see Table 2).

Exclusion criteria for both groups included (a) contraindication to fMRI, specifically, having a pacemaker, claustrophobic, mental or electrical implants, accidents leaving metal in the eye, recent surgery, metal dental work (aside from fillings), piercings that cannot be removed, eye glasses or insufficient vision for viewing the tasks, (b) abnormalities in their structural MRI scan, (c) meeting DSM criteria for an Axis I disorder using the C-DISC, (d) current use of prescription medication with central nervous system effects including psychotropic medication, as well as reported regular use of illicit drugs (defined as once a month or more) including amphetamines, crack, cocaine, heroin, mushrooms, hashish, lysergic acid, steroids, solvents and tranquilizers, (e) testing positive for cocaine, opiates or amphetamines in their urine, (f) history of seizures, diabetes requiring insulin treatment, heart attack, stroke, blood clots, high blood pressure, cancer, brain injury and chronic pain, (g) first language other than English and (h) left-handedness. In addition, all participants signed informed consent prior to being imaged. The study was approved by the Ottawa Hospital Research Ethics board.

Measures

Visuospatial 2-Back Task. A visuospatial 2-Back task was used to measure visuospatial working memory processes while in the scanner. The visuospatial 2-Back paradigm involves a

circle presented in white on a black background at one of 9 different positions on the screen (see Figure 1). The circle was displayed in this position for 75 milliseconds before being relocated to one of the other positions, with an interstimulus interval of 1925 milliseconds. The task was a block design and included two conditions: a control condition (Match to Centre) and a visuospatial working memory condition (Visuospatial 2-Back). The control condition began with the instruction "Match to Centre" on the screen for 4 seconds. In this condition, participants were required to press a button with their right index finger each time the circle was presented in the middle of the screen. The visuospatial working memory condition began with the instruction "Press for 2-Back" on the screen for 4 seconds. In this condition, participants were required to press a button with right their right index finger each time the circle reappeared in the same position that it was in two presentations prior. There was also a rest period of 30 seconds at the beginning, middle, and end of the entire task. During this rest period a white box was presented on the screen and no response was required. Both the control (Match to Centre) and visuospatial working memory condition (Visuospatial 2-Back) were comprised of 16 stimuli, presented every 2 seconds, for a total duration of 32 seconds. Each condition was presented six times. The total duration of the task was 9 minutes and fifteen seconds. The order of blocks was counterbalanced. This presentation allowed for the manipulation of visuospatial working memory load by changing the instructions while maintaining all other features of the task the same, including number of stimuli, number and type of response. This ensured that following the subtraction of the neural activity during the control condition (Match to Centre) from that during the visuospatial working memory condition (Visuospatial 2-Back) only the neural activity involved in visuospatial working memory processing was observed in the statistical parametric maps. The visuospatial 2- Back task has been shown to reliably initiate visuospatial working

memory activation in healthy controls (Carlson et al., 1998; Pfefferbaum et al., 2001; Postle et al., 2000).

The task was presented to the participants on a back projection screen, located at the foot of the MRI table, via a mirror attached to the head coil. All lighting was turned off and button responses were recorded via a MRI-compatible fiber optic device (Light-wave Medical, Vancouver, British Columbia).

Drug History Questionnaire (DHQ). The DHQ was used to measure participants' present usage of both licit and illicit substances (see Appendix A). Substances assessed included the regular use of amphetamines, crack, cocaine, heroin, mushrooms, hashish, lysergic acid, steroids, solvents, tranquilizers, marijuana, nicotine and alcohol (as defined above). Both the amount and the number of years of use were recorded among regular users. This survey was adapted from the Monitoring the Future Survey which was used as a national survey focused on the lifestyles, attitudes, and preferences of American youth (Johnston et al., 2003). The substance use portion of the survey has been shown to have good psychometric properties (O'Malley, Bachman, & Johnston, 1983). Group differences for the amount of nicotine, marijuana and alcohol consumed among regular users was computed by ANOVA using SPSS v. 20, with a statistical threshold set at $p<0.05$.

Urine Sample. Current drug use was verified through a urine sample which was tested for amphetaminies, opiates, cocaine, cannabis, creatinine and cotinine (metabolite of nicotine). All metabolite concentrations were adjusted for creatinine to control for urine dilution. Group differences for marijuana and cotinine urine values were computed by ANOVA using SPSS v. 20, with a statistical threshold set at $p<0.05$.

Imaging Parameters. All imaging was performed using a 1.5 Tesla Siemens Magnetom Symphony MR scanner. Participants lay supine with their head secured in a standard MRI head holder. A conventional T1-weighted spin echo localizer was acquired and used to align the slice orientation for the fMRI scans such that the anterior commissure-posterior commissure (AC-PC) line in the sagittal view were at right angles to the slice selection gradient. Whole brain fMRI was performed using a T2*-weighted echo planar pulse sequence (TR/TE 3000/40ms, flip angle 90^{o}, FOV 24 x 24 cm^{2}, 64 x 64 matrix, slice thickness 5 mm, 27 axial slices, bandwidth 62.5 kHz).

Behavioural Performance Parameters and Analysis. For the visuospatial 2-Back task, reaction time for each response, errors of commission and omission were recorded. Errors of commission included any response following the presentation of a non-target stimulus within 900 milliseconds of stimulus presentation. Omission errors were defined as a failure to respond to a target stimulus within 900 milliseconds. Mean reaction times were calculated for all accurate responses occurring within 900 milliseconds of stimulus presentation. Errors of omission, errors of commission and mean reaction times for both the control condition (Match to Centre) and the visuospatial working memory condition (Visuospatial 2-Back) were calculated. Group differences were computed by multivariate analysis of covariance using SPSS v. 20, with a statistical threshold set at $p<0.05$.

Whole Brain Analysis. Statistical Parametric Mapping 8 (SPM8) was used to post-process the fMRI data and to perform the statistical analysis. For post-processing, functional images from the first 9 s of the initial rest block were discarded to ensure that longitudinal magnetic relaxation (T1 effects) had stabilized. The remaining functional images were realigned to correct for motion by employing the procedures of Friston et al. (1995). The motion

correction did not exceed 1 mm for any subject. Images were spatially normalized, due to differences in human brain morphology, to match the echo planar imaging (EPI) template provided in SPM8. Following spatial normalization, images were smoothed to eliminate noise interference in the data, with an 8mm full-width at half-maximum Gaussian filter.

Individual participant first-level fixed effects analyses were performed for the comparison of the visuospatial working memory condition (Visuospatial 2-Back) minus the control condition (Match to Centre). One contrast image was created per person, and these images were then used for second-level random effects analyses that were corrected for multiple comparisons. Random effects analyses eliminate highly discrepant variances between and within individuals in constructing an appropriate error term for hypothesis testing and generalizability to the population. Multiple regression was used to assess the relationship between prenatal nicotine exposure and neural activity during visuospatial working memory, at a statistical threshold set at $p<0.05$, both with and without covariates. Prenatal nicotine exposure was entered as a continuous variable. Prenatal marijuana exposure, prenatal alcohol exposure, current alcohol use, current marijuana exposure (e.g. marijuana urine), current nicotine exposure (e.g. cotinine urine), family income and WISC-III Full Scale IQ scores were all used as continuous variables and were entered as covariates. Each covariate was included in the regression separately to maintain power and to determine if these variables were contributing to the neurophysiological results. Covariates that were not found to be significantly associated with brain activity were removed from the final analysis.

Procedure

Upon arrival to the hospital, informed consent was attained and MRI compatibility was confirmed. Then participants provided a urine sample which was immediately sent to the

Hospital Laboratory for testing. Prior to commencing imaging, participants were required to view the visuospatial 2-Back task outside the scanner and performed a practice session of 10 trials of each condition. This ensured that all participants were able to perform the task accurately. Participants then entered the scanner and task instructions were repeated including to press the response pad as quickly as possible and, if they made a mistake, to continue without thinking about the mistake. Once imaging was completed, participants filled out a drug history questionnaire. Completing the drug questionnaire after scanning ensured the blindness of the fMRI researcher.

Results

Drug history questionnaire and urine sample data

Regular marijuana use was reported by three participants from the prenatally exposed group and two participants from the control group. The average number of years of regular marijuana use for the 3 prenatally exposed participants was 5 years (range 4 to 6 years), while the average number of years of regular marijuana use for the 2 non-exposed participants was 3.5 years (range 2 to 5 years). No participant reported smoking marijuana at least 24 hours prior to testing. There were no significant differences between groups for current marijuana use or urine cannabis (see Table 1). All participants from both groups reported regular alcohol use. However, no participant from either group reported drinking more than 8 alcoholic drinks per week on a regular basis. In addition, no participant from either group reported drinking alcohol at least 24 hours prior to testing. No significant differences between groups emerged on current alcohol use (see Table 1). Regular tobacco use was reported by four participants from the prenatally exposed group and two participants from the control group. The average number of years of regular tobacco use for the 4 prenatally exposed participants was 4.7 years (range 1 to 6

years), while the average number of years of regular tobacco use for the 2 non-exposed participants was 5.5 years (range 5 to 6 years). No significant group difference emerged for current tobacco use or cotinine values (see Table 1). No participant from either group reported using illicit drugs on a regular basis or within the month prior to testing.

Behavioural Performance Data

There were no significant performance differences between participants prenatally exposed to nicotine and non-exposed controls on reaction time, errors of omission and errors of commission. This was analysed while controlling for cotinine values (see Table 3).

