An investigation into the effects of cigarette and nicotine consumption during pregnancy and the effects on fetal and infant neurobehaviour.

SUZANNE LISA FROGGATT

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Suzanne Lisa Froggatt, BSc (Hons), MSc

Abstract

The research presented in this thesis is an examination of the relationship between cigarette and e-cigarette use on both fetal and infant behaviour, maternal understanding of risks associated with these products and the association between fetal and infant behaviour. Smoking during pregnancy is well known to lead to an array of negative health and behavioural outcomes, with very few studies assessing the impact on fetal behaviour. Chapters 1-3 introduce the topic alongside in-depth methodology sections. Chapter 4 discusses research partially replicating a pilot study conducted by Reissland et al. (2015) assessing the impact of cigarette exposure on fetal mouth movements, with the addition of separated cigarette groups (light and heavy smoking) and an e-cigarette exposed group. The findings indicate that there were no significant differences in frequency of fetal mouth movements between the four exposure groups, but generally a decline in mouth movement across the gestational ages. Chapter 5 is a meta-analysis that indicated that prenatal cigarette exposure was associated with worse neurobehavioural outcomes up to one year of age, with results from Chapter 6 also indicating negative neurobehavioural effects at one month of age. These effects were not only evident for the cigarette exposed infants but also for infants who were prenatally exposed to e-cigarettes. Research suggests that fetal facial movements, in particular mouth movements are indicative of brain functioning. However, the findings reported in Chapter 7 found no significant

relation between fetal mouth movements and infant neurobehaviour. Chapter 8 provides an account of maternal perceptions of risks associated with both cigarette and e-cigarette use during pregnancy. Results indicate that women provide several justifications for continued smoking and that e-cigarettes were regarded as a riskier option. The thesis concludes with a general discussion of the main findings, implications for policy and a critique of the research.

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The PEN Study: Prenatal Effects of Nicotine

Suzanne Lisa Froggatt, BSc (Hons), MSc.

Thesis submitted for the Degree of Doctor of Philosophy

Department of Psychology
Durham University
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Abbreviations

2D/3D/4D	2-dimenstional/3-dimenstional/4-dimenstional
ASH	Action on Smoking and Health
ASQ	Ages and Stages Questionnaire
ATNAT	Amiel-Tison Neurodevelopmental Test
BMI	Body Mass Index
CCG	Clinical Commissioning Group
CNS	Central Nervous System
CO	Carbon Monoxide
COHb	Carboxyhemoglobin
CT	Computed Tomography
DSM-IV	Diagnostic and Statistical Manual
ECG	Electrocardiogram
EEG	Electroenecephogram
FACS	Facial Action Coding
FCOHb	Fetal Carboxyhemoglobin
FENS	Fetal Neurobehavioural Assessment Scale
FGR	Fetal Growth Restriction
FOMS	Fetal Observable Movement System
HADS	Hospital Anxiety and Depression Scale
HG	Hyperemesis Gravidarum
ICU	Intensive Care Unit
IUGR	Intrauterine Growth Restriction
KANET	Kurjak's Antenatal Neurodevelopmental Test
MRI	Magnetic Resonance Imaging
nAChRs	Nicotinic acetylcholine receptors
NBAS	Neonatal Behavioural Assessment Scale
NHS	National Health Service
NICE	The National Institute for Health and Care Excellence
NICU	Neonatal Intensive Care Unit
NNNS	NICU Network Neurobehavioural Scale

NRT	Nicotine Replacement Therapy
PHE	Public Health England
PSS	Perceived Stress Scale
PWS	Prada Willi Syndrome
SATOD	Smoking at time of delivery
SGA	Small for gestational age
SIDS	Sudden Infant Death Syndrome
SNAP	Smoking and Nicotine in Pregnancy
WHO	World Health Organisation

Declaration

I confirm that none of the material presented in this thesis has been submitted elsewhere for any other qualification, and unless referenced, all work is my own. This work was funded by the Northern Ireland and North East Doctoral Training Partnership (Economic and Social Research Council, grant number ES/P000762/1) and in collaboration with The James Cook University Hospital (South Tees NHS Foundation Trust) in which the 4D ultrasound scans were provided as an in-kind contribution.

Copyright

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Chapter 5, 6 and 8 are presented as they were submitted, with the exception of a few additional references requested as examiner corrections, referencing style and numbering of both tables and figures in order to be consistent throughout the thesis. Chapter 4 has additional analysis (individual mouth movement analysis) that is not part of the published work.

Dedication

To Mum and Dad, I wouldn't be where I am today without you both.

Chapter 1
Introduction

"The tobacco epidemic is one of the biggest public health threats the world has ever faced" (World Health Organization, 2020, Page1).

The overarching aim of the current research studies is to provide a better understanding of how smoking status during pregnancy impacts both fetal and infant neurobehaviour. The main inspiration for the thesis is derived from a pilot study indicating that cigarette exposed fetuses display a different behavioural profile in comparison to non-exposed fetuses (Reissland, Francis, Kumarendran, & Mason, 2015). The motivation to develop this study was to examine what prenatal behavioural differences may mean for later infant behaviour. The group of e-cigarette exposed fetuses adds to the wider debate regarding the controversary surrounding e-cigarette use in pregnancy, with the behavioural effects largely neglected and thus not assessed. The key aims are to assess whether smoking status leads to different behavioural profiles for the fetus and infant, to establish the relationship between these two time points and to gain an understanding of maternal perceptions of risk associated with cigarette and e-cigarette use during pregnancy.

In this introduction, an in-depth discussion of cigarette smoking and the implications from preconception through to infancy is provided. The focus will then turn toward cigarette smoking alternatives such as nicotine replacement therapy (NRT) and e-cigarette use. Next, given that fetal behavioural psychology is a relatively new field of

psychology, a detailed discussion is provided. Finally, the key aims are addressed, followed by an outline of the thesis chapters.

Smoking

Across the world each year, tobacco exposure, as a result of cigarette smoking, kills on average 8 million people, with 7 million of those being a direct result of smoking and 1.2 million associated with secondhand smoke exposure (World Health Organization, 2020). It is estimated that there are approximately 1.3 billion tobacco users worldwide, with the most common form of tobacco intake coming from cigarette smoking, with 80% of smokers living in low to middle income countries (World Health Organization, 2020). Death and illness as a result of cigarette smoking is 100% preventable. In 2019, the prevalence of smoking in adults (+18 years) was 13.9% across England, ranging from 12.2% in the South East to 15.7% in the Yorkshire and Humber regions (Public Health England, 2020).

Cigarette smoking affects the entire body, not just the lungs and heart, although it is known to cause heart disease, respiratory disease and lung cancer, there are a number of other cancers that have been associated with cigarette smoking; for example, mouth, bladder, stomach and cervical cancers (ASH, 2020a). The common medical diseases associated with smoking include chronic obstructive pulmonary disease (COP), coronary heart disease (CHD) and lung cancer. According to Action on Smoking and Health (ASH), for the year 2015, 27% of cancer deaths, 35% respiratory deaths and 13% of circulatory deaths were caused by smoking (ASH, 2020a).

For 2017/2018 there were 489,300 hospital admissions across the UK in which the cause of admission was associated to cigarette smoking, with 22% for respiratory disease, 15% for circulatory disease and 9% for cancer. Of the 9% for cancers, 47% of those were reported to be directly caused by smoking, with the same being true for 39% of those who were admitted due to respiratory disease (NHS Digital, 2019). Examining mortality rates in the UK as a result of cigarette smoking, in 2017, 33% of deaths were associated to conditions that occur as a result of cigarette smoking (NHS Digital, 2019).

There are over 60 well-known carcinogenic compounds in cigarettes including Nitromethane, Arsenic, Cadmium, Polycyclic aromatic hydrocarbon (PAH), Nicotine-derived nitrosamine ketone (NNK), Benzene and Lead. With both PAH and NNK thought to play a critical role in the development of lung cancer, mouth cancer and cervical cancer (Hecht, 2006). In addition, benzene is thought to play a role in the development of leukaemia in cigarette smokers (Hecht, 2006), highlighting the many risks posed by a variety of different compounds in cigarettes and the development toward cancer. The two most prominent and well-known carcinogenic compounds in cigarettes are carbon monoxide (CO) and nicotine.

Carbon Monoxide

Carbon monoxide is present in the environment including in homes because of cleaning products, central heating and cooking equipment, and outside, mainly through car pollution. In the US for example, 75% of CO in the air outside the home is due to car exhaust emissions (Levy, 2015).

When CO is inhaled it affects body tissue and oxygen levels in numerous ways. CO binds to haemoglobin creating carboxyhaemoglobin (COHb), therefore affecting the bodies transportation of oxygen in the blood, because CO binds quicker than oxygen to haemoglobin cells (Sandilands & Bateman, 2016). The problem arises when CO enters the blood stream exceeding the natural outdoor concentrations of the environment (e.g., higher levels of car exhaust fumes in cities or cigarette smoking) (Levy, 2015). This becomes more problematic during pregnancy, as CO is in maternal blood, it crosses through the placenta and into the fetal circulatory system and thereby affecting fetal brain development (Levy, 2015). A central concern regarding fetal brain function is fetal hypoxia, because as the level of COHb increases slowly, fetal levels of COHb (FCOHb) are roughly 10-15% higher than that of the mother. Furthermore, levels decrease slower in the fetus than the mother, with the effect overall increasing the exposure to CO in the fetus (Sandilands & Bateman, 2016).

Nicotine

Nicotine is a highly addictive substance that is carcinogenic and toxic. It can cause an increase in blood pressure and heart rate, with a stimulating effect on the central nervous system (CNS) (Holbrook, 2016). Such effects occur as nicotine activates the nicotinic acetylcholine receptors (nAChRs) (Holbrook, 2016). The nAChRs are expressed throughout the nervous system, with nicotine binding to these receptors after crossing the placenta leading to elevated levels during the critical periods of development and therefore affecting the regulation of fetal brain maturation (England, Bunnell, Pechacek, Tong, & McAfee, 2015). When metabolised nicotine turns into cotinine, a by-product of nicotine which can be measured in urine, salvia, blood and

hair samples. The process of metabolising nicotine to cotinine happens within minutes and the problem is that it has a long plasma half-life, i.e., the amount of time it takes to be expelled from the body (15-19 hours), which in turn affects the development of the fetal brain and maturation processes (Demirhan, 2017; Dempsey & Benowitz, 2001; England et al., 2015). Research has identified that levels of cotinine in the newborn are associated with behaviour, with increased irritability, and growth restriction from birth through to five years old (Mansi et al., 2007; Ng et al., 2019).

Nicotine consumption through cigarette smoking, is the most commonly used toxic substance used during pregnancy (Forray & Foster, 2015). There are well known detrimental outcomes associated with cigarette smoking, including cancer and death. As reducing the consumption of cigarette smoking amongst the whole population will drastically reduce such negative outcomes linked to the many carcinogenic and toxicants in cigarettes, the consideration of nicotine and the dangers are often absent from public health debates and effects of nicotine per se are considered to be of minor importance (England et al., 2015). It is often reported that public perception of the use of e-cigarettes and nicotine replacement therapy (NRT) are relatively safe and beneficial without proper research being conducted. This has resulted in advice not taking into account the special circumstances of the mother and fetus during pregnancy (England et al., 2015). This is particularly important in the case of e-cigarette use during pregnancy, which is advised as being safer than cigarette use. However, there is little to no research to suggest e-cigarettes are safe; in fact, there is no evidence to suggest that any amount of nicotine is safe during pregnancy (Holbrook, 2016).

Referring to animal models to assess the impact of nicotine, thus NRT and e-cigarettes, on pulmonary function, a review of the literature indicates that pure nicotine has comparable effects to cigarettes on lung development and disease (Spindel & McEvoy, 2016). As a result of nicotine exposure, there is a decrease in expiratory airflow, a thickening of the airway walls leading to narrow airways which changes how nAChR is expressed and is likely to lead to immune function problems and an increase of incidence of a wheeze and asthma. The authors of this paper concluded that due to the lack of epidemiological research, e-cigarette should not be recommended as a safe alternative to cigarette smoking (Spindel & McEvoy, 2016).

Preconception and early pregnancy

The first hurdle that many couples face as a result of smoking is infertility, which is defined as a failure to conceive after 12 months of intercourse, without the use of protection (Royal College of Physicians, 2010). A review article assessing lifestyle factors and reproductive health indicates that smoking can lead to fertility problems in both males and females, with couples who smoke taking longer to conceive. In women, this is linked to not only the hormone production, but also the uterine environment and function of the ovaries. In males, infertility is linked to the effects cigarette smoking has on decrease in sperm count and fertilising capacity (Sharma, Biedenharn, Fedor, & Agarwal, 2013). A systematic review assessing preconception maternal lifestyle and the effect on the development and function of the placenta found smoking in the preconception period resulted in reduced placental weight and an increase in alteration of the placental villi, leading to disrupted development and function of the placenta (Reijnders, Mulders, van der Windt, Steegers, & Steegers-Theunissen, 2019). It is recommended that women stop smoking at least one month

prior to conception as this is a critical time for placental development, with the additional problem associated with the cumulative effect of cigarette smoking, and therefore also the amount smoked prior to conception (Stephenson et al., 2018). Stopping smoking in the preconception period, defined as six months prior to conceiving, is most beneficial to prevent any potential epigenetic changes that may occur as a result of cigarette exposure (Amoako, Nafee, & Ola, 2017).

Following successful conception, an ectopic pregnancy could occur. This is where the embryo implants in the abdominal areas of the women, outside of the uterus, with 90% of cases being within the fallopian tubes (Azeez, Prasad, Kantor, Arora, & Kaushik, 2020). The embryo cannot survive outside of the uterus; therefore, an ectopic pregnancy can lead to haemorrhages and can endanger the woman's life (Azeez et al., 2020). Research has indicated that cigarette smoking leads to changes of both structure and function within the fallopian tube which contributes to an increased risk of an ectopic pregnancy (Horne et al., 2014). Across eleven case control studies, 10 studies had found a relationship between cigarette smoking and risk of ectopic pregnancy, with the incidence increasing with the number of daily cigarettes smoked (Dekeyser-Boccara & Milliez, 2005).