Whole Brain Analysis

A first-level fixed effects group analysis of all participants, at a *p* value corrected for cluster level at 0.001, for the comparison of the visuospatial working memory condition (Visuospatial 2-back) minus the control condition (Match to Centre), confirmed the task was activating areas of the brain typically observed during visuospatial working memory. These areas included the middle, superior and inferior frontal gyri, the precuneus, the inferior parietal lobe, the middle occipital gyrus, the cuneus, the cerebellum, the caudate and the thalamus.

When examining the relationship between prenatal nicotine exposure and neural activity during the visuospatial working memory task in the second-level random effects regression analysis without covariates, there was a significant positive relationship between prenatal nicotine exposure and brain activity. Current nicotine (cotinine values) was the only variable to contribute to this relationship when added as a covariate and thus was included in the final analysis. Prenatal marijuana, prenatal alcohol, current alcohol, current marijuana, IQ and family income did not impact the results and were not included.

The most robust effect of the study was a significant positive relationship between the amount of prenatal nicotine exposure and neural activity, while controlling for cotinine values, during the visuospatial working memory condition (Visuospatial 2-Back) minus the control condition (Match to Centre), at a *p* value corrected for cluster level <0.05 in a large cluster of 1316 voxels, in the following regions: the left middle frontal gyrus, the left inferior frontal gyrus, the medial frontal gyrus bilaterally, the left superior frontal gyrus and the left anterior cingulate (see Table 4 & Figure 2).

There was also a significant negative relationship between the amount of prenatal nicotine exposure and neural activity during the same contrast at the same *p* value in a large cluster of 1129 voxels, in the following regions: the right middle frontal gyrus, the right inferior frontal gyrus and the superior frontal gyrus bilaterally; and in a large cluster of 2251 voxels, in the following regions: the left precuneus, the superior occipital gyrus bilaterally and the left thalamus (see Table 5 & Figure 3). All coordinates are in Montreal Neurological Institute (MNI) space.

Discussion

The purpose of the present study was to examine the long-term neurophysiological effects of prenatal nicotine exposure on visuospatial working memory in young adults from the OPPS, using fMRI. Despite similar task performance, greater prenatal nicotine exposure was associated with significantly greater BOLD response in a large set of left hemispheric brain regions and significantly less BOLD response in a large set of right hemispheric brain regions.

The most interesting effect of the study was that greater prenatal nicotine exposure was associated with significantly less activity during spatial working memory in predominately right hemispheric regions including the dorsolateral prefrontal cortex. The right dorsolateral prefrontal

cortex has consistently been implicated in visuospatial working memory processes (Manoach et al., 2004; Owen et al., 2005; Pfefferbaum et al., 2001; Schweinsburg et al., 2008) due to the critical role it plays in the manipulation and maintenance of information within working memory (Barbey et al., 2012; Glahn et al., 2002; Owen et al., 1999). Studies examining the relationship between working memory difficulty and prefrontal cortical activation typically demonstrate that increasing working memory load produces increasing brain activity in prefrontal regions including the dorsolateral prefrontal cortex (Glahn et al., 2002; Linden et al., 2003), suggesting that harder tasks require more neural resources to adequately maintain information in working memory. However, when this brain region is taxed beyond capacity, an opposite pattern tends to emerge. For example, Callicott et al. (1999) found evidence of an inverted-U shaped prefrontal neurophysiological response, particularly in the dorsolateral prefrontal cortex, to increasing working memory load in healthy participants who became hypofrontal as they were taxed beyond their working memory capacity. Thus, the decrease in activity of right prefrontal regions that typically subserve spatial working memory exhibited by participants with greater prenatal nicotine exposure, suggests that they were close to reaching working memory capacity and needed to recruit additional prefrontal regions to successfully complete the task. Although in the present study there were no performance differences between exposed and non-exposed participants, most likely due to the relative simplicity of the task, if challenged with a harder 3-Back task, young adults with greater amounts of prenatal nicotine exposure may not be able to compensate and performance may suffer.

The greater activity exclusively in the left dorsolateral and ventrolateral prefrontal cortices is not typical of tasks involving spatial working memory, which tend to show a predominance for right hemispheric prefrontal regions (Jennings et al., 2006; Manoach et al.,

2004; Owen et al., 1999; Owen et al., 2005; Smith, Jonides, & Koeppe, 1996). This shift in laterality during spatial working memory among individuals with greater prenatal nicotine exposure may reflect strategic differences in the approach taken to perform the task. In fact, the left ventrolateral prefrontal cortex is typically associated with the rehearsal of verbal information held in mind at a subvocal level (i.e. inner speech) (Awh et al., 1996; Geva et al., 2011; Jonides et al., 1997; Li et al., 2012), suggesting that participants with greater prenatal nicotine exposure likely used rehearsal strategies to a greater extent. Although there were no verbal items, the visual images used in the n-back task were circles and thus, could lend themselves to verbal coding. Another explanation for the imbalance between the left and right prefrontal regions likely reflects a compensatory mechanism for dysfunction within prefrontal networks that subserve spatial working memory, leaving participants with greater prenatal nicotine exposure to recruit homologous contralateral brain regions to successfully complete the task.

Dysfunction in the neural circuitry subserving spatial working memory can come about through several mechanisms since nicotine acts on nicotinic acetylcholine receptors (nAChRs), which are expressed in the brain early in development (Hellström-Lindahl et al., 1998) and play a critical role in many aspects of brain development (for review see Dwyer, McQuown, & Leslie, 2009). First, animal research has shown that prenatal nicotine exposure alters several neurotransmitter systems including, decreasing both neural cell number and neural activity of cholinergic and serotonergic pathways located within the cerebral cortex (Slotkin et al., 2006; 2007; Xu et al., 2001). The serotonergic system is considered important for learning and memory, with serotonin depletion impairing memory consolidation and retrieval in humans (Riedel et al., 1999). Similarly, blocking acetylcholine receptors has led to deficits in working memory, visual attention and psychomotor speed, implicating an important role for the

cholinergic system in modulating general cognitive functioning in humans (Ellis et al., 2006). Thus, damage to these neurotransmitter systems may contribute to dysfunction within neural circuitry subserving visuospatial working memory, leading to altered neural functioning. Second, prenatal tobacco exposure has been shown to alter white matter maturation. Specifically, research using diffusion tensor imaging has found decreased fractional anisotropy in projections within the premotor cortex and supplementary motor areas in adolescents with in utero tobacco exposure, an indicator of myelin dysfunction and degradation (Liu et al., 2011). Similarly, Jacobsen and colleagues (2007b) found disturbed white matter integrity in both left and right frontal regions of adolescents prenatally exposed to tobacco smoke. Myelination is directly involved in accelerating communication throughout the brain (for review see Luna & Sweeney, 2004) and increases in white matter have been shown to correlate with better performance on cognitive tasks (Mabbott et al., 2006). Thus, perturbed maturation of white matter can lead to reduced efficiency of neural circuitry subserving spatial working memory, leading participants with greater nicotine exposure to recruit additional neural resources to successfully complete the task.

Less activity among participants with greater prenatal nicotine exposure was also found within more posterior brain regions including the precuneus and the occipital gyrus. The involvement of the parietal-occipital association cortex in the performance of spatial working memory tasks has previously been indicated in several imaging studies (Courtney et al.,1996; Jennings et al., 2006; Pfefferbaum et al. , 2001; Riccardi et al., 2006; Ventre-Dominey et al., 2005) due to their role in visual-spatial attention, visual-spatial updating, visual-spatial imagery and form and object processing (Jahn et al., 2012; Larsson & Heegar, 2006; Lepsien et al., 2005; Thompson et al., 2009). In addition, activity within the precuneus and occipital gyrus has also

been found during the maintenance of visuospatial information in working memory (Sala et al., 2003; Umla-Runge et al., 2011). Less activity in these posterior regions during spatial working memory among participants with greater prenatal nicotine exposure suggests another form of altered neural processing. Interestingly, a recent imaging study examining brain morphology among children prenatally exposed to tobacco found cortical thinning within the occipital cortex, compared to non-exposed children (Derauf et al., 2012). Structural abnormalities within regions of the brain important for visual processing may contribute to altered neural functioning among participants with greater prenatal nicotine exposure.

Further evidence of prenatal nicotine exposure impacting the spatial working memory circuitry is that those participants with greater prenatal nicotine exposure also exhibited less activity in the thalamus. The thalamus plays an important role in mediating cortical and subcortical structures (Haber & Calzavara, 2009) and is typically involved during working memory processes (Manoach et al., 2004) Like the dorsolateral prefrontal cortex, however, the thalamus has also been shown to exhibit a capacity-constrained response, resulting in an inverted-U shaped neurophysiological response from lowest to highest load (Calicott et al., 1999). These results, once again, suggest that participants in our study with greater prenatal nicotine exposure were working close their capacity.

The one right hemispheric region which showed greater activity with increased amounts of prenatal nicotine exposure was the medial frontal gyrus, corresponding to the frontal pole. Although its exact function in cognition remains controversial, this area has consistently been shown to be activated during working memory tasks (Carlson et al., 1998; Manoach et al., 2004; Owen et al., 2005). The frontal pole is believed to contribute to establishing and maintaining an attentional set that is employed during tasks that demand a high level of control (not easily

automatic) (Velanova et al., 2003). Spatial working memory, as assessed using the visuospatial 2-Back task, is a good example of a task involving high levels of control due to its many processing demands, including maintenance, manipulation, active processing of stored information and a selection of a response based on active processing, with each of these cognitive processes demanding a high level of attention to be adequately performed. Thus, greater activity in this region among participants with greater prenatal nicotine exposure suggests that they needed to work harder at sustaining their attention so that they could successfully complete the task.