A miscarriage is a naturally occurring event in which the embryo/fetus dies in the uterus before the 23rd gestational week (NHS, 2018a). Approximately 1 in 4 pregnancies result in a miscarriage across the UK, with 1 in 100 women experiencing recurrent miscarriages defined as women having three miscarriages in a row in the

first trimester of pregnancy (Tommy's, 2021). The risk of a miscarriage after 12 weeks gestation decreases to approximately 1 to 2 in 100 (Tommy's, 2021). In a study of 697 Australian women who did not experience any symptoms, the risk of a miscarriage declined with each gestational week, however smoking status was not reported in this study. The risk of a miscarriage at 6 weeks was 9.4%, at 7 weeks 4.2%, at 8 weeks 1.5%, at 9 weeks 0.5% and at 10 weeks 0.7% (S. Tong et al., 2008). Miscarriages can occur regardless of maternal smoking status, however there is a clear and associated link with smoking, with the risk increasing by 1% per cigarette smoked per day, based on 112 studies (Pineles, Park, & Samet, 2014). Additionally, the results indicated that there is an 11% increased risk of miscarriage as a result of second-hand smoke exposure.

Smoking in pregnancy (the mother)

Data from 2018/2019 for rates of smoking early in pregnancy indicate that in England the average rate is 12.8%, ranging from between 6% in London to 18.6% in the North East (Public Health England, 2020). Reducing rates of smoking during pregnancy is of paramount importance due to the devastating effects smoking has on fetal and infant outcomes. Hence it is a key public health aim to reduce smoking at time of delivery (SATOD) to at least 6% by 2022 (NHS Digital, 2020). Some success was recorded from the period of 2018/2019 to 2019/2020 with an overall reduction in England by 2.4%. The 2019/2020 data indicates that the SATOD rate in England is 10.4%, ranging across England from 4.8% in London to 15.2% in the North East (Public Health England, 2020). Due to maternal smoking in pregnancy, it has been estimated that

there are between 3,000 to 5,000 miscarriages per year, 300 perinatal deaths and 2,200 premature births (Royal College of Physicians, 2010).

Smoking during pregnancy costs the NHS approximately between £20-£87.5 million per year, attributable to both maternal and infant health effects (Godfrey, Pickett, Parrott, Mdege, & Eapen, 2010). More specifically it is thought that approximately £21 million per year is spent on dealing with miscarriages, ectopic pregnancies, low birth weight, rupture of membranes and placenta previa (Royal College of Physicians, 2018).

In order to reduce rates of smoking during pregnancy, a number of initiatives have been employed in NHS Trusts. All women are required to undergo a CO breath test at their initial booking appointment to determine which mothers either smoke or are exposed to secondhand cigarette smoke, as well as ascertaining whether there are any additional household smokers. If the reading on the breath test indicates 4 parts per million (ppm) of CO or above, in line with the National Institute for Health and Care Excellence (NICE) guidance, the woman should be referred to specialist stop smoking services, and at each subsequent antenatal appointment the CO breath test should be applied (Saving Babies' Lives Care Bundle, 2019).

In addition to the measures outlined in the NICE guidance and Saving Babies' Lives Care Bundle, the babyClear© approach has an element of risk perception intervention, which aims to further reduce the number of women smoking during pregnancy. Women are shown using a doll and fabric placenta how toxins affect the fetus, with the CO breath test being linked to a computer. Here, a fetal avatar changes colour from green, to amber to red dependent upon amount of CO in the mothers system (Fendall, Griffith,

Iliff, & Radford, 2012), with research suggesting this type of intervention has a positive effect on the women's quitting attempts (Fergie, Coleman, Ussher, Cooper, & Campbell, 2019).

One reason why women may continue to smoke has been linked to the perceived notion that smoking can be used as a method of weight management (White, 2012). A study assessing 183 women who had quit smoking during pregnancy found that by 24 weeks postpartum, 65% had relapsed with results suggesting weight concerns were significantly associated with smoking relapse (Levine, Marcus, Kalarchian, Houck & Cheng, 2010). Whilst women may view smoking as a positive action during pregnancy to avoid weight gain, there are a number of negative pregnancy related outcomes discussed below.

Smoking effects in pregnancy

Placental abruption is when the placenta either fully or partially separates from the uterus before the birth of the infant, leading to an increased risk of both maternal and fetal mortality (Shobeiri, Masoumi, & Jenabi, 2017). A meta-analysis of 27 studies indicated that smoking is a risk factor for placental abruption (Shobeiri et al., 2017). In a study identifying 189 women who had a placental abruption, 10% were smokers (Kaminsky et al., 2007). Although the aetiology of this is still unknown, it is most likely linked to the placenta's structure changing as a result of smoking (Kaminsky et al., 2007). Research indicates that there is an increased risk by 40% of placental abruption for women who smoked at least one pack of cigarettes per day throughout pregnancy,

and for when abruption did occur mortality rate was higher overall for those women in comparison to non-smokers (Raymond & Mills, 1993).

Fetal growth restriction (FGR) is a well-established consequence of maternal smoking during pregnancy (Sabra, Gratacós, & Roig, 2017). A problem with FGR is that this can lead to miscarriage, stillbirth, preterm delivery, low birth weight and hypoxia leading to infant brain injury. FGR is defined as a fetus measuring below the 10^{th} centile once adjusted for gestational week (Sabra et al., 2017). The most common explanation of FGR is linked to the reduced oxygen-carrying capacity of fetal blood as a result of cigarette exposure (Sabra et al., 2017). This is likely to be related to nicotine itself, as it is a vasoconstrictor leading to a reduction of blood flow, with a key concern being that both nicotine and CO can cross the membrane barrier of the placenta (Royal College of Physicians, 2010).

The fetal origins hypothesis developed by David Barker, a physician and epidemiologist, who argued that the uterine environment can affect the fetus leading to later adulthood disease (Barker, 1995). Although early work on the fetal origins hypothesis mainly assessed coronary heart disease, the scope of research has since been widened to assess a range of maternal health related behaviours including diet, exercise, drug use and cigarette smoking. Research based on the fetal origins hypothesis can be traced back to the thalidomide episode, in which thalidomide was prescribed to reduce morning sickness but with devastating fetal consequences including missing limbs (Almond & Currie, 2011). Prior to this point, it was largely accepted that the placenta was a filter mechanism preventing harmful substances from reaching the fetus and therefore rending it acceptable for the mother to drink alcohol

and smoke during pregnancy. Fetal Alcohol Syndrome is characterised as a pattern of abnormalities including facial malformations, short stature and CNS abnormalities, which are a result of heavy maternal alcohol drinking during pregnancy, also reflecting the fetal origins hypothesis (Almond & Currie, 2011).

Smoking effects on birth outcomes

Preterm birth is defined as when the infant is born less than 37 weeks gestational age in which there is natural onset of labour either with or without rupture of the membranes or induced delivery as a result of maternal or fetal compromise (Goldenberg, Culhane, Iams, & Romero, 2008). Research has suggested there is a window of opportunity to quit smoking during the first trimester of pregnancy. Women smoking in the first trimester had only a slightly increased risk of preterm delivery similar to the level of risk for those who did not smoke at all. The risk increased when women continued to smoke beyond the first trimester, regardless of intensity of smoking, i.e., half a pack or a full pack of cigarettes (Kondracki & Hofferth, 2019). However, a large-scale analysis of over 25 million mother-infant pairs found that even smoking 1 or 2 cigarettes per day in the first and second trimester was associated to increased risk of preterm birth. In contrast mothers, who quit in the months leading up to conception, regardless of whether they were low or high intensity smokers, their risk for preterm delivery was similar to those mothers who did not smoke at all (Lui et al., 2020), providing further support for the advice that women should quit smoking at least six months prior to conception (Amoako et al., 2017).

Small for gestational age (SGA) is defined as an infant who is born with a weight that is below the 10th centile based on their gestational age (Schlaudecker et al., 2017). There is an increase in SGA which occurs in a dose-response manner from non-daily smoking to daily smoking with an increase in the number of cigarettes smoked per day, highlighting that even very low levels of cigarette consumption still increases the risk of SGA (V. T. Tong, England, Rockhill, & D'Angelo, 2017). Low birth weight (LBW) is defined as an infant born weighing less than 2500g, very low birth weight (VLBW) less than 1500g and extremely low birth weight (ELBW) is less than 1000g (World Health Organization, 2010). A meta-analysis of 30 studies indicated that maternal smoking during pregnancy was associated to low birth weight (Pereira, Da Mata, Figueiredo, de Andrade, & Pereira, 2017).

Sudden infant death syndrome (SIDS) typically occurs when the infant is asleep and under six months of age, with the cause of death unexpected and unknown (NHS, 2018b). Each year in the UK, SIDS accounts for approximately 200 deaths, with the consensus being that there are problems associated with how the infant responds to environmental stressors, including cigarette smoke exposure, or due to vulnerabilities including preterm birth (NHS, 2018b). Research from the Unites Sates highlights that approximately 22% of SIDS cases are thought to be directly associated to maternal smoking during pregnancy, with the risk of SIDS doubling for any amount of smoking throughout pregnancy. There is also a linear relationship from one cigarette to 20 cigarettes per day in relation to increased risk of SIDS (Anderson et al., 2019). Additionally, it has been suggested that risk of SIDS is 4.09 times greater for infants born to smoking mothers, with the risk increasing further if the father also smoked (Mitchell, Ford, Stewart, Taylor, Becroft, Thompson…& Roberts, 1993). The Triple

Risk Hypothesis has been suggested as a way of explaining SIDS, whereby there are underlying vulnerabilities of the infant including genetic, abnormalities of serotonin neurons alongside pregnancy related stressors (Mitchell, 2009). The cardiovascular functioning is likely to be affected by maternal smoking leading to a loss of blood pressure, bradycardia and ultimately death of the infant because of maternal smoking during pregnancy (Mitchell, 2009). Further research supports claims that cardiovascular functioning is altered because of reprogramming of the infant blood pressure control systems as a result of smoke exposure, leading to increased vascular, cardiac and blood pressure in infants who have been prenatally exposed to cigarettes (Cohen, Vella, Jeffery, Lagercrantz & Katz-Salamon, 2008; Cohen, Jeffery, Lagercrantz & Katz-Salamon, 2010).

Smoking on infant behavioural outcomes

A number of studies have used a variety of assessments to assess infant neurobehaviour including the Neonatal Intensive Care Unit (NICU) Neurobehavioural Network Scale (NNNS) (Lester & Tronick, 2004) and the Neonatal Behavioural Assessment scale (NBAS) (Brazelton & Nugent, 2011). There are a number of behaviours that are affected as a result of cigarette smoking during pregnancy including infant attention, excitability, lethargy, stress, irritability, muscle tone, affect, orientation, regulation and temperament (Godding et al., 2004; Hernández-Martínez, Val, Subías, & Sans, 2012; Mansi et al., 2007; Pickett, Wood, Adamson, DeSouza, & Wakschlag, 2008). Chapter 5 fills an important gap in the literature outlining a meta-analysis of studies demonstrating negative behavioural effects of prenatal cigarette exposure up to one year of age.

The results of such studies are likely to be a result of the toxins in cigarettes, namely CO and nicotine (Ekblad, Korkeila, & Lehtonen, 2015). The exposure to CO leads to COHb which reduces the amount of oxygen to fetal organs and tissue, with nicotine affecting the brain structure by influencing cell replication and differentiation. This potentially leads to fetal hypoxia further affecting brain development. A lack of oxygen supply to the fetus is linked to fetal hypoxia, and as cited in Dubovický, research has highlighted that this is linked to cognitive, learning and memory deficits later in life (Dubovický, 2010).

In attempts to reduce the number of devastating risks associated with cigarette smoking during pregnancy, as outlined above, the use of NRT is often recommended during pregnancy (NHS, 2019).

Nicotine Replacement Therapy

In addition to the recommendations outlined in the NICE guidance, Saving Babies' Lives Care Bundle and the babyClear© initiative, NRT can be prescribed. During pregnancy the NHS states that it is safe for women to use a range of nicotine replacement therapies including patches, gum, inhalators, sprays, or lozenges, all of which can be prescribed free of charge by the woman's GP or by a stop smoking advisor (NHS, 2019). However, there are caveats to prescribing NRT during pregnancy. For example, NRT must only be prescribed if quitting attempts have not been successful without the use of NRT and should only be prescribed for a 2-week period, unless, once re-assessed, the woman is not smoking and this is likely to be a result of the prescribed NRT (NICE, 2010).

Nicotine consumption is problematic during pregnancy because of the impact it has on the brain maturational processes (England et al., 2015). However, when assessing levels of cotinine when using NRT, these women have lower levels of cotinine in comparison to cigarette smokers, thus their fetus is receiving less nicotine exposure (Hickson et al., 2019). A cross sectional study of 220,630 pregnant women found that, when adjusted for potential confounders (diabetes, pre-pregnancy BMI, maternal age and socioeconomic status), there was a 41% increased risk of still birth for those who smoked during pregnancy, however, there was no significant difference in risk between those using NRT and non-smokers (Dhalwani, Szatkowski, Coleman, Fiaschi, & Tata, 2019). In both of the reviews outlined above, e-cigarettes were not included in their NRT grouping.

A large-scale study involving 1,050 pregnant women across England who were smoking at least five cigarettes per day were recruited into a trial assessing adherence to nicotine patches, as part of the Smoking and Nicotine in Pregnancy Study (SNAP) (Coleman et al., 2012; Cooper et al., 2014). Between 12 to 24 weeks gestational age, women were either randomly allocated to an 8-week treatment of nicotine patches (15mg per 16 hours) or a placebo, with both groups receiving smoking cessation behavioural support (Cooper et al., 2014). Results indicate that there were no significant differences between the two groups for rate of abstinence, and compliance was low, with only 7.2% of NRT group and 2.8% of the placebo group continuing to use the patches beyond one month. Comparing the birth outcomes, including congenital abnormalities, birth weight and preterm birth, these were similar across the two groups (Coleman et al., 2012). These same women and infants were followed up following the

birth. Parental questionnaires were sent out to participants at 6, 12 and 24 months of age assessing maternal smoking, infant health and the Ages and Stages Questionnaires (ASQ). 88% of respondents in both groups returned their questionnaires when their infant was two years of age. However, by two years of age, only 3% of the NRT group and 2% in the placebo group were abstinent from smoking cigarettes. Results of this study indicate that those exposed to NRT in pregnancy were more likely to survive without developmental impairment in comparison to the placebo group and additionally when analysing separate domains of the ASQ, the only significantly different domain was the personal social domain, with the NRT group scoring slightly higher. There were no significant differences between the two groups for reported respiratory problems. The authors involved in the SNAP trail claim that due to similar outcomes, there is no evidence to suggest nicotine patches cause harm, but due to low compliance, women may opt to use alternative forms of NRT, including e-cigarettes. One problem with this study is that these infants were not compared to a group of infants who were not exposed to any nicotine at all during pregnancy, only assessing differences between cigarettes and NRT which is unlikely to tell us anything of significance in terms of the effects that NRT has on the infant. We only know that effects of NRT use are comparable to cigarette smoking in pregnancy with the exception of survival without impairment (yet this was not definite impairment but based on parental report and thus not an objective assessment) and in the personal social domain of the ASQ.