Finally, greater activity in the left anterior cingulate was also associated with increasing amounts of prenatal nicotine exposure. The anterior cingulate has consistently been shown to be involved in visuospatial working memory tasks (Carlson et al., 1998; Glahn et al., 2002; Honegger et al., 2011; Riccardi et al., 2006). This region of the brain is believed to serve as an index of task difficulty and increased effort (for review see Botvinick et al., 2004), with tasks that involve greater effort and increasing difficulty leading to greater activity in the anterior cingulate (Barch et al., 1997; Paus et al., 1998). Again, greater activity in the anterior cingulate, suggests that young adults with more prenatal nicotine exposure likely found the task more difficult.

Comparing the results from the current paper with the Smith et al. (2006) study of the long-term effects of prenatal marijuana exposure on executive functioning provides interesting information on the effects of these two drugs. Consistent with our results, Smith et al. (2006) also found that prenatal marijuana exposure was associated with significantly less activity in a relatively large set of right prefrontal regions including the dorsolateral prefrontal gyrus and medial frontal gyrus and significantly greater activity in a relatively large set of left prefrontal

regions during the same visuospatial working memory task as was used in the present study (see Table 6 & 7). These findings suggest that both prenatal nicotine and prenatal marijuana exposure affect the same areas of the brain, leading to similar alterations in neural functioning during visuospatial working memory. In addition, the effects of both drugs continue into adulthood. These results are interesting given that neurocognitive studies examining the effects of these two drugs within the OPPS population have found unique cognitive effects on the offspring. However, executive functioning and visual perceptual difficulties were noted in both groups (Fried et al., 1992; 2000) and these are the types of processing that seem to be impacted into young adulthood.

The strength of this study is the use of the participants from the OPPS, where information on prenatal nicotine exposure, as well as exposure to other prenatal substances, was gathered prospectively and offspring were followed from infancy to adulthood. This allowed for the measurement of an unparalleled number of lifestyle variables across the lifespan. The unique methodology strengthens the validity of the results and provides outcomes that can shed light on the long-term effects of prenatal nicotine exposure on neural processing, with greater control than previous studies. Despite these strengths, several limitations should be addressed. First, the prenatal exposed group was also exposed to marijuana and alcohol in utero as was the control group. Despite examining their effect on neural activity and finding that these substances did not contribute to differences in neural activity between groups, future studies should endeavor to examine participants with only prenatal nicotine exposure. Second, the results cannot be generalized to other ethnic or socioeconomic status populations as the OPPS is primarily a Caucasian, middle class population. Third, a block design was used. A block design does not permit the separation of working memory from other cognitive processes. However, the design

of the task, including the same motor output and sensory input for both conditions, ensured as much as possible that the only difference between the two cognitive tasks was working memory. In addition, a block design does not examine the effects of performance on brain activity. An event-related study may have helped to decipher working memory from other cognitive processes and would have allowed us to take into consideration performance on brain activity. Fourth, although the sample size is generally in keeping with other imaging studies, it remains small and future studies should replicate the results with a greater number of participants.

In conclusion, this was the first fMRI study of the long term impact of prenatal nicotine exposure on visuospatial working memory. Smoking during pregnancy continues to remain a worldwide public health concern and this study demonstrates that prenatal nicotine exposure produces long lasting alterations in neural processing which continues into young adulthood. Greater prenatal nicotine exposure was associated with significantly less activity in predominately right hemispheric prefrontal regions but also in posterior regions including the precuneus and the occipital gyrus. These regions of the brain are typically associated with visuospatial working memory. Less activity in these regions suggests that participants with greater prenatal nicotine exposure likely reached working memory capacity. In addition, more prenatal nicotine exposure was also associated with significantly greater activity in a relatively large set of left prefrontal regions. This widespread left lateralized activity is suggestive of compensation for right hemispheric prefrontal dysfunction. Working memory is an important executive functioning skill required for much of everyday living. Thus, awareness of the continued long-term neurophysiological effects of prenatal nicotine exposure is critical.

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Table 1: Prenatal and Current Drug Exposure for Prenatally Exposed and Non-Exposed Participants

Drug Exposure	**Prenatal Nicotine Exposure Group (n=12) M (SD)**	**Non Exposed Control Group (n=13) M (SD)**	**ANOVA**
Prenatal Marijuana (joints/week)	6.39 (10.80)	3.13 (5.59)	$F(1, 23)=0.92$ ($p=0.35$)
Prenatal Alcohol (AA/day)	0.10 (0.08)	0.21 (0.22)	$F(1, 23)=3.03$ ($p=0.11$)
Current Nicotine (cigarettes/day)	2.08 (3.52)	1.35 (3.33)	$F(1, 23)=0.30$ ($p=0.60$)
Cotinine Urine Values (ug/L)	327.47 (623.02)	224.52 (595.03)	$F(1, 23)=0.18$ ($p=0.69$)
Current Marijuana (joints/week)	1.79 (3.82)	0.23 (0.60)	$F(1, 23)=2.12$ ($p=0.16$)
Marijuana Urine Values (ug/L)	63.41 (134.72)	18.23 (49.16)	$F(1, 23)=1.28$ ($p=0.27$)
Current Alcohol (drinks/week)	2.39 (2.28)	2.24 (2.88)	$F(1, 23)=0.02$ ($p=0.88$)

Table 2: Demographic, IQ and Externalizing and Internalizing Characteristics for Prenatally Exposed and Non-Exposed Participants

Measures	**Prenatal Nicotine Exposure Group (n=12) M (SD)**	**Non-Exposed Control Group (n=13) M (SD)**	**ANOVA**
WISC Full Scale IQ	110.46 (10.44)	115.70 (9.57)	F(1, 23)=1.51 (*p*=0.23)
Conner's (Learning Problems)	0.12 (1.13)	0.05 (0.76)	F(1, 23)=0.19 (*p*=0.67)
Conner's (Psychosomatic Problems)	0.56 (1.22)	0.44 (1.50)	F(1, 23)=0.05 (*p*=0.83)
Conner's (Conduct Problems)	0.23 (0.97)	0.12 (0.81)	F(1, 23)=0.50 (*p*=0.36)
Conner's (Anxiety)	0.44 (1.12)	0.25 (1.01)	F(1, 23)=0.19 (*p*=0.67)
Conner's (Hyperactivity)	-0.16 (0.80)	-0.20 (0.88)	F(1, 23)=0.01 (*p*=0.91)
Family Income	29,400 (15,223)	37,040 (17,279)	F(1, 23)=1.25 (*p*=0.28)
Mother`s Education (Total Years)	14.545 (0.72)	15.25 (0.69)	F(1, 23)=0.50 (*p*=0.49)

Table 3: Performance data for the two conditions of the Visuospatial 2-Back Task for Prenatally Exposed and Non-Exposed Participants, while controlling for cotinine.

Performance Measure	**Prenatal Nicotine Exposure Group (n=12) M (SD)**	**Non-Exposed Control Group (n=13) M (SD)**	**MANCOVA**
Errors of Omission (Match to Centre)	0.11 (0.32)	0.08 (0.29)	F(2, 22)=0.04 (p=0.84)
Errors of Omission (Press for 2-Back)	3.00 (6.40)	2.56 (3.20)	F(2, 22)=0.65 (p=0.43)
Errors of Commission (Match to Centre)	0.30 (0.67)	0.49 (1.16)	F(2, 22)=0.20 (p=0.67)
Errors of Commission (Press for 2-Back)	0.98 (1.33)	0.60 (0.90)	F(2, 22)=1.25 (p=0.28)
Reaction Time (s, Match to Centre)	0.52 (0.10)	0.45 (0.06)	F(2, 22)=2.98 (p=0.15)
Reaction Time (s, Press for 2 Back)	0.55 (0.10)	0.51 (0.07)	F(2, 22)=0.58 (p=0.46)

Table 4: Significant positive relationship results between the amount of prenatal nicotine exposure and neural activity, during the visuospatial working memory condition (Visuospatial 2-Back) minus the control condition (Match to Centre), while controlling for cotinine.

Region	Coordinates	z value
L middle frontal gyrus	-45 39 -5	4.30
L inferior frontal gyrus	-48 -45 -15 -51 21 5	4.01 3.64
L medial frontal gyrus	-12 57 -5	4.00
R medial frontal gyrus	3 57 10 (Brodmann area 10)	3.28
L superior frontal gyrus	-27 33 35	3.00
L anterior cingulate	-3 33 0	2.80

Table 5: Significant negative relationship results between the amount of prenatal nicotine exposure and neural activity, during the visuospatial working memory condition (Visuospatial 2-Back) minus the control condition (Match to Centre), while controlling for cotinine.

Region	Coordinates	z value
R middle frontal gyrus	33 0 65 (Brodmann area 6) 36 27 30	3.08 3.05
R inferior frontal gyrus	51 12 20	3.00
R superior frontal gyrus	27 54 0 (Brodmann 10)	3.16
L superior frontal gyrus	-9 6 70 (Brodmann 6)	3.70
L precuneus	-27 -63 50 (Brodmann area 7) -24 -54 45	3.73 3.07
R superior occipital gyrus	42 -75 15	4.30
L superior occipital gyrus	-33 -78 25	3.79
L thalamus	-18 -24 0	3.24

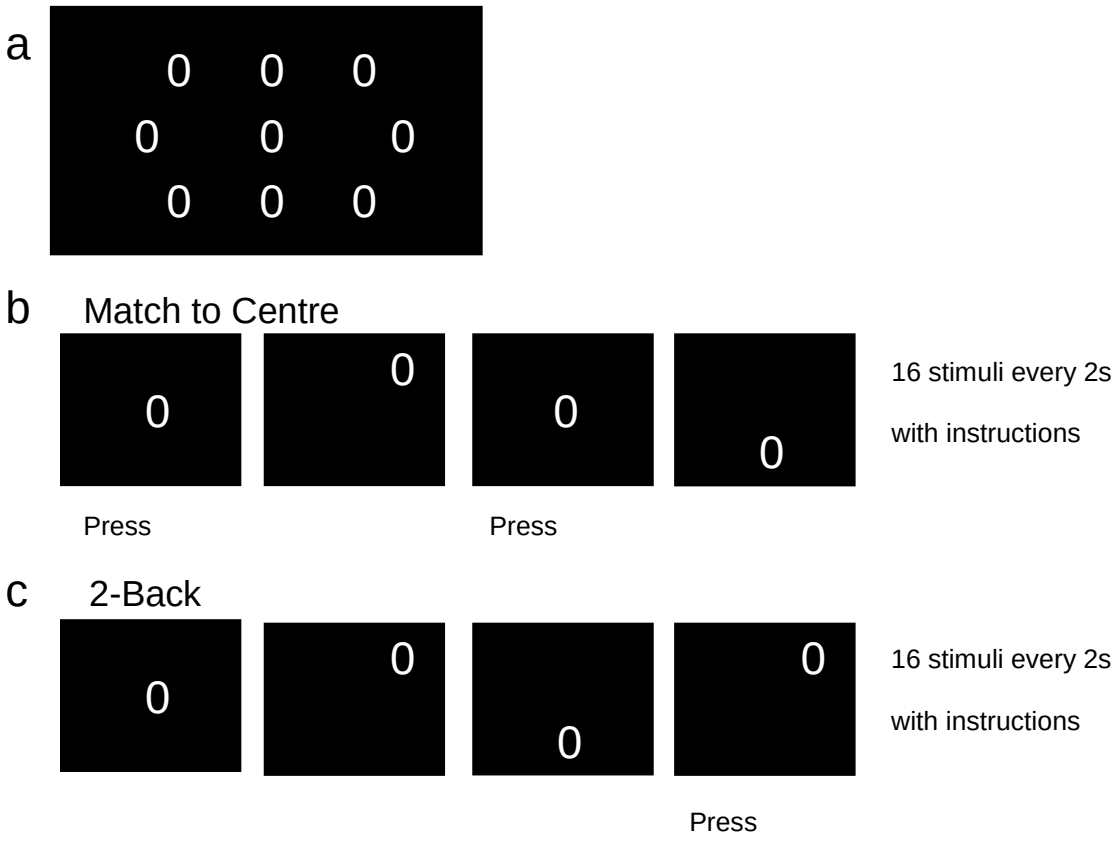
Table 6: Significant positive relationship results between the amount of prenatal marijuana exposure and neural activity, during the visuospatial working memory condition (Visuospatial 2-Back) minus the control condition (Match to Centre)

Region	Coordinates	Z value
L medial frontal gyrus	-12 12 -15	5.78
L inferior frontal gyrus	-45 18 20	3.24
L cerebellum	-33 -48 -45	5.13
R cuneus	9 -81 20	5.27

Table 7: Significant negative relationship results between the amount of prenatal marijuana exposure and neural activity, during the visuospatial working memory condition (Visuospatial 2-Back) minus the control condition (Match to Centre)

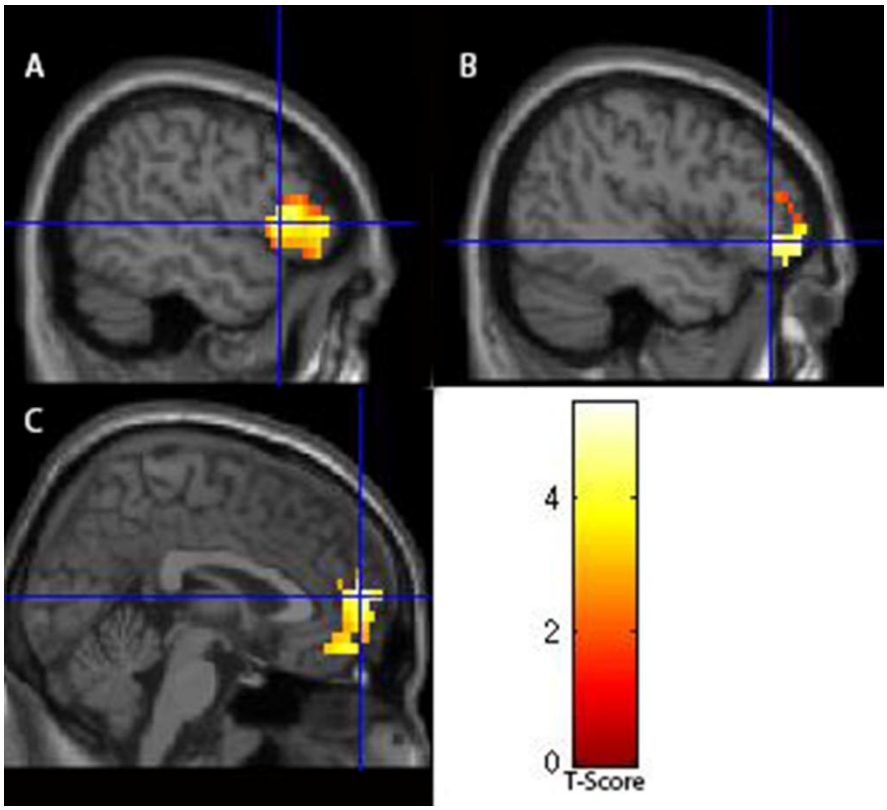
Region	Coordinates	Z value
R dorsolateral prefrontal gyrus	27 18 35	3.07
R medial prefrontal gyrus	9 63 5	3.31
R ventrolateral prefrontal cortex	45 33 -5	2.86
R parahippocampul gyrus	24 -9 -25	4.63
L presupplimentary motor area	-9 30 50	3.69
L putamen	-27 3 0	4.2
R precentral gyrus	45 -6 45	6.80

Figure 1: Visuospatial 2-Back Task



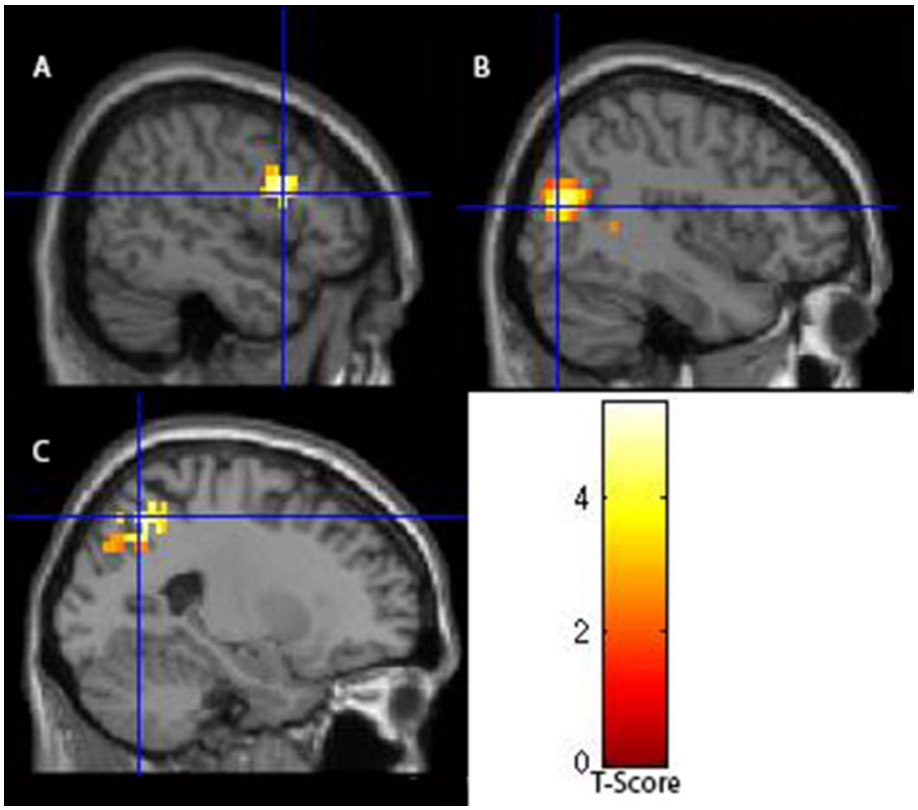
a) The nine positions where the 0 stimulus was presented, one at a time. b) An example of 4 stimulus presentations for the Match to Centre condition with 'Press' indicating where an appropriate response should occur. c) An example of 4 stimulus presentations for the visuospatial 2-Back condition with 'Press' indicating where an appropriate response should occur.

Figure 2: Blue Cross hairs indicate where increasing amounts of prenatal nicotine exposure was associated with greater activity during the visuospatial working memory condition (Visuospatial 2-Back) minus the control condition (Match to Centre), while controlling for cotinine.



A) Left inferior frontal gyrus (x,y,z= -48, -45, -15) B) Left middle frontal gyrus (x,y,z= -45, 39 -5) C) Right Frontal pole (x,y,z= 3, 57, 10)

Figure 3: Blue Cross hairs indicate where increasing amounts of prenatal nicotine exposure was associated with less activity during the visuospatial working memory condition (Visuospatial 2-Back) minus the control condition (Match to Centre), while controlling for cotinine.