As it appears from the SNAP trial, birth outcomes do not appear to differ between nicotine patch exposed and non-patch exposed. In addition, a meta-analysis assessing six studies including both placebo controlled and non-placebo controlled trials

assessing the safety of NRT, found there was no adversity associated with NRT use in relation to fetal and infant health outcomes, including miscarriage, still birth, birth weight, low birth weight, preterm delivery, congenital abnormalities, NICU admission and infant death (Taylor et al., 2021). However, the authors of this meta-analysis did indicate that the evidence was not sufficient, due to individual study limitations, to indicate whether or not NRT is safe to use during pregnancy. Furthermore, newborn and infant behavioural outcomes associated with NRT use during pregnancy are not reported in the literature.

Whilst nicotine patches are a common alternative in the UK, snuff is an alternative form of NRT that is often used in countries such as Sweden. Snuff is a form of smokeless tobacco but does have higher levels of nicotine in comparison to cigarettes. Research indicates that whilst there is a strong association between smoking during pregnancy and asthma and wheeze, these associations are weaker for mothers using snuff (Lundholm, Gunnerbeck, D'Onofrio, Larrson, Pershagen & Almqvist, 2020). Such results indicate that nicotine might not cause the respiratory outcomes observed. However, snuff use has been associated with a higher incidence of neonatal apnea, therefore NRT use cannot be recommended as a safe alternative to cigarette smoking during pregnancy (Gunnerbeck, Wikström, Bonamy, Wickström & Cnattingius, 2011).

Studies assessing the effects of NRT (as an umbrella term) do not reflect the use of e-cigarettes during pregnancy, despite 4.8% of women opting to use these with an additional 3.5% of women using both an e-cigarette and continue to smoke cigarettes during pregnancy (Bowker et al., 2020).

E-cigarettes

In the adult population in the UK, there are approximately 3.2 million users of e-cigarettes, with 61.7% ex-smokers and 38.3% dual users of both cigarettes and e-cigarettes (ASH, 2020b). E-cigarettes vary in the flavourings, substances, and amount of nicotine that they contain (6-20 mg per ml), the maximum without a medical license is 20mg per ml, similar to a cigarette and are often cheaper (ASH, 2020b; Carlsen, Skjerven, & Carlsen, 2018; England et al., 2015). E-cigarettes contain Glycerine or propylene glycol which is a liquid that is heated by a battery to create aerosol (England et al., 2015). Although primarily containing nicotine, there are concerns that a number of other toxic substances are present in e-cigarettes for example nickel, cadmium, manganese and lead (Hess et al., 2017). Furthermore, there are a number of concerns associated with the labelling of e-cigarette products (Kong, Derrick, Abrantes, & Williams, 2018). The study by Kong et al. (2018) reviewed a number of e-cigarette packaging that they had ordered online to assess the labelling on the products. Out of 125 orders, results indicated that only 60% of e-cigarettes labelled the content, and only 44.6% included a health warning regarding the use and safety of e-cigarettes. In addition to this, some of the e-cigarettes included labels with unsupported claims regarding the health effects of their use (Kong et al., 2018). This study highlights the variety of e-cigarettes that are available to purchase online and therefore leading to difficulties associated with conducting research on the effects that e-cigarettes may have due to the varying content.

Due to the belief that e-cigarettes are safe, they are currently being used in homes and vehicles with the suggestion that e-cigarettes do not produce second-hand smoke and therefore are not damaging to a bystander. However, studies have indicated that

nicotine can be deposited on surfaces and thus absorbed by a non-user (England et al., 2015). Nevertheless, they are considered a harm reduction tool and promoted by organisations such as ASH and Public Health England (PHE). Despite being determined as safe to use by ASH and PHE, the World Health Organisation (WHO) claims that use of e-cigarettes increases the risk of heart and lung diseases (World Health Organization, 2020). Although often considered a safe way to reduce harm of smoking cigarettes during pregnancy, there are still a number of concerns associated with e-cigarette use generally. Although e-cigarettes do not contain tobacco, such as traditional cigarettes, they do contain nicotine.

A research study of non-pregnant individuals involving five differing smoking status' including cigarettes only, dual cigarette and NRT use, dual cigarette and e-cigarette use, NRT only and e-cigarette only, assessing levels of nicotine, carcinogenic and toxicant exposure (Shahab et al., 2017). Results from this study indicate that levels of nicotine, measured by urine and saliva cotinine, between cigarette use, e-cigarette use and NRT were comparable, however, the e-cigarette only and NRT only groups had reduced levels of toxicants and carcinogenic compounds compared to cigarette smokers, as identified by urine and saliva samples (Shahab et al., 2017). Authors attribute the results for similar levels of nicotine as cigarette smokers as a positive that both e-cigarette and NRT use satisfies the nicotine cravings without the toxicants that are in cigarettes. This result could be beneficial to the general population, but their benefit is questionable during pregnancy.

Whilst not offered through the NHS, e-cigarettes are becoming increasingly popular not only in the general population, but also by pregnant women (ASH, 2020b). Due to

the use of CO monitors in antenatal clinics, identifying cigarette smokers can be considered easy, however, it can be difficult to obtain accurate numbers of women using an e-cigarette due to no quick method of measuring nicotine during pregnancy. Nonetheless, a recent survey study of hospitals across England and Scotland found that out of a sample of 3360 women, 15.3% were cigarette smokers only and 4.8% used e-cigarettes, with 3.5% of those being a dual-user (Bowker et al., 2020). Little research has been conducted on the effects of e-cigarettes during pregnancy; rather they are often considered safe to use, based on health research in the general adult population (ASH, 2020b), disregarding the effects nicotine has on the developing brain of the fetus. The use of e-cigarettes during pregnancy is a contentious issue. However, whilst e-cigarettes might be beneficial to the non-pregnant user, we cannot use this research to inform us about the use of e-cigarettes during pregnancy.

The considerations of the harm associated with nicotine exposure during pregnancy is often absent from public health debates, with nicotine being considered as a minor importance, due to the other carcinogenic and toxicants absent in e-cigarettes, therefore leading to a reduction in many health-related outcomes associated with cigarette smoking (England et al., 2015). It is often thought that due to the reduced number of toxins in e-cigarettes that they are safe and beneficial, however there is a lack of sound scientific research during pregnancy supporting such claims (England et al., 2015). Despite the increasing popularity of e-cigarettes during pregnancy, there are only a handful of studies assessing the risk, with studies providing contrasting results with some research suggesting birth outcomes of e-cigarette users are no different to non-nicotine exposed fetuses (McDonnell, Bergin, & Regan, 2019), whereas a recent large scale survey study of 53,971 participants, indicated that low birth weight, preterm

delivery and small for gestational age were more likely for e-cigarette users in comparison to controls and similar to cigarette smokers (Kim & Oancea, 2020).

One concern is that nicotine is a highly addictive carcinogenic substance, which is a pressing issue for the developing fetus, particularly in the 3rd trimester of pregnancy when the brain is most sensitive to the effects of nicotine (Holbrook, 2016). There is no research proving the use of e-cigarettes during pregnancy is safe, indeed, there is no evidence to suggest any amount of nicotine during pregnancy is safe (Holbrook, 2016), with research highlighting the impact nicotine has on brain development including impaired cognition, attention, and processing difficulties (Makadia, Roper, Andrews, & Tingen, 2017).

Given the known related behavioural outcomes associated with cigarette smoking including increased irritability, poorer reflexes, regulation and attention for example [1](Froggatt, Covey, & Reissland, 2020a; Froggatt, Reissland, & Covey, 2020b), it is imperative that the behavioural outcomes of e-cigarette exposure are assessed to ensure women are not recommended to use one product potentially causing harm in replacement of another harmful product (cigarettes). Chapters 4 and 6 explore the use of e-cigarettes on both fetal and infant neurobehaviour in order to address the clear gap within the literature.

Maternal views of e-cigarette use

In order for public health to address the high rates of smoking, a study (England et al., 2016) assessing a range of different alternatives to cigarettes such as dissolvable

[1] See Chapters 5 and 6.

products (strips that dissolve in the mouth), NRT (patches, gum, lozenges) and e-cigarettes assessed the perception of these products. Results indicated that women felt dissolvable products would be ideal for reducing stigma surrounding smoking during pregnancy, but many did not like the idea of NRT as they believed it was ineffective. Regarding e-cigarettes, although some women feared that due to no natural stopping point in use, like with a cigarette which you 'finish', that this would lead to overusing the product. However, e-cigarettes were viewed favourably amongst the women due to the perceived health benefits in comparison to cigarettes, reduced cost and ability to use them in a smoke free area (England et al., 2016). These views were collected from women who smoked during pregnancy, as well as women who had quit and were pregnant and women who smoked and were planning a pregnancy.

Analysing online forums regarding whether or not it is safe to use an e-cigarette during pregnancy, three key themes across 13 different online forums were identified via google search (e.g., baby centre, pregnancy forum and vaping underground). Results indicated that individuals in these discussion groups felt that quitting cigarette smoking 'cold turkey' was unsafe and therefore led to the second theme that e-cigarette use during pregnancy is a 'lesser of two evils'. However, an alternative theme that was discussed regarded the risks associated with e-cigarette use and with some women claiming that use during pregnancy is not worth the risk (Wigginton, Gartner, & Rowlands, 2017).

A systematic review (McCubbin, Fallin-Bennett, Barnett, & Ashford, 2017) was carried out regarding the perceptions of e-cigarettes for use during pregnancy, comprising of seven studies. Results indicated two key outcomes. Firstly, that e-

cigarettes are viewed as a safer alternative to cigarettes during pregnancy and secondly that the most common reason for use is a way of harm reduction and as a tool for smoking cessation. Authors of this paper argue, despite maternal perceptions, that there is a lack of evidence-based research to support the use during pregnancy (McCubbin et al., 2017).

In attempts to address the lack of knowledge regarding e-cigarette use, and due to e-cigarettes posing risks, a study assessed the impact of anti-smoking messages and changed them to be related to the use of e-cigarettes. Results indicated that when educated about the harmful chemicals present in e-cigarettes, this led to a negative emotional reaction and was found to be the most effective way to communicate the risks, whilst all other messages including information about the ingredients, harms and cost were also found useful and did lead to reduced intentions to use e-cigarettes (Owusu, Massey, & Popova, 2020).

In this thesis, Chapter 8 further explores maternal perceptions of both cigarette and e-cigarette use during pregnancy, for women who were undergoing a risk-based educational intervention as part of their routine antenatal care.

Fetal behaviour

As discussed above, one way in which the effects of nicotine can be established is to assess infant behaviour. With the growing advancement in ultrasound technology, this has led to the ability to assess fetal behaviour. At present there is limited fetal behavioural research examining the effects of nicotine consumption. Outlined in this

section is prior research using fetal behavioural assessment measures to provide an indication of fetal differences as a result of cigarette use and an explanation of fetal behavioural differences that may occur.

The health effects for both the mother, fetus and subsequently the child are well documented throughout the literature, including fetal growth restriction (Sabra et al., 2017), placental abruption (Shobeiri et al., 2017), preterm birth (Kondracki & Hofferth, 2019), and childhood asthma for example (Neuman et al., 2012). One of the ways in which the health effects are manifested is through fetal behaviour, with research into this topic area just beginning to emerge (see Reissland et al., 2015; Stroud, Bublitz, Crespo, Lester, & Salisbury, 2020; Stroud, McCallum, & Salisbury, 2018). For medical research, fetal wellbeing can be determined by maternal blood tests, Doppler assessments for fetal growth (Dipak, Kumar, Reddy, & Tiwari, 2021), 3/4-dimensional (3/4D) ultrasound (Bergh & Bianco, 2020) and nuchal translucency assessments for chromosomal abnormalities (Nicolaides, 2004) for example. Whereas for psychological research assessing fetal behaviour, the majority of research use methodologies that focus on gross fetal body movement, breathing and heart rate (e.g., Kurjak et al., 2008; Salisbury, Fallone, & Lester, 2005). Early research highlighted that fetal breathing movements were reduced after a mother smoked one cigarette (Gennser, Maršál & Brantmark, 1975), with the suggestion that this was a result of nicotine exposure (Manning & Feyerabend, 1976). Nicotine chewing gum has been found to have comparable levels of nicotine to cigarettes, which leads to a rise in maternal plasma nicotine concentrations, with both products leading to a reduction in fetal breathing movements (Manning & Feyerabend, 1976). However, new assessment methods are emerging focusing on the fine-grained analysis of fetal movements, in

particular facial movement, such as the Fetal Observable Movement System (FOMS) (Reissland, Francis, & Buttanshaw, 2016).

In recent years, a number of studies have demonstrated a range of fetal behavioural differences of fetuses who have been exposed to cigarette smoke. Habek (2007) assessed three groups of pregnant women, non-smokers, light smokers (average of 10 cigarettes per day) and heavier smokers (20 cigarettes per day) between 10 to 20 weeks gestational age. Results indicated that the rates of brisk and sluggish movements differed significantly between the three groups, with heavier smoke exposed fetuses displaying greater amounts of sluggish (slow) movements compared to fetuses of non-smokers showing brisk (strong) movements. This differed significantly to non-exposed and light exposed fetuses. Additionally, heavier smoke-exposed fetuses displayed fewer upper body movements of the head and arms in comparison to the other two groups (Habek, 2007).