A) Right dorsolateral prefrontal cortex (x,y,z= 51, 12, 20) B) Right occipital gyrus (x,y,z= 42, -75, 15, z=4.30) C) Left precuneus (x,y,z= -27, -63, 50)

Chapter 5

General Discussion

General Discussion

The three original manuscripts presented in the current dissertation are the first imaging studies to investigate the long-term neurophysiological effects of prenatal nicotine exposure on executive functioning in a sample of young adults. Participants were members of the Ottawa Prenatal Prospective Study, a longitudinal study that collected a unique body of information on participants from infancy to young adulthood, this allowed for the measurement of an unprecedented number of potentially confounding drug exposure variables. Exposed and non-exposed participants completed 3 executive functioning tasks while neural activity was quantified using fMRI. In manuscript 1, participants completed a response inhibition task, in manuscript 2 participants completed a verbal working memory task and in manuscript 3 participants completed a visuospatial working memory task. Taken together, results from all three manuscripts showed that prenatal nicotine exposure leads to altered neural functioning during executive functioning processing that continues into young adulthood.

In manuscript 1, participants with prenatal nicotine exposure compared to non-exposed controls and in manuscripts 2 and 3 participants with increasing amounts of prenatal nicotine exposure all demonstrated significantly greater activity in regions of the brain that are typically associated with each of the three tasks performed, despite no performance differences. Greater activity has been primarily attributed to reduced neural efficiency. According to the neural efficiency theory postulated by Haier and colleagues (1988; 1992) participants performing a difficult task well, tend to use a limited number of brain circuits and fewer neurons, while poor performers use more circuits and more neurons. Evidence for increased activity being primarily attributed to decreased neural efficiency has come from a large body of imaging research examining brain activity after practice effects on executive functioning tasks. For example,

Garavan et al. (2000) examined the effects of practice on functional activation patterns during visuospatial working memory task performance. After only a relatively brief 20 minute practice session, the researchers found activation decreases in the majority of areas activated during the initial, unpracticed performance of the task including the prefrontal, parietal and occipital cortex. In addition, improvements in reaction time were also noted with practice. According to the authors, these findings suggest an increase in neural efficiency, which likely underlies improvements in task performance that were observed over the training session.

These findings are consistent with those of Sayala, Sala & Courtney (2006) who used fMRI to examine functional changes during performance of an object and spatial working memory task both before and after a 30 minute training session. The results found decreased activity in regions of the brain that subserve object and spatial working memory after task practice. Decreases in functional activations accompanied by improved behavioural performance have also been observed in a study with an even longer training period (Schneiders et al., 2011). For example, after two weeks of training on a visual or auditory working memory task, Schneider and colleagues (2011) found decreased activations in the middle frontal gyrus and the posterior parietal lobule during performance of a visual n-back task. Additional decreases in frontal regions during performance of the task were also found among participants with visual working memory training only. These decreased activations were accompanied by large performance gains among participants with visual working memory training compared to those with auditory training or no training. Decreased activations have also been observed after practice of more complex executive functioning tasks such as the Tower of London (Beauchamp, Dagher, Aston, & Doyon, 2003). Taken together, these studies provide support for the hypothesis that increased brain activity is associated with less efficient neural networks.

Therefore, the greater activity found among participants with prenatal nicotine exposure suggests reduced neural efficiency as they had to work harder in order to successfully perform the task, as evidenced by the recruitment of greater neural resources in regions of the brain that subserve executive functioning processes, likely due to the greater difficulty they experienced with the task.

A region of the brain that showed significantly greater activity among participants with prenatal nicotine exposure compared to non-exposed controls in manuscript 1 and among participants with increasing amounts of prenatal nicotine exposure in manuscript 2 and 3 were large regions of the prefrontal cortex. This is consistent with the role of the prefrontal cortex in mediating executive functioning processes, particularly those associated with response inhibition, verbal working memory and visuospatial working memory (Aron et al., 2004; Barbey et al., 2012; Collette et al., 1999; Glahn et al., 2002; Jolles et al., 2011; Owen et al., 2005). In fact, in manuscript 1, participants with prenatal nicotine exposure exhibited significantly greater activity in the right inferior frontal gyrus; crucial for the suppression of inappropriate responses (Aron et al., 2004). In manuscript 2, increasing amounts of prenatal nicotine exposure was associated with significantly greater activity in the left ventrolateral and dorsolateral prefrontal cortex; critical for the manipulation and maintenance of verbal information in working memory (Barbey et al., 2012; Collette et al., 1999; D' Esposito et al., 1999; Li et al., 2012; Narayanan et al., 2005; Owen et al., 2005; Veltman et al., 2003). In the third manuscript, increasing amounts of prenatal nicotine exposure was associated with significantly greater activity in the right frontal pole; important for sustained attention (Velanova et al., 2003). In addition, greater activity was also observed in the left dorsolateral and ventrolateral prefrontal cortex. Although the left hemispheric dominance is not typical of visuospatial working memory tasks (Jennings et al.,

2006; Smith, Jonides, & Koeppe, 1996), these regions of the brain are implicated in working memory tasks (Awh et al., 1996; Narayanan et al., 2005; Owen et al., 2005).

For manuscript 3 only, increasing amounts of prenatal nicotine exposure was associated with decreased activity in right hemispheric prefrontal regions. The widespread hypofrontality, suggests that this region of the brain could not keep up with task demands as they were close to reaching working memory capacity limits and needed to recruit compensatory contralateral homologous prefrontal regions in order to successfully complete the task. The greater difficulty experienced with the visuospatial task among participants with increasing amounts of prenatal nicotine exposure is corroborated by performance data which shows that of the two working memory tasks performed, reaction time for the visuospatial working memory task was longer than for the verbal working memory task. The greater difficulty experienced with the visuospatial task is likely reflective of the task requiring two cognitive processes that have been shown to both be weak in offspring with prenatal nicotine exposure, visuospatial processing and working memory abilities (Fried, Watkinson, & Gray, 1998; Fried & Watkinson, 2000; Fried & Watkinson, 2001).

Similar to manuscript 3, the recruitment of additional brain regions not typically associated with the task was also observed in manuscript 1, whereby the prenatally exposed group showed greater activity in more posterior regions of the brain including large sections of the cerebellum, compared to the non-exposed group. The cerebellum has been shown to be important for motor response preparation and inhibition (Mostofsky et al., 2003; Simmonds et al., 2007). However, widespread diffuse activity within cerebellar regions is not typical of tasks involving response inhibition, as inhibitory control is often associated with greater activity within frontal regions (Liddle et al., 2001; Menon et al., 2001).

Taken together, greater activity within the cerebellum for manuscript 1 and in the left prefrontal cortex for manuscript 3, suggests that participants with prenatal nicotine exposure likely found the response inhibition and visuospatial working memory task more difficult than the response inhibition task and compensated for this difficulty by recruiting additional brain regions to successfully complete the task. Consistent with this hypothesis, an examination of performance data shows that of the three tasks performed, prenatally exposed participants made more errors of commission on the response inhibition and visuospatial working memory task, compared to the verbal working memory task.

In addition to the prefrontal cortex, several other regions of the brain typically implicated with each of the executive functioning tasks also showed greater activity among participants with prenatal nicotine exposure compared to non-exposed controls in manuscript 1 and participants with increasing amounts of prenatal nicotine exposure in manuscript 2. In manuscript 1, participants with prenatal nicotine exposure exhibited significantly greater activity in the basal ganglia, shown to be crucial for motor suppression (Aron & Poldrack, 2006; Li et al., 2008). In manuscript 2, increasing amounts of prenatal nicotine exposure was associated with greater activity in the left precentral gyrus, important for inner speech (Awh et al., 1996; Li et al., 2012) and the left inferior parietal lobe, critical for the short-term storage of verbal information in working memory (Chen & Desmond, 2005). Together, the greater activity exhibited by prenatally exposed participants in regions of the brain that are typically associated with both response inhibition and verbal working memory, suggests that they had to work harder to successfully perform the task, as evidenced by the recruitment of greater neural resources.

In all three manuscripts, prenatal nicotine exposure was associated with significantly greater activity in the anterior cingulate. This is not surprising since the anterior cingulate has

been shown to play a role in the detection of conflict (for review see Botvinick et al., 2004; Braver et al., 2001; Menon et al., 2001; Weissman et al., 2003). In fact, the greater the conflict, the greater the activation within the anterior cingulate, suggesting that conflict may serve as an index of task difficulty (for review see Botvinick et al., 2004; Paus, et al., 1998). Given that prenatally exposed participants exhibited greater activity in the anterior cingulate for all three tasks, provides further evidence that they likely found the tasks more difficult and compensated for this difficulty by recruiting greater neural resources within regions of the brain responsible for response inhibition, verbal working memory and visuospatial working memory.

The altered neural functioning found among participants with prenatal nicotine exposure is reflective of dysfunction in the neural circuitry subserving executive functioning. This dysfunction can come about through several mechanisms since nicotine acts on nicotinic acetylcholine receptors (nAChRs), which are expressed in the brain early in development (Hellström-Lindahl et al., 1998) and play a important role in many aspects of brain development (for review see Dwyer, McQuown, Leslie, 2009). First, animal and human research has shown that prenatal nicotine exposure alters several neurotransmitter systems including, serotonin, dopamine, acetylcholine, and glutamate (Navarro et al., 1988; Parameshwaran et al., 2012; Slotkin et al., 2006; 2007; Xu et al., 2001). Second, prenatal tobacco exposure has been shown to alter white matter maturation in children (Liu et al., 2011; Jacobsen et al., 2007). Third, structural abnormalities in several brain regions have been found among participants with prenatal nicotine exposure (Derauf et al., 2012; Toro et al., 2008). Any of these mechanisms either alone or in combination could explain the greater difficulty experienced by the prenatal nicotine group on executive functioning measures leading them to have to work harder to

perform the task, as evidenced by the recruitment of greater neural resources in regions of the brain that subserve each of the three tasks or in compensatory brain regions.