In order to assess fetal behaviour using a refined prenatal assessment method, research has demonstrated a different pattern of fetal behaviour in relation to prenatal maternal smoking (Stroud et al., 2018). Mothers in this study smoked on average 7 cigarettes per day and the fetuses were examined between 32 to 37 weeks gestational age (m=35.1 weeks). The Fetal Neurobehavioural Assessment System (FENS) was used, which assesses rates of different body movements including mouth movements, isolated limb and head movements, breathing movements and quality of movements, as well as fetal actocardiograph measures (see Chapter 3 for further information). Fetuses exposed to maternal cigarette smoking showed a greater number of isolated movements and an increase in overall fetal activity, opposed to more complex body movements, in which

a number of movements are coordinated. A cross sectional study involving fetuses between 24 to 37 weeks gestational age assessed fetal motor reactivity, which is defined as isolated limb, head and trunk movements as well as complex body movements where these movements occur together, found that in younger fetuses (M=28 weeks), those exposed to maternal cigarette smoking had a higher baseline score of motor activity in comparison to non-exposed fetuses (Stroud et al., 2020). Furthermore, in response to stimulation, motor activity increased in the later gestational ages of the smoke exposed fetuses in comparison to the non-exposed fetuses whereby motor reactivity was consistent throughout.

A study of fine grained fetal mouth movements and facial self-touches assessed using 4D ultrasound technology, indicated that fetuses who were prenatally exposed to cigarettes displayed greater rates of mouth movements and self-touches compared with non-exposed fetuses. The pilot study carried out by Reissland et al. (2015) is the inspiration and most significant piece of research for this thesis. The pilot study assessed 20 fetuses (four cigarette exposed and 16 non-exposed) at 24-, 28-, 32- and 36-weeks gestational age. The differences in movement for the two exposure groups widened and become more statistically significant at the later gestational ages, 32- and 36-weeks. The pilot study indicated greater levels of significance for mouth movement differences in comparison to self-touches.

A significant advance in this series of research studies is the behavioural assessment following up the fetuses who have been prenatally exposed to e-cigarettes into early infancy. These studies are the first known pieces of research to examine the effects of exposure to e-cigarettes prenatally, therefore identifying a key gap within the literature.

The novelty of such research was to assess the implications of e-cigarette use during pregnancy and the effect this has on infant behaviour at one month of age. Behavioural implications of e-cigarette use during pregnancy has not yet been widely studied, nor the effects of e-cigarette use on postnatal behaviour. In order to assess postnatal effects of prenatal cigarette and e-cigarette exposure, a neurobehavioural assessment of the infants was carried out after birth at one month of age (Chapter 6).

Explanation of behavioural differences

Differences in prenatal behaviour can be explained by the fetal programming hypothesis, whereby the intrauterine development leads to a range of physiological adaptations explaining how maternal psychological state and toxin exposure can influence such behaviour (Talge, Neal, Glover, & Early Stress, 2007). Both intrinsic and extrinsic factors lead to the changes in fetal development and are central for preparing the fetus for their postnatal environment (Rotem-Kohavi, Williams, & Oberlander, 2020). The epigenetic process that occurs can lead to changes at various points of fetal development as a result of an interaction between a variety of environmental exposures, which ultimately determines how adaptable one is to future life events (Bale, 2015). Maternal stress signals, for example, are detected from the mother and transferred through to the fetus via the hypothalamo-pituitary-adrenal axis and placenta as a result of maternal stress, anxiety or toxin exposure, with the trajectory of development changing due to fetal vulnerability (Sandman, Davis, Buss, & Glynn, 2012). Stress signals in turn lead to an accelerated release of corticotropin-releasing hormone, in a dose-response manner, with increased levels circulating within the fetal environment which can lead to negative pregnancy related outcomes, such as preterm delivery (Sandman et al., 2012).

There is abundant support for the fetal programming hypothesis. According to this hypothesis, both intrinsic and extrinsic factors lead to functional changes in the fetus in anticipation of life after birth. However, when the anticipated environment is not as expected, this leads to normally adaptive processes being dysfunctional (Pluess & Belsky, 2011). An example of this can be seen in obesity research for example, where infants with a low birth weight, due to poor prenatal maternal nutrition, are programmed and are anticipating a 'thrifty' environment, when in contrast, after birth they are exposed to enriched fatty food, leading to later adulthood obesity (Jornayvaz et al., 2016; Simmons, 2008). Research assessing maternal mood for example, has shown through a variety of methods including neuroimaging, ultrasound scanning and behavioural assessments how the uterine environment elicits changes within the structure and function of the developing brain leading to behavioural differences in some children (Rotem-Kohavi et al., 2020). Using such assessments allows for an assessment of fetal behaviour that is thought to be directly linked to development of the CNS (Hata, 2016).

To further support claims that prenatal behavioural assessments can provide an insight into CNS function is by assessing fetuses with known structural brain abnormalities such as ventriculomegaly, which is a dilation of lateral ventricles. Of 140 fetuses diagnosed with ventriculomegaly, 34.9% had an abnormal prenatal score, as assessed by Kurjak's Antenatal Neurodevelopmental Test (KANET; see Chapter 3 for assessment details), in comparison to 6% out of 100 control fetuses. In addition, lower KANET scores were present for those with a greater severity of ventriculomegaly (Talic et al., 2012). Results of such study highlight that fetuses with a structural

abnormality also had functional abnormalities, which were assessed by examining facial and body movement activity. Again, further indicating that it is possible to assess functionality of the brain and CNS maturation and development. This leads to the suggestion that examining fetal behaviour can provide an insight into CNS maturation (Kurjak, Barišić, Antsaklis, Stanojević, & Medjedovic, 2020). Furthermore, those fetuses with a normal KANET score showed typical scores on a neurological assessment both at birth and three months post birth (Honemeyer & Kurjak, 2011). As reported in Kurjak et al. (2020) assessing fetus' using the KANET and the Amiel-Tison Neurological Assessment at Term (ATNAT; see Chapter 3 for assessment details) postnatally, of three infants who had a pathological ATNAT score, all three had a significant reduction in fetal facial movements. Such results further support the notion that brain development can be identified prenatally. In sum, it can be concluded that neurological impairment can be identified prenatally and confirmed through a postnatal assessment. Further support comes from studies assessing continuity of behaviour, with the first study to assess this found that fetal movement patterns, as measured by an actocardiotocograph, at 36 weeks gestational age correlated with neonatal motor activity (e.g. spontaneous active movements, crawling and head raising) and irritability (e.g. amount of crying during the assessment), with 36 weeks gestational age also associated to activity level at one year (for boys) and inhibition at two years of age (DiPietro et al., 2002).

Research examining the development of fetal facial movements indicates that gestational age is linked to development. For example, at 25 to 27 weeks mouthing and yawning occur at significantly higher rates than all other facial movements. By 28 to 34 weeks only mouthing occurs more, with the frequency of all facial movements

decreasing toward the end of pregnancy (Hata, 2016). Despite the number of facial movements decreasing throughout pregnancy, the complexity of facial movements increases (Reissland, Francis, & Mason, 2013). Further, fetal yawning studies suggest that fetal facial movements are related to CNS maturation (Reissland, Francis, & Mason, 2012). Therefore, using ultrasonography may help identify typical and atypical development of the CNS. Additionally, evidence supporting the claim that fetal movements can represent CNS development comes from studies demonstrating a decline in movements throughout the gestational weeks, as it is thought that a decline in movement is a result of cortical control increasing toward term reflecting optimal neuronal development (DiPietro et al., 2002). It has therefore been argued that through assessing fetal movement patterns it can provide a good indication of neurobehavioral functioning and can provide an insight into brain and CNS development (Kurjak et al., 2020).

Such research provides insight into how the brain and CNS are influenced by a range of factors with the possibility of identifying postnatal functional problems prenatally. Findings from the current research can potentially be applied by clinicians, researchers and those in clinical settings developing interventions, specifically for smoking. Although birth outcomes are well known and associated with the health of the newborn, the behavioural outcomes of the effects of smoking are less well known. A large-scale review (Flemming, Graham, Heirs, Fox, & Sowden, 2013) highlighted that mothers continued to smoked whilst pregnant as it was embedded with their lives and that quitting attempts were transient for the period of pregnancy, with cutting down is seen as a positive. If such views are addressed in smoking cessation interventions, further success may be possible. If information regarding behavioural

outcomes and scan differences were highlighted to pregnant women and their partners, this may lead to an increase in success in interventions aimed at reducing smoking during pregnancy and improve maternal psychological state, through a reduction of stress, depression and anxiety.

Given that the implications of prenatal mouth movement differences are currently unknown, this research aim is to explore the association between such prenatal mouth movements and postnatal neurobehaviour, addressing a significant gap in the literature. This will be discussed in further detail in Chapter 7, pre-to-postnatal behaviour. With the growing field of fetal development and behaviour, it is important to consider what these behavioural differences, specifically prenatal mouth movements observed, indicate in terms of postnatal behaviours of the infant.

At the time of planning the current research studies for this thesis (2016/2017), there were no published studies assessing how these two periods of behavioural development were related, other than research assessing continuity of mouth movements and eye blinks for example (Kurjak et al., 2004). However, since then, it has been shown that prenatal behaviour (as assessed via gross body movement) and postnatal behaviour are related (Stroud et al., 2018). However, to date there is no research assessing fine-grained facial movements in the prenatal period and how this relates postnatal behaviour. If this method is to be used to provide meaningful insight into fetal behaviour and postnatal development, then this needs to be further assessed, hence this is a key aim of the thesis. In order for fetal psychology to progress, we must understand what prenatal behavioural differences mean for postnatal behaviour.

Research aims

The research in this thesis employs a variety of methodological assessments to evaluate the impact of nicotine exposure on fetal and infant behaviour, meta-analysis to observational data and interview data assessing maternal perceptions of cigarette and e-cigarette use during pregnancy. There are a number of key objectives for this research.

1) A partial replication study of Reissland et al. (2015). The pilot study found that fetuses exposed to cigarettes via maternal smoking had a greater number of mouth movements in comparison to non-exposed fetuses when assessed using 4D ultrasound scans. Therefore, the initial aim of this thesis was to build upon this with a larger sample and separate smoking groups into light (<10 cigarettes per day) and heavy (11-20 cigarettes per day) exposure, based on findings highlighted by Habek (2007) indicating differences between these two exposure groups. In addition to Reissland et al. pilot study, fetuses of women using e-cigarettes will be assessed due to the growing trend within recent years and lack of scientific evidence regarding the safety and use during pregnancy (Holbrook, 2016).
2) To conduct a meta-analysis assessing the impact of prenatal cigarette exposure on infant behavioural outcomes up to one year of age. The literature examining the effects of smoking was conducted covering the dates between 1950-2018. The aim of this review was to provide an overview and analysis of the research studies assessing neurobehavioural outcomes of infants as a result of prenatal cigarette exposure up to one year of age. Other reviews have examined behaviour beyond 1 year of age (Cornelius & Day, 2009) missing out the early

period of development; in contrast the focus is on the first year as it is a critical time point in development (Stettler, 2007), a key gap within the literature.

3) To assess whether broader smoking status (non-smokers, cigarette smokers, e-cigarette users) has an impact on infant birth and neurobehavioural outcomes at one month of age. Similar to the prenatal study, to date it has only been birth outcomes that have been assessed as a result of e-cigarette use during pregnancy. Given the already known association between cigarette exposure and neurobehaviour (Froggatt, et al., 2020a), it is essential to assess the impact of e-cigarettes, in order to guide future policy on the use during pregnancy. This is the first known study to assess the impact of prenatal e-cigarette use on infant neurobehaviour.
4) To assess whether there is a relationship between prenatal behaviour, as defined by fetal mouth movements, and postnatal behaviour, defined by scores on the NBAS, regardless of smoking status. This is to assess what prenatal mouth movements mean for postnatal behaviour. The FOMS is being used for prenatal research (e.g., Reissland et al., 2015; Reissland, Makhmud, & Froggatt, 2019; Reissland et al., 2020a), yet an understanding of what differences may mean is currently unknown.
5) To understand maternal risk perceptions of cigarette and e-cigarette use during pregnancy, a view from cigarette smokers. Can an understanding of risks, for women who are prime targets due to already undergoing risk education, help aid future smoking cessation interventions? This novel research is important as it assesses maternal understanding of risks in women already receive a risk-based intervention as part of their routine antenatal care in attempts to aid smoking cessation.

Outline of thesis

The introduction has outlined research on a number of devastating health and behavioural effects that can occur as a result of cigarette exposure during pregnancy, and lack of research on e-cigarettes in which this research will fill an important gap within the literature. Furthermore, the significance of prenatal mouth movement will be assessed in relation to postnatal behaviour. Chapter 2 includes an overview of the methods used in the current series of studies. Chapter 3 will provide an in-depth discussion of the main assessment measure used in this research; the Fetal Observable Movement System. Following this, there are five chapters (Chapters 4-8) which report the meta-analysis, three observational studies and the interview study. The last chapter of the thesis (Chapter 9) concludes with a discussion of the research conducted, the implications for policy, limitations and directions for future research.

Chapter 2

Method

The studies reported in the thesis were designed to assess the relationship between maternal smoking and e-cigarette use during pregnancy and both fetal and infant behaviour. The research adopts a number of different methodological approaches that have been used to gain multiple perspectives of the issue of maternal smoking during pregnancy. Five studies were conducted:

- The prenatal study in Chapter 4. The effect of pregnant women's smoking status and e-cigarette use on fetal mouth movements.
- The meta-analysis in Chapter 5. Infant neurobehavioural consequences of prenatal cigarette exposure: A systematic review and meta-analysis.
- The postnatal study in Chapter 6. The effects of prenatal cigarette and e-cigarette exposure on infant neurobehaviour: A comparison to a control group.
- The pre-to-postnatal study in Chapter 7. The association between prenatal mouth movement frequency and postnatal behaviour at one-month post birth.
- The interview study in Chapter 8. Risk perception of cigarette and e-cigarette use during pregnancy: A qualitative postpartum perspective.

The prenatal study reported in Chapter 4 is a partial replication of Reissland et al.'s (2015) pilot study, in which mouth movements were coded as outlined in the Fetal Observable Movement System (FOMS) along with self-touches. This small pilot study was conducted at James Cook University Hospital (JCUH), Middlesbrough, UK in 2015. Twenty mother-fetal pairs underwent 4D ultrasound scans at approximately 24-, 28-, 32- and 36-weeks gestational age. In this pilot study 16 non-

exposed and four cigarette exposed (two light and two heavily exposed) fetuses were included. Relative frequency of mouth movements and self-touches were recorded at each gestational age, with results indicating that cigarette exposed fetuses displayed significantly more mouth movements and self-touches in comparison to the non-exposed fetuses at the later gestational time points (i.e., 32- and 36- weeks).