For all three tasks, no performance differences were found between participants with prenatal nicotine exposure and non-exposed controls. This is interesting in light of the fact that brain activation patterns clearly differed for participants with prenatal nicotine exposure compared to non-exposed control in study 1 and for increasing amounts of prenatal nicotine exposure in studies 2 and 3. Thus, simply relying on neurocognitive testing would yield quite different conclusions about the long-term effects of prenatal nicotine exposure on executive functioning in young adulthood. Moreover, being able to complete a task does not reveal how the brain is functioning in order to do so. Our study highlights both the importance of and sensitivity of fMRI to provide valuable information about underlying neurophysiological differences that could not be identified with behavioural data alone. In addition, our findings may also offer an explanation for the mixed results found among neurocognitive studies about the effects of prenatal tobacco exposure on executive functioning during the adolescent period. For example, in the OPPS sample, at 13 to 16 year olds, a working memory factor was negatively associated with maternal cigarette smoking, such that heavily exposed adolescents performed worse on working memory tasks compared to lightly exposed and non-exposed adolescents (Fried & Watkinson, 2001). In addition, an impulsivity factor, which was mainly derived from the CPT, was associated with prenatal tobacco exposure, but only among 13 year old adolescents, not 14 to 16 year olds (Fried & Watkinson, 2001). In contrast, using a more extensive executive functioning battery, with the same cohort, at the same age, neither perseverative errors on the WCST or interference on the Stroop was associated with maternal smoking in utero (Fried, Watkinson & Gray, 2003). It is possible that traditional neurocognitive

measures may not be sensitive enough to detect subtle cognitive impairments and the use of fMRI may help uncover these underlying neurobiological changes.

The three tasks used in the manuscripts were designed to be relatively easy so that all participants would complete the tasks, yet participants with prenatal nicotine exposure demonstrated significantly greater activity in regions of the brain that subserve each of the three tasks or in compensatory brain regions. It is likely that if given a harder task, participants with prenatal nicotine exposure may not be able to compensate and performance may suffer. Future studies should endeavour to include tasks of varying difficulty level. Second, the prenatal exposed group was also exposed to marijuana and alcohol in utero as was the control group. Despite examining their effect on neural activity and finding that these substances did not contribute to differences in neural activity between groups, future studies should aim to examine participants with only prenatal nicotine exposure. Third, some participants in both groups were also regular users of tobacco. Despite controlling for the effect of this substance on neural activity, future studies should replicate the results with participants who do not use tobacco. Fourth, the results cannot be generalized to other ethnic or socioeconomic status populations as the OPPS is primarily a Caucasian, middle class population. Fifth, although the sample size is generally in keeping with other imaging studies, it remains small and future studies should replicate the results with a greater number of participants. Sixth, a block design was used. A block design does not permit the separation of the processes of interest from other cognitive processes. However, the design of the task, including the same motor output and sensory input for both conditions of all three tasks, ensured as much as possible that the only difference between the two cognitive tasks was the process of interest. In addition, a block design does not examine the effects of performance on brain activity. An event-related study may have helped to

decipher response inhibition and working memory from other cognitive processes and would have allowed us to take into consideration performance on brain activity. Seventh, this study was a correlation design and as such, inferences about the cause of this relationship cannot be made.

Clinical Implications

Given the findings that prenatal nicotine exposure leads to altered neural functioning during executive functioning processing that continues into young adulthood, interventions aimed at helping pregnant women quit smoking should be of the utmost importance. Interestingly, smoking cessation interventions in pregnant women have often included nicotine replacement therapy (NRT). The rational for using NRT is that it delivers only nicotine, while eliminating the remaining 4000 chemicals in cigarette smoke (Osadchy, Kazmin, & Koren, 2009). In addition, NRT has been shown to be a highly effective smoking cessation aid in the general population (Kralikova, Kozak, Rasmussen, Gustavsson & Le Houezec, 2009). However, research examining the efficacy and safety of NRT among pregnant women has been both limited and mixed. Wisborg, Henriksen, Jespersen, & Secher (2000) compared smoking cessation rates, preterm delivery and infant birth weight among pregnant women randomly assigned to receive nicotine patches to those assigned to receive placebo patches. Overall, 28% of women in the nicotine group and 25% of women in the placebo group reported smoking abstinence during their final prenatal visit, with no differences between the groups. There were also no group differences in the number of cigarettes smoked per day or cotinine values. In addition, no group differences emerged on rate of preterm delivery, although babies born to women who received nicotine patches had a slightly higher weight at birth, compared to the placebo group. These results are consistent with those of Coleman et al. (2012) who randomly

assigned 521 tobacco smoking pregnant women to receive behavioural cessation support in addition to nicotine patches, with the remaining 529 women receiving behavioral support with matched placebo patches. The researchers found no significant differences in the rate of self-reported abstinence from quit date until time of delivery, validated by salivary cotinine concentrations between the nicotine and placebo groups, with both groups having extremely low rates (less than 10% in each group). There were also no differences between groups on several birth outcomes including rates of preterm birth, low birth weight or congenital abnormalities. However, women in the nicotine group were found to have higher rates of delivery by means of caesarian section. The most striking finding in the study, however, was the low compliance with the usage of the patches. Only 7.2% of women assigned to the nicotine group and 2.8% assigned to the placebo group used patches for more than 1 month. Several other studies have also shown that the addition of nicotine patches to cessation interventions that involve relatively brief supportive counselling for pregnant women does not significantly increase the rate of abstinence or increase compliance to the intervention (Hotham, Gilbert, & Atkinson, 2006; Kapur, Hackman, Selby, Klein, & Koren, 2001). In addition, the safety of NRT has been called into question when one study was prematurely discontinued after an interim analysis found a higher rate of negative birth outcomes among offspring whose mothers were enrolled in the counselling plus NRT group compared to the offspring of women who were enrolled in the counselling only group (Pollack et al., 2007).

In contrast to studies with nicotine patches, low dose nicotine gum has been shown to reduce nicotine and cotinine concentrations among pregnant women compared to regular cigarette smoking (Oncken, Hatsukami, Lupo, Lando, Gibeau, & Hanson, 1996). In a later study, Oncken et al. (2008) found that low dose nicotine gum with counselling reduced the

number of cigarettes per day and cotinine concentrations among pregnant smoking women, compared to women who received counselling with a placebo. In addition, birth weight and gestational age was also greater in the NRT group, compared to that of the placebo group. However, cessation rates were not significantly different between the two groups.

As a whole, the limited effectiveness of NRT in increasing cessation rates, the questionable safety, its low compliance and the numerous animal studies that have shown that nicotine is the main neurotoxin, strengthens the case for discouraging NRT as an intervention strategy. In addition, although NRT administered via gum has proven slightly more successful in decreasing nicotine levels among pregnant women, they do not eliminate it. Thus, implementing interventions that are more effective at increasing cessation rates among pregnant women without the use of NRT should be top priority. However, providing effective intervention strategies is contingent on understanding the characteristics of women who continue to smoke during pregnancy. A consistent body of research has shown that smoking cessation is less likely among pregnant women who have a low income, have less than a high school education, have had previous births, are highly addicted and have a husband or partner who smokes (Ockene et al., 2002; Ma, Goins, Pbert, & Ockene, 2005). As such, these risk factors should be taken into consideration during the development of intervention programs to help pregnant women quite smoking.

Conclusion

The aim of the present dissertation was to shed light on the long-term neurophysiological effects of prenatal nicotine exposure on three different executive functioning processes by assessing participants in young adulthood, using functional magnetic resonance imaging (fMRI). The dissertation consists of three separate original manuscripts. In manuscript 1, participants

completed a response inhibition task, in manuscript 2 participants completed a verbal working memory task and in manuscript 3 participants completed a visuospatial working memory task. Taken together, results from all three manuscripts showed that prenatal nicotine exposure leads to altered neural functioning during executive functioning processing. These manuscripts are the first to show that the effects of prenatal nicotine exposure on executive functioning continue into young adulthood. Executive functions have been shown to be critical for academic, social, behavioural and emotional functioning. Thus, impairments in executive functioning skills could leave children with prenatal nicotine exposure at risk for failure in many of these domains. Therefore, these findings highlight the need for continued educational programs and public awareness campaigns to reduce tobacco use among pregnant women.

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Appendix A

Drug History Questionnaire: Booklet A

Cigarette Smoking Questionnaire

1. Please indicate the day of the week today:

Mon. ☐ Tues. ☐ Wed. ☐ Thurs. ☐ Fri. ☐ Sat. ☐ Sun. ☐

2. Today's date is _____ _____ _____
day month year

3. Are you now or have you ever been exposed to secondhand smoke from cigarettes on a regular basis, i.e., typically at least one hour everyday?

No: Insert an **N** in box and skip to question 10 (page 3).

Yes: Insert a **Y** in box and continue. ☐

4. How many years, in the past five years, have you been exposed regularly to secondhand smoke? Insert answer (in years) in box. ☐

5. Are you **now** regularly exposed to secondhand smoke, i.e., typically at least one hour everyday (at home, workplace etc.)?

No: Insert an **N** in box and skip to question 10 (page 3).

Yes: Insert a **Y** in box and continue. ☐

6. Are you exposed on a regular basis to secondhand smoke in your home, i.e., in the same room as the person smoking?

No: Insert an **N** in box and skip to question 8 on the next page

Yes: Insert a **Y** in box and continue. ☐

7. How many hours were you exposed to secondhand smoke at home (in the same room as the person smoking) today and in each of the last 7 days? Insert the number of hours in boxes.

Last Sat. ☐ Last Sun. ☐ Last Mon. ☐ Last Tues. ☐

Last Wed. ☐ Last Thurs. ☐ Last Fri. ☐ Today ☐

8. Are you exposed to secondhand smoke outside your home (e.g., friend's home, a car, a bar)?

No: Insert an **N** in box and skip to question 10 (page 3).