There were a number of key differences between the current study and Reissland et al.'s pilot study. Firstly, due to the results indicating that the later gestational time points found significant differences between smoke exposed and non-exposed fetuses, 4D scans were only conducted at approximately 32- and 36-weeks gestational age. Secondly, a larger sample was recruited, with refined cigarette exposure groupings; light smokers smoking less than 10 cigarettes per day and heavier smokers smoking 11-20 cigarettes per day. In addition, women using e-cigarettes were included and formed their own subgroup. In comparison to the pilot study, the focus was on mouth movements, given this type of behaviour was significant to a greater extent than self-touches in the pilot study. Additional research also indicates that facial movement can provide an insight into the developing brain and CNS (Antsaklis, Kurjak, & Izebegovic, 2013).

The empirical research conducted for the thesis also extended Reissland et al.'s (2015) pilot study by investigating three related issues alongside assessing the impact of cigarette and e-cigarette exposure on fetal mouth movements. Firstly, the postnatal study in Chapter 6 assessed the relationship between maternal smoking or e-cigarette use and the postnatal neurobehaviour of infants at one month of age using the Neonatal Behavioural Assessment Scale (NBAS). Secondly, the pre-to-postnatal

study in Chapter 7 assessed the longitudinal relationship between prenatal mouth movement of fetuses and the postnatal neurobehaviour at one month of age regardless of maternal smoking status or e-cigarette use. And thirdly, the interview study reported in Chapter 8 assessed mothers' understanding of the perceived risks associated with both prenatal cigarette and e-cigarette exposure at one-month post birth.

Ethical approval was granted via the NHS (REC reference, 11/NE/0361) and Durham University (17/12; PSYCH-2018-05-08T11:27:21-flbm2).

Recruitment

The criteria for recruitment were based on the Reissland et al. (2015) pilot study. The criteria were in place to ensure that mothers were similar allowing for a better comparison between the fetuses and avoid extraneous variables. The eligibility for the research was as follows:

- Maternal age between 18-40 years old.
- Pre-pregnancy BMI between 18-25
- Not under the care of the consultant for pregnancy complications and a low-risk pregnancy.
- Not currently taking any medication.
- Not diagnosed with medical or mental health condition that would affect the fetus.
- Not taking any recreational drugs or drinking alcohol.

The 4D ultrasound scans took place at either JCUH, Middlesbrough or The Friarage Hospital, Northallerton, dependent upon the woman's geographical location, hospital which provided their antenatal care and availability of scan appointments. Both hospitals are within the South Tees NHS Hospital Foundation Trust, with the same sonography team. Pregnant women were identified by the sonographers at the hospitals at their 20-week anomaly scan. Maternity notes were screened to ensure eligibility for the study. Leaflets (appendix 1) containing study information and contact details were given to the women after their 20-week scan. Women who expressed an interested in the study were invited to an informal discussion and then were asked if they would want to sign up to the project. Some women took the leaflet home and were in contact at a later date to ask questions and sign up. Due to initial slow up take for women smoking cigarettes, the head sonographer provided a list of women who were eligible for the research and also smoked during their pregnancy. These women were then contacted via phone between 20-29 weeks gestational weeks. In line with departmental ethics, some women were recruited via the fetal and infant lab group social media page.

Table 2.1. Number of women involved in each phase of research.

Nicotine group	**Recruited**	**Scans analysed at 32 weeks**	**Scans analysed at 36 weeks**	**Follow up at one month**
Non-smokers	54	46	34	44
Cigarette smokers (<10)	38	32	27	*(Cigarette smokers were combined)* 29
Cigarette smokers (11-20)	15	13	12	
E-cigarette users	16	15	14	10
Total	123	106	87	83

Despite recruiting 123 pregnant women and conducting the 4D ultrasound scans at 32 weeks gestation, not all scans could be analysed (see table 2.1). This was due to poor position of the fetus, with the fetal face not visible despite attempts from the sonographers to gain a clear fetal facial image (N=16). In addition, on one occasion, the 4D scan was not recorded due to a technical error. These same reasons also applied for the 36-week time point (N=20), however with the additional reason of drop out. Drop out occurred due to birth of the infant prior to 36 weeks gestational age (N=2) or could not attend the appointment (N=13). Some women were unable to attend the appointment due to a schedule clash or unable to organise childcare. At both hospitals, children are not permitted to attend any scan appointment. The antenatal ultrasound department at both hospitals were very busy, with scans typically carried out between 7-9am and 3.30-5.30pm, and only occasionally mid-day. For this reason, scans were not able to be re-done as the department had routine scans to conduct alongside the research. Only one mother-infant pair was excluded from the research following the birth of the baby, due to a postnatal diagnosis of septo-optic dysplasia at 6 hours old. This is a condition that occurs in 1 in 10,000 births when two or more of the following issues occurs, including defects in the midline brain, optic nerve hypoplasia and pituitary gland abnormalities (Webb & Dattani, 2010) and due to this mother smoking cigarettes during her pregnancy, we did not want to include the fetal results as this may have influenced the overall findings.

In comparison to the cigarette and non-exposed groups of women, the e-cigarette group was small. Due to women categorised as either cigarette smokers or non-smokers in their maternity notes, it was difficult to identify any NRT users. In addition, since opportunity sampling was used, as NRT users do not identify as either

smokers or non-smokers, they may have felt they were not eligible to participate in the research. For the 16 women recruited in this group, they volunteered as non-smoking participants and it was only through questioning that it was discovered they used e-cigarettes. Interestingly, despite NRT (patches and inhalators) being prescribed free of charge, none of the women using these products volunteered, only women using e-cigarettes agreed to participate in the study.

Materials

Smoking assessment

This assessment was designed to identify whether the women smoked during their pregnancy, whether anyone else in the household smoked, whether she did smoke but has since stopped, whether she used NRT previously, as well as e-cigarettes and mg of nicotine in the product, whether she has been referred to smoking cessation and whether she has considered stopping smoking. This assessment was used in the aforementioned pilot study conducted by Reissland et al. (2015). Alongside the questionnaire asking about smoking status, a CO breath test was conducted. There were no women recruited who used traditional NRT such as patches or inhalators. See appendix 2.

CO monitoring

In hospitals across the North East of England, CO monitoring is carried out at antenatal appointments using the Bedfont Smokerlyser PicoBaby™, which provides a reading of amount of CO for both mother and fetus to determine smoking status. The same approach was used in the present series of studies. A breath test for establishing

CO exposure is non-invasive and provides an immediate indication of smoke exposure. NICE guidance recommends a cut-off point of 3 parts per million (ppm) for an indication of whether an individual smokes cigarettes (NICE, 2010). There are a number of issues using CO to indicate smoking as environmental factors such as car emissions may lead to a higher reading. Equally, low levels of smoking may not be detected given that CO levels decrease rapidly e.g., they can decrease by 50% within 4 hours of last smoking a cigarette (NICE, 2010), therefore it is important to use alongside a questionnaire and at each time point. However, to use as an indication of smoking, CO breath tests have been suggested valid and reliable method of assessing exposure to cigarettes (Christensen et al., 2004). The questionnaires and CO breath test were conducted at each time point; 32 weeks gestation, 36 weeks gestation and at the postnatal one month follow up.

Attachment scale

There were two attachment scales. The antenatal attachment scale (Condon & Corkindale, 1997) (appendix 3) given at the 32 and 36 week scan. Women were asked to choose the most appropriate response to a range of statements. Statements include 'Over the past two weeks I have thought about or been preoccupied with the baby inside me'. The postnatal attachment scale (Condon, 2015) (appendix 4) is given at the one-month follow assessment. Similar to the antenatal scale, women were asked to choose the most appropriate response to a range of statements. Statements include 'When I am caring for the baby, I get feelings of annoyance or irritation'. These scales were included to assess whether attachment scores had any relation to either fetal mouth movement or NBAS score postnatally. Maternal-fetal attachment may be important here, as research indicates that those who have a higher

attachment are more likely to reduce or quit smoking during pregnancy (Jussila et al., 2020). Additionally, attachment is thought to play a role in later infant and child development and behaviour (e.g., Branjerdporn, Meredith, Strong, & Garcia, 2017).

Perceived stress scale (PSS)

The PSS (S. Cohen, Kamarck, & Mermelstein, 1983) (appendix 5) assesses levels of perceived stress over the last month by rating ten questions, such as 'how often have you been able to control irritations in your life?' on a Likert scale, from never (0) to very often (4). Scores range from 0-40, the higher the score, the higher level of perceived stress. The scale has appropriate levels of reliability and validity (S. Cohen et al., 1983). When the PSS was used in the research for this thesis Cronbach's alpha demonstrates a high level of reliability (32 weeks = .871, 36 weeks = .846). Measuring stress is important as indicated by a number of studies demonstrating that stress has an impact on fetal behaviour (e.g., Reissland, Francis, Kumarendran, & Mason, 2015).

Hospital anxiety and depression scale (HADs)

The HADs (Zigmond & Snaith, 1983) (appendix 6) involves rating a number of responses relating to anxiety and depression as to how the individual has been feeling over the past week. Statements are such 'Worrying thoughts go through my mind' and 'I have lost interest in my appearance'. Separate scores are created for both anxiety and depression. The scale has been shown to have excellent reliability and validity for both patients in hospital and the general population (Bjelland, Dahl, Haug, & Neckelmann, 2002; Martin & Thompson, 2002). When the HADs was used in the research for this thesis Cronbach's alpha demonstrates a high level of reliability

for the research presented in this thesis (32 weeks = .834, 36 weeks = .835). Previous research has highlighted differential effects of depression and anxiety on fetal behaviour (e.g., Reissland et al., 2015; Reissland, Froggatt, Reames, & Girkin, 2018).

These measures were used to assess the pregnant women's mental health at the 32- and 36-weeks scans and at the postnatal follow up. Research has indicated that maternal mental health can have an impact on the developing fetus (e.g., Reissland et al., 2018) and have later life consequences for the infant (e.g., Deave, Heron, Evans, & Emond, 2008). Mothers who score highly on measures of depression have higher levels of cortisol and lower dopamine and serotonin levels (Field et al., 2004). This in turn can affect fetal growth, as in pregnant women with high cortisol and higher levels of depressive symptoms their fetus had a reduction in head circumference, abdominal circumference, fetal weight and were more likely to be premature and have a lower birth weight (Field et al., 2006). Alongside fetal growth being affected, level of fetal activity appears to change for those mothers experiencing depressive symptoms. For example, mothers who were depressed, their fetus showed a difference in fetal activity, with an increase in gross body movement and isolated limb movements between 20 to 28 weeks gestational age (Dieter et al., 2001). However, not all studies agree. The pilot study by Reissland et al. (2015) indicated that mothers with higher levels of depression, their fetus showed a decrease in frequency of mouth movements. Whereas fetal mouth movement frequency increased by 1% for every one-point increase in the mothers' stress score. These two research studies demonstrate the differing effects depression and stress may have at different gestational ages and the type of activity being assessed. Therefore, it is essential to

take maternal mental health into account when assessing how the behaviour of the fetus is related to maternal smoking and e-cigarette use.

Prenatal maternal anxiety and depression can also lead to a range of negative infant health outcomes. Research indicates that maternal anxiety and depression accounts for some of the variance in general infant wellbeing (10.7%), respiratory illness (9.3%), skin conditions (8.9%) and the need for the infant to take antibiotics (7.6%) within the first year of life (Beijers, Jansen, Riksen-Walraven, & de Weerth, 2010). The authors suggest that stress and anxiety lead to an increase in cortisol of which the fetus is subjected to and therefore leads to abnormal programming of the immune system prenatally, leading to greater susceptibility to illness later in early infancy (Beijers et al., 2010).

Furthermore, prenatal maternal stress leads to negative effects on the infant as assessed by the NBAS, for example scoring lower on measures of orientation and state regulation (Rieger et al., 2004). Assessing the impact of maternal prenatal depression using the NBAS, higher depressive symptoms led to worse outcomes of habituation, orientation, automatic stability, range of states and motor maturity and a greater number of abnormal reflexes (Field et al., 2004; Field et al., 2006), hence the importance of assessing prenatal maternal mental health and infant behavioural outcomes.

Prenatal phase

4D ultrasound scans

Following recruitment, women were invited to a 4D ultrasound scan appointment at JCUH or the Friarage at approximately 32-weeks and then again at 36-weeks gestational age. Firstly, mothers were asked to complete the consent form (see appendix 7), questionnaires and CO breath test. Mothers were asked to drink cold water prior to the scan and briefly walk the corridor in attempts to 'wake' the fetus, this was determined based on whether the mothers felt active fetal movements. The mother and one other adult attended the scan which lasted approximately 15-20 minutes with an NHS qualified sonographer. At the beginning of the scan, the sonographer briefly checked the well-being of the baby by assessing whether there was a heartbeat and movement. Other measures of well-being or fetal growth were not conducted, and mothers were aware that these were not medical scans, but for the purpose of research. Should the sonographer notice anything untoward, this was investigated further, and women were provided with a medical appointment. The scans took place typically outside of busy routine hours, with most scans conducted between 7-9am and 3.30-5.30pm, and only occasionally throughout the rest of the day. Exact time of day the scan was conducted was recorded to include in the analysis as this may influence level of fetal activity (Raynes-Greenow, Gordon, Li, & Hyett, 2013). The 4D scan focused on the fetal face. Should there be a poor view of the fetal face mothers were asked to change position or take a brief walk.

The hospitals followed The British Medical Ultrasound Society guidance regarding safety, temperature, timing and exposure (Society and College of Radiographers and British Medical Ultrasound Society, 2019). The scans were conducted using the GE Voluson E10™. The full length 4D ultrasound was recorded to a DVD in order for

accurate offline coding of the fetal scans. Mothers received a copy of their 32- and 36-weeks scans after the 36-week appointment.

Length of the scan was determined based on three factors. Firstly, the safety of using ultrasound, secondly, the amount of time that the sonographers were able to dedicate to the project and thirdly, based on previous research and how long the scans would need to be in order to capture the amount of information necessary for the research hypotheses. Ultrasound scans for the Reissland et al. (2015) study were approximately 15-20 minutes in total. See Chapter 4 for prenatal study.

Coding

The 4D ultrasound scans were coded offline using the Observer XT, using the mouth movements outlined in the FOMS (see Chapter 3 for further details). Figure 2.1 displays a screengrab image of what the observer coding screen looks like. Blind coding of the 4D ultrasound scans was carried out by the primary researcher (SF), with the test-retest reliability on 10% of the scans indicated a mean Cohens Kappa (J. Cohen, 1960) of .97 ranging between .92-1. Inter-rater reliability was carried out by another researcher blind to the study conditions on 10% of the scans, and the mean Cohens Kappa was .86, ranging between .75-.98, demonstrating overall excellent reliability.

The method of coding was similar for both the Reissland et al. (2015) pilot study and the research in this thesis. Relative frequency of mouth movements was used to determine differences between exposure groups. Relative frequency is the total number of mouth movements shown per minute over the total time the fetal mouth

was visible throughout the scan. For the pilot study, only the initial 600 seconds (10 minutes) of codable scan were used. In contrast, for the PhD, it was decided that the full scan would be coded given that the data was available. The scans for both the pilot study and the research presented in this thesis were conducted with the same NHS Foundation Trust using the same machine, therefore the quality of the scans are comparable. Further details are provided in the data analysis section.

At 32 weeks' gestation, there was just over 15 hours (878.08 minutes) of codable scan recording in which the fetal face was visible, with 3,075 mouth movements being coded (i.e., 3.5 mouth movements per minute). At 36 weeks' gestation, there was just over 12 hours (708.23 minutes) of scan recording where the fetal face was visible, with 1,725 mouth movements coded (i.e., 2.4 mouth movements per minute). Length of time the mouth area was visible varied across the scans (32 weeks M= 8.04 minutes, S.D.= 5.08 minutes and 36 weeks M= 8.14 minutes, S.D.= 4.24 minutes). Each 15–20-minute scan took approximately 7 hours to code, using frame by frame coding.

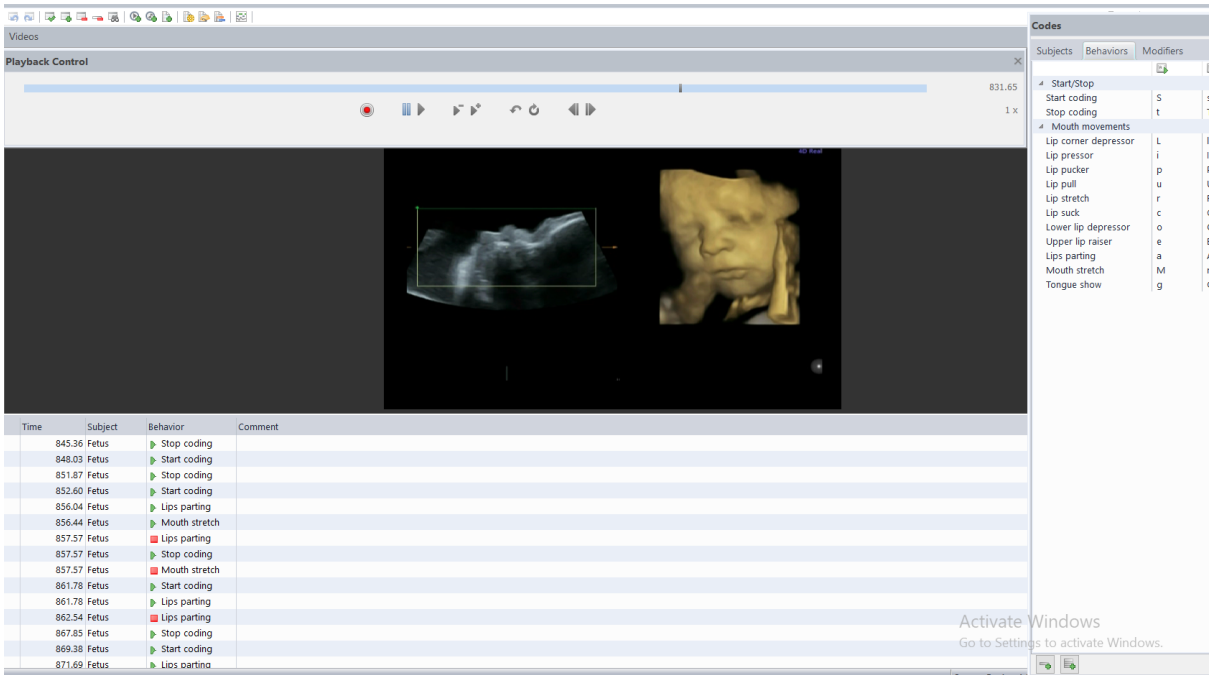

Figure 2.1. Observer screen layout.

Birth outcomes

The majority of the women who participated in the research attended JCUH or the Friarage Hospital for all their antenatal care and birth, therefore their birth records were obtained from their hospital. For the small portion of women who were recruited via social media pages, their birth records were obtained via their health visitor 'red books', where this information was recorded by their own hospital. A number of birth outcomes were relevant and recorded for this research. Gestation at birth, gender, birthweight, head circumference, Apgar scores, type of delivery and first feed were noted, see table 2.2. Birth outcomes were recorded to assess whether there were any differences across the exposure groups and to include into the analysis to see if there is an association to postnatal neurobehaviour.

Interestingly, in the current sample of women there was a higher rate of caesarean section deliveries in the cigarette smoking groups. Research has indicated a higher incidence of c-sections for women who smoke, possibly as a result of placenta previa as a result of cigarette smoking (Sharma & Choudhary, 2014), with smokers 1.24 times more likely to need an instrumental delivery (Lurie, Ribenzaft, Boaz, Golan & Sadan, 2014). It is important to record such information as research indicates there is a relationship between c-section deliveries and neurodevelopmental disorders, in particular attention deficit hyperactivity disorder (ADHD) and intellectual disability at 16 years of age (Zhang, Brander, Mantel, Kuja-Halkola, Stephansson, Chang…& de la Cruz, 2021). Such findings have been shown in a large meta-analysis of 61 studies finding a greater risk of ADHD and Autism as a result of C-section delivery (Zhang, Sidorchuk, Sevilla-Cermeño, Vilaplana-Pérez, Chang, Larsson, ... & de la Cruz, 2019).

Table 2.2. Birth outcomes split by smoking status.

	Gestation at birth (weeks)	**Gender**	**Birthweight (grams)**	**Head circumference (cm)**	**Apgar scores at 1 minute**	**Type of delivery**	**First feed**
Non-smokers N= 54	Mean – 39.231 Standard deviation – 1.262	Male – 25 (46.3%) Female – 29 (53.7%	Mean – 3410.179 Standard deviation – 533.724	Mean – 34.635 Standard deviation – 1.119	Mean – 8.800 Standard deviation - .613	Vaginal – 42 (77.8%) C-section – 5 (9.3%) Missing – 7(12.9%)	Breastfed – 27 (50%) Formula- 19 (35.2%) Not recorded – 8 (14.8%)
Light smokers (<10 per day) N= 36	Mean – 38.973 Standard deviation – 1.568	Male – 19 (50%) Female – 19 (50%)	Mean – 3093.864 Standard deviation – 499.246	Mean – 33.968 Standard deviation – 1.572	Mean – 8.552 Standard deviation – 1.420	Vaginal – 29 (76.3%) C-section – 6 (15.8%) Missing – 3 (7.9%)	Breastfed – 10 (26.3%) Formula- 20 (52.6%) Not recorded – 8 (21.1%)
Heavier smokers (11-20 per day) N= 15	Mean – 38.846 Standard deviation -1.188	Male – 9 (60%) Female – 6 (40%)	Mean – 2936.875 Standard deviation – 408.999	Mean – 33.103 Standard deviation – 1.324	Mean – 8.722 Standard deviation - .546	Vaginal – 9 (60%) C-section – 4 (26.7%) Missing – 2 (13.3%)	Breastfed – 4 (26.7%) Formula- 8 (53.3%) Not recorded – 3 (20%)
E-cigarette users N= 16	Mean – 39.695 Standard deviation - .698	Male – 4 (25%) Female – 12 (75%)	Mean – 3340.784 Standard deviation – 265.015	Mean – 34.182 Standard deviation - .758	Mean – 8.828 Standard deviation - .263	Vaginal – 10 (62.6%) C-section -1 (6.3%) Missing – 5 (31.3%)	Breastfed – 8 (50%) Formula- 3 (18.8%) Not recorded – 5 (31.3%)

Series means estimates were used to replace missing values. Due to unobtainable birth records some data is missing for type of delivery.

Postnatal follow up

Following the birth of their infant, mothers received a phone call to arrange the postnatal follow up assessment (see appendix 8 for information leaflet), at approximately one month of age (M=32.6 days, S.D.=5.33). Out of the 123 women involved in the prenatal phase of the research, 83 infants were eligible to participate at one month. Nine infants were not eligible due to gestation at birth or medical complications, six women could not be contacted, and 25 did not want to participate. Inclusion criteria was the same as for the prenatal study with the addition of infants to be born at term (>37 weeks), healthy and no neonatal intensive care unit (NICU) admission. The one month follow up took place in the infant's home and lasted between 40-60 minutes dependent on the infant's state (e.g., sleeping, feeding, changing schedule). Mothers completed a range of questionnaires including the PSS, HADS, postnatal attachment questionnaire, smoking questionnaire and CO breath test.

Prenatal cigarette exposure has been found to be correlated with infant irritability, attention, hypertonicity and decreased response to auditory stimuli (Mansi et al., 2007; Stroud, Paster, Goodwin, et al., 2009). Additionally, those infants exposed to cigarette smoke prenatally have a greater need for handling, demonstrate lower self-regulation (Stroud, Paster, Papandonatos, et al., 2009) and lack of focused attention (Wiebe et al., 2009). Motor behaviour is also impacted as a result of cigarette exposure, and early motor development is thought to be directly associated with cognitive and behavioural outcomes (Hitzert, Roze, Van Braeckel, & Bos, 2014). Chapter 5 of this thesis further outlines the effects of prenatal cigarette exposure on

infant neurobehaviour (Froggatt, Covey, & Reissland, 2020a). Hence, the research aim is to replicate these findings alongside understanding the impact that e-cigarette exposure may have on infant behaviour. The NBAS was chosen due to the vast amount of previous research conducted assessing a variety of different factors including the effects of smoking (e.g., Hernandez-Martinez, Arija Val, Escribano Subias, & Canals Sans, 2012), preterm birth (Wolf et al., 2002) and maternal mental health (Rieger et al., 2004), with the research suggesting the NBAS has good predictive validity (Canals, Hernandez-Martinez, Esparo, & Fernandez-Ballart, 2011). The NBAS was conducted with the infant by the primary researcher (SF) following a period of training and certification. At the end of this phase mothers were given a debrief sheets (appendix 9) and some women provided feedback on the project (see appendix 10).

The Neonatal Behavioural Assessment Scale (NBAS)

In the earliest period of the Twentieth Century, it was commonly thought that infants were entirely passive, with the emphasis focused on their environment and parental interactions (Brazelton & Nugent, 2011), however this is no longer accepted (Lagercrantz, 2009). Early assessment measures of infant behaviour were mainly based on Apgar scores at birth and primitive reflexes. However, in the early 1960s new research emerged that demonstrated the complexities of newborns, with classic research including newborn face-like preferences (Fantz, 1961) and newborns ability to orientate to sound (Wertheimer, 1961). In contrast to the early assumptions of infants, the NBAS was developed on the premise that newborns have a predisposition to interact with caregivers for survival purposes, and therefore the assessment was developed based on an interaction between the infant and examiner. The NBAS was

developed in 1973 and then revised in 1995 (Hawthorne, 2005), the assessment is centralised on the development of the infant, with an additional focus on the family, including diagnosis and intervention for parents. The assessment includes observation of the newborn's reaction to aversive and non-aversive stimulation (Hawthorne, 2005) and is a holistic assessment of the infant (Başdaş, Erdem, Elmali, & Kurtoğlu, 2018). It is understood that the examiner can facilitate and elicit newborn responses to develop an accurate and comprehensive picture of newborn functioning by highlighting strengths and areas for improvement (Brazelton & Nugent, 2011). The NBAS, unlike earlier behavioural assessment scales, is based on the notion that newborns are complexly organised with social abilities that allow them to be active in their development from birth (Brazelton, Brazelton, & Nugent, 1995). Similarly, after extensive research when developing the assessment scale, it has been highlighted that behaviour is not solely genotypic, but in fact also phenotypic with influences such as maternal mental state, nutrition and drug use having an effect throughout pregnancy (Brazelton & Nugent, 2011).

Reliability and validity has been established when using the NBAS in other cultures. In a Turkish sample, researchers found that when the NBAS was conducted on 380 newborns at 1-3 days old and then a repetition of the test with 60 of these newborns between 52-55 days old, Cronbach's alpha was .974. This suggests that the NBAS is a valid, stable and reliable measure to assess the profile of a newborn (Başdaş et al., 2018). In preterm and low birth weight infants, the NBAS has shown to have good validity and internal consistency (Lizarazo Medina, Ospina Díaz, & Manrique Abril, 2012).

As outlined in the NBAS manual (Brazelton & Nugent, 2011), there are four key domains of neurobehavioural functioning, with hierarchical progression. This can provide an insight into the infant's current development and provide an indication where the infant may need further support. The domains are outlined below.

1) Automatic/physiological regulation. This is the most basic observation of the newborn and it reflects their ability to regulate their autonomic systems including breathing, temperature control, tremors, startles and body colour changes.
2) Motor organisation. Providing the infant has a stable autonomic system, they will be able to control their motor and muscle movements.
3) State organisations and regulation. This refers to the infant's sleep/wake cycles and whether they have the ability to reduce disturbance from outside stimuli. An infant who has stable states can provide an indication that the infant is able to deal with stress and have self-consoling abilities.
4) Attention/social interaction. This reflects the infant's ability to attend and orientate socially to others and objects, which is essential for caregiver interactions.

In order to become a certified NBAS examiner, following a period of pre-course preparation, there is an intensive two-day training course. Following the two-day training course, there is a phase of self-training which involves developing a portfolio of 20 NBAS assessments and then an examination. The training covers brain development, regulatory behaviours, the transition into parenthood, infant and adult mental health issues in the postpartum period, how to deliver the NBAS, a practical

session using dolls, demonstration with an infant and how to score the NBAS for example (Brazelton & Nugent, 2011).