Yes: Insert a **Y** in box and continue. ☐

9. How many hours were you exposed to secondhand smoke outside your home today and in each of the last 7 days? Insert the number of hours in boxes.

Last Sat. ☐ Last Sun. ☐ Last Mon. ☐ Last Tues. ☐

Last Wed. ☐ Last Thurs. ☐ Last Fri. ☐ Today ☐

10. Among your friends, how many have tried cigarettes?

none ☐ some ☐ most ☐ all ☐ don't know ☐

11. Among your friends, how many smoke cigarettes on a regular basis (i.e., at least once a day)?

none ☐ some ☐ most ☐ all ☐ don't know ☐

12. Have **<u>you</u> ever** smoked (beyond just trying out one or two cigarettes)?

No: Insert an **N** in box and skip to <u>Booklet B.</u>

Yes: Insert a **Y** in box and continue. ☐

13. How old were you when you first smoked cigarettes (beyond just trying out one or two)?

Insert age in years in box. ☐

14. Have you ever smoked on a **regular** basis, i.e., at least once a day?

No: Insert an **N** in box and skip to <u>Booklet B.</u>

Yes: Insert a **Y** in box and continue. ☐

15. How old were you when you started to smoke on a regular basis?

Insert age in years in box. ☐

16. How many years have you been smoking (or did you smoke) on a regular basis? Insert answer in box.

17. Are you smoking **now** on a regular basis, i.e., at least once a day?

No: Insert an **N** in box and skip to Booklet B.

Yes: Insert a **Y** in box and continue.

18. How many cigarettes do you smoke on average per day?

Insert answer in box

19. How many cigarettes did you smoke on each of the last three days?

Insert answers in boxes.

today:  yesterday:  day before yesterday: 

20. After waking up in the morning, how many minutes pass before you usually have your first cigarette? Insert answer in box.

21. Do you usually smoke the same brand?

No: Insert a **N** in box: Skip to Question 23 on next page

Yes: Insert a **Y** in box and continue.

22. What brand (including size [e.g., king] and strength [e.g., light]) do you usually smoke?

_____

23. Have you ever tried to cut down on the use of cigarettes?

Yes ☐ No ☐

24. Have you ever felt unable to cut down on the use of cigarettes?

Yes ☐ No ☐

25. Have you ever felt the need to have more cigarettes to get the same effect?

Yes ☐ No ☐

26. Have you ever felt the need to have cigarettes or felt dependent on cigarettes?

Yes ☐ No ☐

27. Have you ever felt sick because of stopping or cutting down on cigarettes?

Yes ☐ No ☐

Go to Booklet B

Drug History Questionnaire: Booklet B

Marihuana/Hashish Questionnaire

1. Among your friends how many have tried marihuana/hashish?

none ☐ some ☐ most ☐ all ☐ don't know ☐

2. Among your friends how many smoke marihuana/hashish on a regular basis (at least once a week)?

none ☐ some ☐ most ☐ all ☐ don't know ☐

3. Have <u>you</u> ever been exposed to secondhand smoke from marihuana or hashish?

No: Insert an **N** in box and skip to question 6 on next page. ☐

Yes: Insert a **Y** in box and continue.

4. When was the last time you were exposed to secondhand smoke from marihuana or hashish?

more than a month ago ☐ between 1 week and 4 weeks ago ☐

between 2 and 6 days ago ☐ yesterday ☐ today ☐

☐

5. The last time that you were exposed to secondhand marihuana or hashish smoke were you exposed for

more than 3 hours? ☐ between 2 and 3 hours? ☐ between 1 and 2 hours? ☐

less than 1 hour? ☐

6. Have you ever tried marihuana?

No: Insert an **N** in box and skip to question 22 on page 4. ☐

Yes: Insert a **Y** in box and continue.

7. How old were you when you first smoked marihuana?

Insert age in years in box. ☐

8. Are you smoking marihuana **now** on a regular basis, i.e., at least once a week?

No: Insert an **N** in box and skip to question 14 on next page. ☐

Yes: Insert a **Y** in box and continue.

9. When you use marihuana, about how many joints do you usually smoke at a time? If you share reefers how much do YOU usually smoke?

Insert the number (or fraction) in box ☐

10. On average, how often do you smoke this amount?

every day ☐ 3-6 times per week ☐ 1-2 times per week ☐

2-3 times per month ☐ once a month ☐ 6-11 times per year ☐

1-5 times a year ☐ less than once a year ☐

11. How many joints of marihuana do you **now** smoke on average per week? If you share reefers I would like to know how much YOU smoke.

Insert number (or fraction) in box. ☐

12. How many joints of marihuana did you smoke during the past 7 days? If you share reefers I would like to know how much YOU smoked on these days. Insert in boxes.

Last Sat. ☐ Last Sun. ☐ Last Mon. ☐

Last Tues. ☐ Last Wed. ☐ Last Thurs. ☐

Last Fri. ☐ Today ☐

13. Please indicate the day of the week today

Mon. ☐ Tues. ☐ Wed. ☐ Thurs. ☐ Fri. ☐ Sat. ☐ Sun. ☐

Go to Question 15

14.(a) If you are not smoking marihuana regularly now, did you ever smoke marihuana regularly in the past (at least once a week)?

No: Insert an **N** in box and skip to <u>question 16B on next page.</u>

If you are or were a regular user, please continue.

14.(b) When you smoked marihuana regularly in the past, how many joints did you usually smoke per week. If you shared reefers, how much did YOU usually smoke?

☐

15. How old were you when you started to smoke marihuana on a regular basis?

Insert age in years in box. ☐

16.(a) How many years have you smoked (or did you smoke) marihuana on a regular basis?

Insert answer in box. ☐

16.(b) Have you smoked any marihuana in the past year?

☐ ☐

Yes Go to 16.(c) No Go to 17

16.(c) How many days is it since you smoked up? ☐

16.(d) When you last smoked up, how many joints did you smoke?

If you shared reefers, how much did YOU smoke? ☐

17. Have you ever tried to cut down on the use of marihuana?

Yes ☐ No ☐

18. Have you ever felt unable to cut down on the use of marihuana?

Yes ☐ No ☐

19. Have you ever felt the need to have more marihuana to get the same effect?

Yes ☐ No ☐

20. Have you ever felt the need to have marihuana or felt dependent on marihuana?

Yes ☐ No ☐

21. Have you ever felt sick because of stopping or cutting down on marihuana?

Yes ☐ No ☐

22. Have you ever tried hashish?

No: Insert an **N** in box and skip to Booklet C. ☐

Yes: Insert a **Y** in box and continue.

23. How old were you when you first smoked hashish?

Insert age in years in box. ☐

24. Are you smoking hashish **now** on a regular basis, i.e., at least once a week?

No: Insert an **N** in box and skip to question 30. ☐

Yes: Insert a **Y** in box and continue.

25. On the days that you use hashish, about how many grams do you usually smoke? If you share, how much do YOU usually smoke?

Insert the number of grams (or fraction) in box ☐

26. On average, how often do you smoke this amount?

every day ☐ 3-6 times per week ☐ 1-2 times per week ☐

2-3 times per month ☐ once a month ☐ 6-11 times per year ☐

1-5 times a year ☐ less than once a year ☐

27. How many grams of hashish do you **now** smoke on average per week? If you share, I would like to know how many grams YOU smoked.

Insert number of grams (use fractions if necessary) in box. ☐

28. How many grams of hashish did you smoke during the past 7 days? If you share, I would like to know how much YOU smoked on these days. Insert in number of grams (use fractions if necessary) in boxes.

Last Sat.		Last Sun.		Last Mon.	
Last Tues.		Last Wed.		Last Thurs.	
Last Fri.		Today			

29. Please indicate the day of the week today

Mon. ☐ Tues. ☐ Wed. ☐ Thurs. ☐ Fri. ☐ Sat. ☐ Sun. ☐

Go to Question 31

30. If you are not smoke hashish regularly now, have you ever smoked hashish on a regular basis, at least once a week or more?

No: Insert a **N** in box: Go to Booklet C. ☐

If you are or were a regular user, please continue.

30.(b) When you smoked hashish regularly in the past, how many grams did you usually smoke per week? If you shared, how much did YOU usually smoke?

☐

31. How old were you when you started to smoke hashish on a regular basis?

Insert age in years in box. ☐

32. How many years have you smoked (or did you smoke) hashish on a regular basis?

Insert answer in box. ☐

32.(b) Have you used any hashish in the past year?

Yes ☐ Go to 32(c) No ☐ Go to 33

32.(c) How many days is it since you used hashish? ☐

17. Have you ever tried to cut down on the use of hashish?

Yes ☐ No ☐

18. Have you ever felt unable to cut down on the use of hashish?

Yes ☐ No ☐

19. Have you ever felt the need to have more hashish to get the same effect?

Yes ☐ No ☐

20. Have you ever felt the need to have hashish or felt dependent on hashish?

Yes ☐ No ☐

21. Have you ever felt sick because of stopping or cutting down on hashish?

Yes ☐ No ☐

Go to <u>Booklet C</u>

Drug History Questionnaire: Booklet C

Drinking Questionnaire

1. Among your friends, how many drink alcohol on a regular basis?

none ☐ some ☐ most ☐ all ☐

2. Compared to your friends do you drink

much less ☐ slightly less ☐ about the same ☐ more ☐ much more ☐

3. Have you ever had a complete drink (i.e., a bottle of beer, a shot of liquor or a glass of wine)?

No: Insert an **N** in box and skip to Booklet D.

Yes: Insert a **Y** in box and continue. ☐

4. How old were you when you first had a complete drink?

Insert age in years in box. ☐

5. On the days that you drink alcohol, about how many drinks do you usually drink? Consider a drink to be a bottle of beer, a shot of liquor or a glass of wine.