The scores on the NBAS can be reduced to a seven-cluster scoring system and this was the approach used in this research (Lester, 1984), see table 2.3 (see appendix 11 for NBAS assessment scoring sheets).

Table 2.3. The seven-cluster scoring measures

Cluster	Assessment measures
Habituation	Light, rattle, bell, pin prick
Orientation	Inanimate visual, inanimate auditory, inanimate visual-auditory, animate visual, animate auditory, animate visual-auditory, alertness
Motor	Tonus, maturity, pull-to-sit, defence, activity
Range of states	Peak excitement, rapidity of build-up, irritability, lability of states
Regulation of states	Cuddliness, consolability, self-quietening, hand-to-mouth
Automatic stability	Tremors, startles, skin colour
Reflexes	Plantar grasp, Babinski, ankle clonus, rooting, sucking, glabella, passive movements arms and legs, palmer grasp, placing, standing, walking, crawling, incurvation, tonic deviation of head and eyes, nystagmus, tonic neck reflex, Moro reflex.

For this research, only six of the NBAS clusters were assessed. Habituation was not included due to the difficulties and lack of consistency in assessing infants. As homes were visited and tight schedule planning for scans dictated by the hospitals, it meant that the allocated time suggested for infant sleep prior to habituation assessment was

not always possible and therefore for the few infants this was carried out on was not a reliable method. Instead, the focus is on the remaining six clusters.

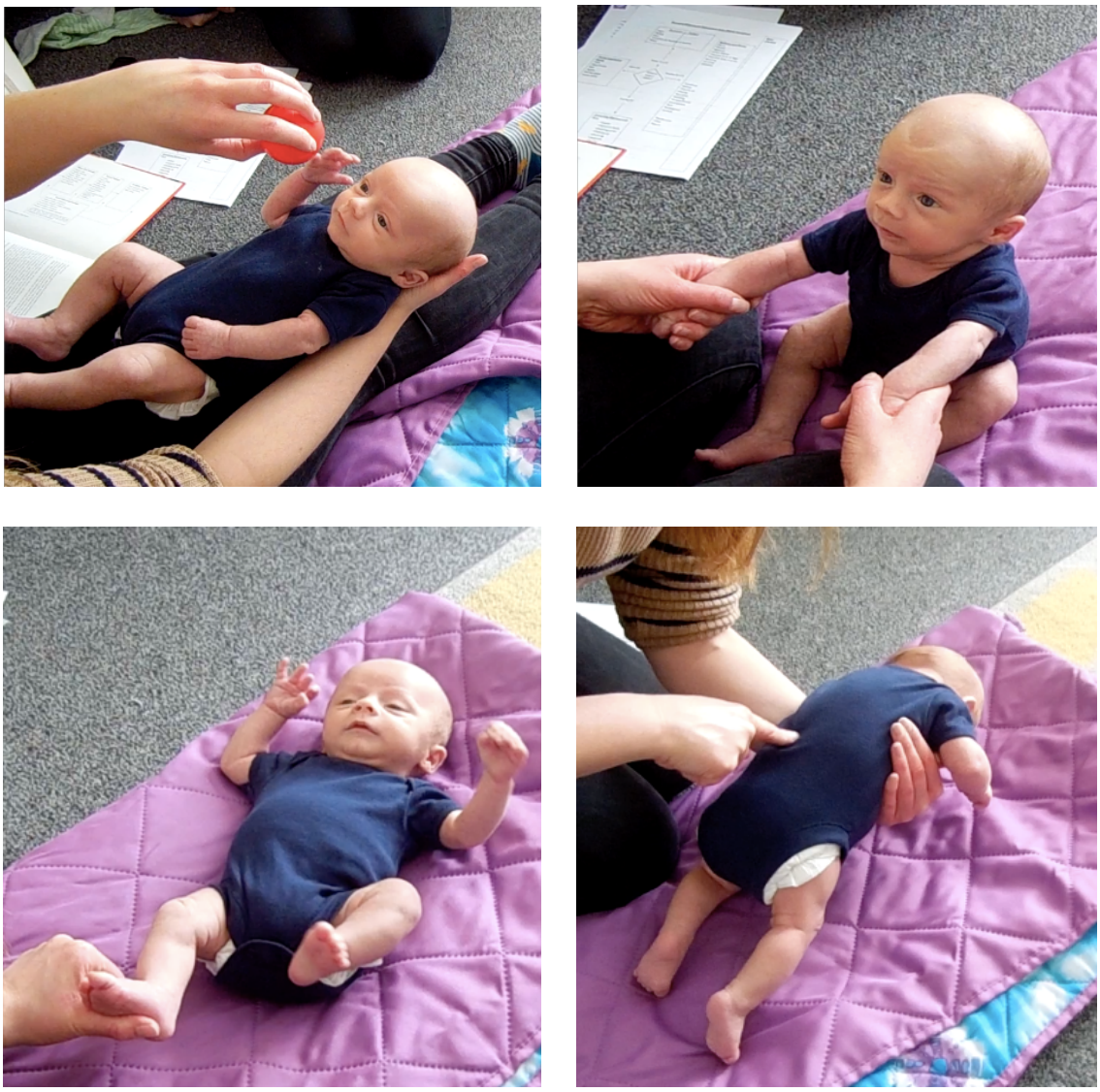

Figure 2.2. NBAS assessment in motion.

Figure 2.2 demonstrates some items from the NBAS assessment being carried out. The top left image demonstrates an item from the orientation package of the infant following an inanimate object. The top right image the item of pull-to-sit from the motor package assessing strength and tone in the infant muscles. The bottom two images show reflexes being carried out, foot reflexes and incurvation of the spine.

Research using the NBAS

The NBAS is used in many different research contexts, including assessing the effects of preterm delivery (Wolf et al., 2002) and maternal mental health (Rieger et al., 2004). For example, research indicated that when assessed 3-5 days postpartum, mothers who self-reported higher levels of chronic stress had infants who scored lower on measures of orientation and state regulation, with lower scores on the supplementary items including quality of alertness, examiner facilitation and robustness and endurance (Rieger et al., 2004).

Studies have assessed the longitudinal predictive nature of the NBAS, for example, one study assessed newborns with low birth weight and/or premature infants who were in the Neonatal Intensive Care Unit (NICU). The NBAS was carried out at three time points, postmenstrual age of 36-38 weeks, 40-42 weeks and 44-46 weeks. These infants were followed up at 5 years of age using neurological exams, MRI and CT scans, EEGS, McCarthy scale of children's abilities and behavioural problems using the DSM-IV. Children in the study were classified into three disability groups: normal, mild disability and severe disability. Results indicated that the NBAS was a good predictor for categorising the children at 5 years of age, with lower behavioural scores on the NBAS (habituation, orientation, motor, range of state, state regulation and automatic stability) and higher scores on the reflexes indicated that the child would subsequently be categorised as having either mild or severe learning disabilities (Ohgi et al., 2003).

A number of studies have also used the NBAS to indicate the effects cigarette smoking has on infant development. Hernandez-Martinez et al. (2012) assessed

infants between 48 to 72 hours old whose mothers were either smokers or exposed to second-hand smoke during pregnancy. The results indicated that for mothers who smoked during pregnancy their infants scored significantly lower on items including state regulation, inanimate visual orientation, peak excitement, liability of states and examiners emotional response. For those infants whose mothers were exposed to second-hand smoke during pregnancy, these infants scored lower on motor systems, examiner facilitation, robustness and endurance and state regulation. All exposed infants (either through maternal smoking or maternal second-hand smoke) had significantly lower habituation responses (Hernández-Martínez, Val, Subías, & Sans, 2012). Mansi et al. (2007) reported that infants who were prenatally exposed to cigarettes scored lower on items including attention, irritability, muscle tone, orientation and regulation (Mansi et al., 2007). It is evident that the NBAS is a suitable method for assessing toxin exposure, in particular cigarettes, and therefore was selected as an appropriate method for assessing the effects of prenatal cigarette and e-cigarette exposure on infant neurobehavioural outcomes at one month of age. See Chapter 6, for the published article.

Pre to postnatal

Until the development of recent methods to assess fetal behaviour, it has been impossible to assess fetal brain development and CNS development. However, with advances in both technology and methodology this provides a window of opportunity to assess such behavioural development. Similarly, tools such as the NBAS have allowed researchers and clinicians to assess infant neurobehavioural development and CNS functioning (Hata, 2016).

Other fetal assessment measures, such as the Kurjak's Antenatal Neurodevelopmental Test (KANET) and the Fetal Neurobehavioural Assessment Scale (FENS) have their origin in postnatal assessments (see Chapter 3). For example, the KANET uses similar principles to the Amiel-Tison Neurological Assessment at Term (ATNAT) (Amiel-Tison, 2002; Kadić et al., 2016). There are some elements that are present in both KANET and ATNAT including "nonreducible adduction of the thumb in a clenched fist…and cranial ridges over each suture or restricted to the squamous suture" (p.181) with these two signs plus a high-arched palate (this cannot be observed via ultrasound) are thought to indicate fetal brain damage (Kadić et al., 2016). The KANET also involves a scoring of general movement, based on postnatal assessments. For example, in preterm infants, there is a greater level of fluctuations, differences in speed and quality of movement in comparison to term infants, which is thought to be an indicator of infant well-being (Prechtl, 1990). The FENS is based on the postnatal assessment of the NICU Network Neurobehavioural Scale (NNNS) (Lester & Tronick, 2004; Salisbury, Fallone, & Lester, 2005). These two assessment measures are similar in some respect as they both assess the three key elements that are indicators of CNS maturation, including neurological, behavioural and stress/reactivity measures (Salisbury et al., 2005).

A recent study using the FENS identified the relationship between prenatal behaviour and postnatal behaviour using the NNNS (Stroud, McCallum, & Salisbury, 2018). Overall fetal isolated movements were associated to infant quality of movement (e.g. number of startles, tremors, jerkiness of movement and motor maturity), fetal complex body movements were associated to infant handling (e.g. the amount of

external soothing required, examiner input to keep the infant in an alter state), and overall fetal activity was associated with attention (e.g. orientating to both animate and inanimate stimuli), handling, lethargy (e.g. low level of motor movement) and regulation (e.g. self-soothing abilities). Additionally, fetal coupling index, the relation between fetal activity and fetal heart rate, was associated with attention, handling and quality of movement.

In sum, such assessment measures have the ability to demonstrate continuity of pre to postnatal behaviour, due to similarities in assessment measures. The postnatal neurological assessments have been used to help develop the prenatal neurological assessments. However, the FOMS is not based on a postnatal infant neurological assessment, therefore at present, it is unknown how prenatal fetal facial or mouth movements relate to postnatal infant behaviour and what the implications of fetal differences mean for the development of the infant postnatally. Given the interest in the pilot study conducted by Reissland et al. (2015) and the questions surrounding what the differences in fetal movement mean, the pre-to-postnatal study presented in this thesis begins to explore such questions, as it is thought that facial movements, in particular mouth movement is linked to CNS function (Hata, 2016). In order to test such theory, the NBAS, a well-established neurological assessment, is used to assess the relationship between the FOMS and postnatal behaviour. This will be the first piece of research attempting to understand what prenatal mouth movement differences mean for postnatal behaviour. Fetal and infant pairs were assessed to evaluate the relationship between these two time points, regardless of cigarette or e-cigarette exposure. The analysis was based on exploring the relationships firstly

between fetal mouth movements at 32 weeks and the NBAS (N=75), and secondly between fetal mouth movements at 36 weeks and the NBAS (N=67).

Interview

As part of the postnatal phase of the research, at the one month follow up, mothers were invited to participate in an additional aspect of the research, namely a semi-structured interview. The purpose of the interview was to assess understanding of risks of cigarette smoking and e-cigarette use, reasons for continuing to smoke during pregnancy, whether anything would help smoking cessation, benefits of 4D ultrasound scanning and perceived differences in a range of fetal movement as a result of cigarette smoking. The questions asked in the interview are listed in full in Appendix 12 and were based on an extensive literature search as part of a Masters dissertation (Froggatt, 2017). However, for this thesis, only questions which provided insights into the perceived risks associated with cigarette and e-cigarette use were analysed. Only cigarette smokers and e-cigarette users were invited to participate in this portion of the research. Overall, 22 women participated in the interview, 14 of which were cigarette smokers. For the purpose of analysis, only the cigarette smokers' views were analysed, given these women are prime targets for smoking cessation interventions and gaining an understanding of their perception of risk may aid development of new smoking interventions during pregnancy.

The main focus of the interview regarded the risk of cigarette and e-cigarette use during pregnancy and the early postpartum period. Questions were as follows:

1) Do you believe there is any harm associated with smoking during pregnancy?

a. Is there a risk to you?
b. Is there a risk to the fetus?
c. Is there a risk once the baby is born?

2) Do you believe there is any harm associated with using e-cigarettes during pregnancy?
    a. Is there a risk to you?
    b. Is there a risk to the fetus?
    c. Is there a risk once the baby is born?

Interviews were audio recorded and transcribed verbatim into Nivo. A six-stage inductive thematic analysis approach was used in order to create themes and subthemes (Braun & Clarke, 2006). See Chapter 8 for the published interview study.

Data analysis

Separate data analysis sections are written for each of the experimental chapters of the thesis. Although data analysis was planned for each study, only two studies were pre-registered and submitted to the Open Science Framework (OSF), this was for the prenatal study (https://mfr.osf.io/render?url=https://osf.io/xn768/?direct%26mode=render%26action=download%26mode=render) and the pre-to-postnatal study (https://osf.io/9c58a). By the time pre-registration was considered, data analysis and write up had already begun for the other three studies; the systematic review and meta-analysis (Chapter 5), the postnatal study (Chapter 6) and the interview study (Chapter 8), hence no pre-registration was conducted for these studies.

Relative frequency of fetal mouth movement was used to establish any differences in fetal behaviour. Relative frequency was measured as each 4D ultrasound scan was not the same in length and there were different amounts of footage which could be coded due to mouth visibility. This allowed easier comparison of movements between each fetal 4D scan. Relative frequency of mouth movement has been used on all other published research articles using the FOMS (Reissland et al., 2015; Reissland, Makhmud, & Froggatt, 2019; Reissland et al., 2020a). This was done for total mouth movements, for each individual mouth movement and clusters of movement. Clusters of movement was an additional measure where individual mouth movements either co-occurred or occurred immediately one after another.