Number of drink. Insert answer in box. ☐

6. On average, how often do you drink this amount?

every day ☐ 3-6 times per week ☐ 1-2 times per week ☐

2-3 times per month ☐ once a month ☐ 6-11 times per year ☐

1-5 times a year ☐ less than once a year ☐

7. Do you have days when you drink more than this amount?

No: Insert An **N** in box: Go to question 10 on this page.

Yes: Insert a **Y** in box and continue. ☐

8. How many drinks do you usually drink then?

Number of drink. Insert answer in box. ☐

9. How often do you drink this amount?

3-6 times per week ☐ 1-2 times per week ☐

2-3 times per month ☐ once a month ☐

6-11 times per year ☐ 1-5 times a year ☐

10. Do you presently drink on a regular basis (at least 2 times a week)?

No: Insert An **N** in box: Go to Booklet D.

Yes: Insert a **Y** in box and continue. ☐

11. At what age did you start to drink on a regular basis? Please enter the answer in years in the box. ☐

12. What alcoholic beverage do you usually drink? If appropriate, check more than one box.

Beer ☐ liquor ☐ wine ☐

13. How much did you drink today and during the past 7 days? Insert the number of drinks in the appropriate boxes. A drink is a bottle of beer, a shot of liquor or a glass of wine.

Last Sat.		Last Sun.		Last Mon.	
Last Tues.		Last Wed.		Last Thurs.	
Last Fri.		Today			

14. Please indicate the day of the week today:

Mon. ☐ Tues. ☐ Wed. ☐ Thurs. ☐ Fri. ☐ Sat. ☐ Sun. ☐

15. Has your drinking decreased, stayed the same or increased in the past 12 months?

decreased ☐ stayed the same ☐ increased ☐

23. Have you ever tried to cut down on the use of alcohol?

Yes ☐ No ☐

24. Have you ever felt unable to cut down on the use of alcohol?

Yes ☐ No ☐

25. Have you ever felt the need to have more alcohol to get the same effect?

Yes ☐ No ☐

26. Have you ever felt the need to have alcohol or felt dependent on alcohol?

Yes ☐ No ☐

27. Have you ever felt sick because of stopping or cutting down on alcohol?

Yes ☐ No ☐

Go to <u>Booklet D</u>

Drug History Questionnaire: Booklet D

Other Drug Use

1. Have you ever used Amphetamines (speed, uppers, ice, bennies, ecstasy)?

No: Insert a **N** in box: Go to Question 10 on the next page. ☐

Yes: Insert a **Y** in box and continue.

2. How old were you (in years) when you first tried them? ☐

3. When was the last time you used (in days)? ☐

4. How many times have you used them in the past month? ☐

5. Have you ever tried to cut down on the use of amphetamines?

Yes ☐ No ☐

6. Have you ever felt unable to cut down on the use of amphetamines?

Yes ☐ No ☐

7. Have you ever felt the need to have more amphetamines to get the same effect?

Yes ☐ No ☐

8. Have you ever felt the need to have amphetamines or felt dependent on amphetamines?

Yes ☐ No ☐

9. Have you ever felt sick because of stopping or cutting down on amphetamines?

Yes ☐ No ☐

10. Have you ever used Crack and/or cocaine?

No: Insert a **N** in box: Go to Question 19 on the next page. ☐

Yes: Insert a **Y** in box and continue.

11. How old were you (in years) when you first tried it? ☐

12. When was the last time you used it (in days)? ☐

13. How many times have you used it in the past month? ☐

14. Have you ever tried to cut down on the use of crack/cocaine?

Yes ☐ No ☐

15. Have you ever felt unable to cut down on the use of crack/cocaine?

Yes ☐ No ☐

16. Have you ever felt the need to have more crack/cocaine to get the same effect?

Yes ☐ No ☐

17. Have you ever felt the need to have crack/cocaine or felt dependent on crack/cocaine?

Yes ☐ No ☐

18. Have you ever felt sick because of stopping or cutting down on crack/cocaine?

Yes ☐ No ☐

19. Have you ever used Tranquilizers (sleeping pills, barbs, downers)?

No: Insert a **N** in box: Go to Question 28 on the next page. ☐

Yes: Insert a **Y** in box and continue.

20. How old were you (in years) when you first tried them? ☐

21. When was the last time you used them (in days)? ☐

22. How many times have you used them in the past month? ☐

23. Have you ever tried to cut down on the use of tranquilizers?

Yes ☐ No ☐

24. Have you ever felt unable to cut down on the use of tranquilizers?

Yes ☐ No ☐

25. Have you ever felt the need to have more tranquilizers to get the same effect?

Yes ☐ No ☐

26. Have you ever felt the need to have tranquilizers or felt dependent on tranquilizers?

Yes ☐ No ☐

27. Have you ever felt sick because of stopping or cutting down on tranquilizers?

Yes ☐ No ☐

28. Have you ever tried Heroin?

No: Insert a **N** in box: Go to Question 37 on the next page. ☐

Yes: Insert a **Y** in box and continue.

29. How old were you (in years) when you first tried it? ☐

30. When was the last time you used it (in days)? ☐

31. How many times have you used it in the past month? ☐

32. Have you ever tried to cut down on the use of heroin?

Yes ☐ No ☐

33. Have you ever felt unable to cut down on the use of heroin?

Yes ☐ No ☐

34. Have you ever felt the need to have more heroin to get the same effect?

Yes ☐ No ☐

35. Have you ever felt the need to have heroin or felt dependent on heroin?

Yes ☐ No ☐

36. Have you ever felt sick because of stopping or cutting down on heroin?

Yes ☐ No ☐

37. Have you ever tried LSD (acid)?

No: Insert a **N** in box: Go to Question 46 on the next page. ☐

Yes: Insert a **Y** in box and continue.

38. How old were you (in years) when you first tried it? ☐

39. When was the last time you used it (in days)? ☐

40. How many times have you used it in the past month? ☐

41. Have you ever tried to cut down on the use of LSD?

Yes ☐ No ☐

42. Have you ever felt unable to cut down on the use of LSD?

Yes ☐ No ☐

43. Have you ever felt the need to have more LSD to get the same effect?

Yes ☐ No ☐

44. Have you ever felt the need to have LSD or felt dependent on LSD?

Yes ☐ No ☐

45. Have you ever felt sick because of stopping or cutting down on LSD?

Yes ☐ No ☐

46. Have you ever tried Solvents (glue, gasoline, paint thinners etc.)?

No: Insert a **N** in box: Go to Question 55 on the next page. ☐

Yes: Insert a **Y** in box and continue.

47. How old were you (in years) when you first tried it? ☐

48. When was the last time you used it (in days)? ☐

49. How many times have you used it in the past month? ☐

50. Have you ever tried to cut down on the use of Solvents?

Yes ☐ No ☐

51. Have you ever felt unable to cut down on the use of Solvents?

Yes ☐ No ☐

52. Have you ever felt the need to have more Solvents to get the same effect?

Yes ☐ No ☐

53. Have you ever felt the need to have Solvents or felt dependent on Solvents?

Yes ☐ No ☐

54. Have you ever felt sick because of stopping or cutting down on Solvents?

Yes ☐ No ☐

55. Have you ever used Steroids?

No: Insert a **N** in box: Go to Question 64 on the next page. ☐

Yes: Insert a **Y** in box and continue.

56. How old were you (in years) when you first tried them? ☐

57. When was the last time you used them (in days)? ☐

58. How many times have you used them in the past month? ☐

59. Have you ever tried to cut down on the use of Steroids?

Yes ☐ No ☐

60. Have you ever felt unable to cut down on the use of Steroids?

Yes ☐ No ☐

61. Have you ever felt the need to have more Steroids to get the same effect?

Yes ☐ No ☐

62. Have you ever felt the need to have Steroids or felt dependent on Steroids?

Yes ☐ No ☐

63. Have you ever felt sick because of stopping or cutting down on Steroids?

Yes ☐ No ☐

64. Have you ever used Mushrooms?

No: Insert a **N** in box: Go to Question 73 on the next page. ☐

Yes: Insert a **Y** in box and continue.

65. How old were you (in years) when you first tried mushrooms? ☐

66. When was the last time you used mushrooms (in days)? ☐

67. How many times have you used mushrooms in the past month? ☐

68. Have you ever tried to cut down on the use of mushrooms?

Yes ☐ No ☐

69. Have you ever felt unable to cut down on the use of mushrooms?

Yes ☐ No ☐

70. Have you ever felt the need to have more mushrooms to get the same effect?

Yes ☐ No ☐

71. Have you ever felt the need to have mushrooms or felt dependent on mushrooms?

Yes ☐ No ☐

72. Have you ever felt sick because of stopping or cutting down on mushrooms?

Yes ☐ No ☐

73. Have you ever used **<u>other</u>** <u>drugs (please specify)</u>? _____

No: Insert a **N** in box: Please turn in booklet.

Yes: Insert a **Y** in box and continue. ☐

74. How old were you (in years) when you first tried _____? ☐

75. When was the last time you used _____ (in days)? ☐

76. How many times have you used _____ in the past month? ☐

77. Have you ever tried to cut down on the use of _____?

Yes ☐ No ☐

78. Have you ever felt unable to cut down on the use of _____?

Yes ☐ No ☐

79. Have you ever felt the need to have more _____ to get the same effect?

Yes ☐ No ☐

80. Have you ever felt the need to have _____ or felt dependent _____?

Yes ☐ No ☐

81. Have you ever felt sick because of stopping or cutting down on _____?

Yes ☐ No ☐

Please turn in this Booklet – if you need another page like this one, please ask.