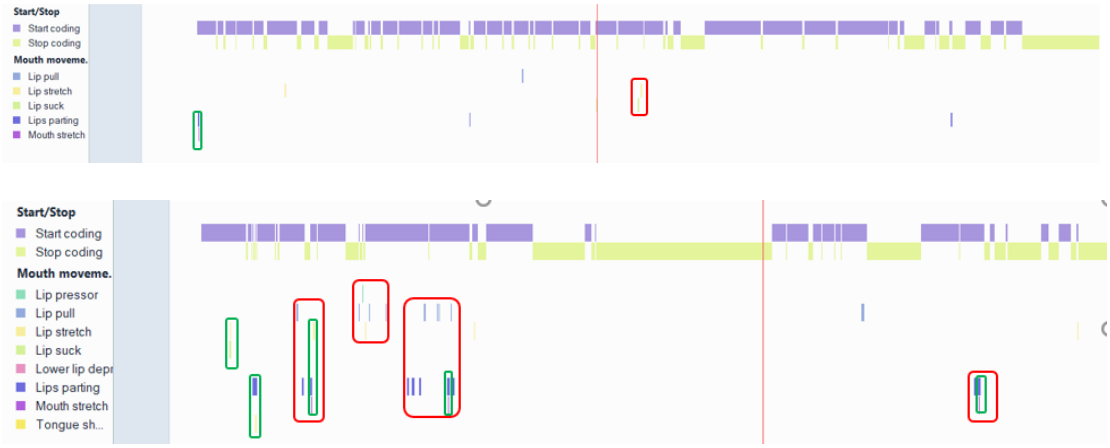

Figure 2.3. Visual graphs for cluster movement analysis.

Figure 2.3 provides two examples of how the clusters of movement were analysed. Individual graphs were created for each fetus, providing a visual display of the different types of movements across the length of the scan. Using a visual method, it can be determined when movements occur at the same time (boxed in green) and

when movements occur immediately after one another (boxed in red). Total number of clusters was used to created relative frequency of clusters of movement.

Chapter 3

The Fetal Observable Movement System (FOMS)

Early ultrasound

Studies dating back to the 1930s focused on fetal physiology (Doyle & Cicchetti, 2018), with a breakthrough occurring in 1958, with the classic paper published in the Lancet by Donaldson et al. with the first fetal ultrasound images being obtained (Campbell, 2013). In recent decades, there has been an influx in research within the field of fetal development due to the advancing ultrasound technology affording the opportunity to analyse fetal behaviour.

Ultrasound images are created by high frequency pulses of sound (Whitworth, Bricker, & Mullan, 2015). Ultrasound scanning during pregnancy is used for a variety reasons including dating the pregnancy, detection of multiple pregnancies and early identification of anomalies. During the later periods of pregnancy, ultrasound scans are used when there are signs of a problem, such as assessing the fetal growth, when maternal bleeding occurs or when the mother suspects a reduction in fetal movements (Whitworth et al., 2015). In sum, ultrasound scans are used to ensure well-being of the fetus (Neilson, 1998). A large-scale systematic review involving 37,505 mother-infant pairs indicated that when routinely scanned, there were no adverse effects on the cognitive or physical development of the child, rendering ultrasound safe during pregnancy to assess the development of the fetus (Whitworth et al., 2015).

Early studies using 2D ultrasonography focused on establishing which movements could be seen and how movements change across the course of gestation. For

example, early research focused on identifying a range of large body movements such as breathing, isolated head and arm movements, startles and hand to face touches (Birnholz, Stephens, & Faria, 1978). Similarly, patterns of movements were identified highlighting that frequency of movements differed throughout the first half of pregnancy. Breathing, jaw opening, swallowing and head rotations increased, whilst arm movements increased until a plateau was reached. Startles and facial touches increased before decreasing (De Vries, Visser, & Prechtl, 1985). The complexity of fetal movement increases across the gestational weeks. At the beginning of the first trimester, only large gross body movements are visible and at the end of this trimester the complexity of head and limb movements increase. During the second trimester the complexity increases further, and the frequency of movement also surges. Facial movements, eye movements, touch behaviours and isolated limb movements are all present during the second trimester. It is toward the later gestational time points in pregnancy where such movements begin to decrease and naturally slow down which is thought to be a reflection of brain and central nervous system (CNS) maturation (Lebit & Vladareanu, 2011).

Prior to the 1980s (Campbell, 2013), ultrasound scanning was not part of routine antenatal care. Early research indicated that when ultrasound was selective in hospitals, women did not approve of the method or its uses. Whereas in a hospital where it was part of routine care, the women were often disappointed as the fetal image could not be clearly seen (Hyde, 1986). Perceptions of ultrasound scans has improved, with studies indicating that regardless whether 2D, 3D or 4D ultrasound are used, there is an increase in attachment, with 3D and 4D scans allowing for clearer images and better recognition of the fetus (de Jong-Pleij et al., 2013).

However, other studies have indicated that it is not the ultrasound scan itself that increases the attachment, but in fact the explanation of the ultrasound scan, therefore the evidence is contradictory regarding the direct increase of attachment as a result of a scan (Cunen, Jomeen, Xuereb, & Poat, 2017).

The advances in ultrasound technology, have allowed the emergent field of fetal psychology to be further developed, with neurological development being established and identified, with the ability to classify what may be considered normal and abnormal development (Hata, 2016). It is thought that through ultrasound examination, brain development and CNS function could be evaluated (Morokuma et al., 2013). Ultrasound technology has provided the opportunity to study the fetal behavioural profile, effects of maternal health behaviours and the relationship to postnatal behaviour and development.

3D & 4D ultrasound

Prior to the development of 3D and 4D ultrasound, 2D scanning was used to assess fetal wellbeing. However, there were a number of issues, such as poor visibility for assessing behaviour, as only the bone structures could be seen (Kadić et al., 2016). 3D ultrasound scanning provides a still image, where 4D ultrasound is a real-time video providing the opportunity to assess fetal movements and subtle rotations, with a clearer view of the anatomy and surface structures, such as the skin of the fetus (Kadić et al., 2016). With the growing use of 3D and 4D ultrasound in clinical practice, a large-scale review indicated that it is useful in detecting facial anomalies (Rotten & Levaillant, 2004), skeletal malformations (Clementschitsch, Hasenöhrl,

Steiner, & Staudach, 2003) and neural tube defects, alongside indicating which fetuses might be at risk and have CNS anomalies by assessing fetal behaviour (Gonçalves, Lee, Espinoza, & Romero, 2005; Kurjak et al., 2007). Timing of when to conduct a 3D or 4D ultrasound is important, as the fetal facial structures are defined by 13-14 weeks gestational age, with facial expressions evident between 15-16 weeks (Piontelli, 2010). However, it would not be appropriate to show families these early gestation scans as the facial structure alone may distort the image of their child weaking the attachment. Therefore, such early scanning would be used mainly for diagnostic purposes. The optimal time for ultrasound scanning, to assess fetal behaviour in particular, is after 23 weeks gestational age, given that most research carried out in order to develop behavioural assessment measures are conducted after this gestational week (Kurjak et al., 2007; Reissland, Francis, & Buttanshaw, 2016).

Using 4D imaging, it has been possible to identify in the fetus subtle behavioural differences when exposed to maternal stress, depression, and anxiety. For example, when assessing eye blink rate in relation to maternal anxiety and depression, there is a 20% increase in eye blink rate for each additional anxiety score, in contrast to a 21% decrease for each additional depression score (Reissland, Froggatt, Reames, & Girkin, 2018).

Assessment measures

A number of fetal behavioural assessment tools have been created including Kurjak's Antenatal Neurodevelopment Test (KANET), the Fetal Neurobehavioral Assessment System (FENS) and the Fetal Observable Movement System (FOMS). Fetal

behavioural assessment methods are thought to be important, as research indicates that normal and abnormal development can be visualised through such behavioural assessments with spontaneous expressions giving an insight into the developing CNS (Reissland, et al., 2015). The two key fetal behavioural assessment measures are the FOMS for facial movement, and KANET for body movements, and up until development of such tools, it was difficult to assess brain development and function of the CNS (Hata, 2016).

The first standardised fetal behavioural assessment method was the KANET, standardised in 2010. The KANET assesses a range of fetal behaviours including mouth openings, eye blinks and isolated arm and leg movements. This assessment is used in the 3rd trimester of pregnancy and takes approximately 20 minutes to administer (Antsaklis, Kurjak, & Izebegovic, 2013). It is based on the postnatal assessment The Amiel-Tison Neurological Assessment at Term (ATNAT) (Kadić et al., 2016). The ATNAT is a postnatal assessment that takes approximately five minutes to administer, from 32 weeks post conceptional age and can be carried out until the child is six years old. It has 10 key domains including a cranial assessment, neurosensory function and spontaneous motor activity, passive muscle tone, axial motor activity, primitive reflexes, palate and tongue assessment, adaptation in the assessment, feeding, medical status and unfavourable circumstances at the assessment (e.g., noise in the environment) (Gosselin, Gahagan, & Amiel-Tison, 2005). The KANET is considered a diagnostic tool in clinical practice due to the potential of detecting neurological impairments prenatally (Antsaklis et al., 2013; Kurjak et al., 2017). Based on scores from this assessment, fetuses are categorised into neurologically normal, borderline or abnormal, with postnatal follow-up

demonstrating the ability of the KANET to identify those fetuses who were born with severe neurological impairment (Kurjak et al., 2008). Research has indicated that those with borderline scores, 66% (12 out of 26 fetuses) were classified as at-risk during pregnancy with 33% (six out of 24 fetuses) in the low risk group (Honemeyer, Talic, Therwat, Paulose, & Patidar, 2013). However, the authors noted that two of the borderline scores in the low-risk group coincided with fetal quiet periods and indicate that there may be issues with the sensitivity of the KANET. However, in a recent large-scale assessment of the KANET of 3,709 fetuses across seven countries, 10.2% were classified as borderline and 3.3% as abnormal (Kurjak et al., 2020). 1,556 of these fetuses were followed up postnatally, with 98.3% experiencing a normal developmental pathway, 0.5% had slight or moderate delay whilst 1.2% were classified as experiencing severe developmental delay. Those infants with moderate to severe developmental delay were more likely to be given an abnormal prenatal KANET score. Authors of the study indicate that a normal KANET score is likely to lead to normal development, whereas if the pregnancy is classed as high risk and the KANET score is borderline or abnormal, there is a higher possibility that the child will have developmental delay (Kurjak et al., 2020).

The KANET took many years to develop and was the cumulation of several different studies in order to establish the parameters of fetal behaviour. It examines a range of fetal behaviours and general movements. In clinical settings the KANET should be repeated until delivery of the infant at intervals of 2 weeks should the fetus be given a borderline or abnormal score (Antsaklis & Antsaklis, 2012). One of the first studies prior to the development of the KANET which was used to inform its development assessed fetuses with intrauterine growth restriction (IUGR) assessing a range of

facial expressions and body movements. This was a prospective study involving 50 healthy and 50 IUGR pregnancies. Results from the study indicated that those with IUGR showed fewer facial expressions and body movements compared to fetuses without IUGR, arguing that assessing behaviour can provide an insight into neurological development (Andonotopo & Kurjak, 2006).

In order to establish whether the KANET was useful in identifying postnatally neurological impaired infants, a retrospective study was carried out assessing the correspondence between the KANET and ATNAT. Infants who experience a low-risk pregnancy had KANET scores between 14 and 20 which was later deemed as optimal neurological development and for those high-risk pregnancies, the postnatally normal infants had KANET scores of between 14-20, those who postnatally were mildly or moderately abnormal had KANET scores between 5-13 and those postnatally who were abnormal had KANET scores of 0-5. Therefore, it can be argued that the KANET is useful for identifying neurological signs of impairment prenatally (Antsaklis & Antsaklis, 2012).

An alternative fetal coding scheme is the FENS, which attempts to chart neurological development. The FENS includes assessment of reactivity, behavioural and neurological measures which is comparable to the postnatal neurobehavioural assessment of the Neonatal Intensive Care Unit (NICU) Network Neurobehavioral Scale (NNNS) (Salisbury, Fallone, & Lester, 2005). There are four key areas that are assessed when using the FENS which include motor activity, behavioural state, heart rate and the fetal response to external uterine stimuli. The test is used for both healthy and at risk fetuses. The FENS is carried out in the 2nd and 3rd trimester and the

authors argue that it provides a clear indication of the development of the CNS. Specific face, chest and body movements are coded along with the quality of the movement, such as how smooth or jerky such movements are. The FENS is a prenatal assessment that is based on the NNNS postnatal assessment assessing the same elements of neurology, behaviour and reactivity (Salisbury et al., 2005). Scores on the FENS are correlated with NNNS (Salisbury et al., 2005). In order to conduct an assessment using the FENS, an ultrasound assessment and fetal actocardiograph are needed to assess both behaviours and a physiological element, which is fetal heart rate. In order to use the FENS, it is first required to establish a baseline of fetal activity that lasts approximately 40 minutes, after which a 3 second vibroacoustic stimulus is used and then up to 30 minutes of further observation. However, the total examination must not exceed 60 minutes. The stimuli can also be a light or sound. The observation is based on the upper part of the body including head, face, trunk, and arms. Firstly, movements of the face and head are coded including eye movements and yawning for example, followed by assessing specific behaviours and movement patterns of the body, such as isolated limb movements, startles, stretches and hiccups. The quality of the movement is also assessed. An initial pilot study assessing the relationship between the FENS and NNNS found that quality of movement, such as smooth movements were correlated to infant self-regulation (Salisbury, Fallone, & Lester, 2005). The scores are defined as percentages of movement or quality of movement. The way in which it is scored appear to be similar to the FOMS (discussed below), however what is being measured differs.

Both the KANET and the FENS assess the behavioural and CNS development continuity from pre to postnatal life given both their origin is in postnatal

neurobehavioural assessments. However, the FOMS was not based on a postnatal neurological infant assessment and therefore it is currently unknown what differences in prenatal movements mean.

The Fetal Observable Movement System (FOMS)

The FOMS is an anatomically based movement coding system, based on the movement of human facial muscles. The development of the FOMS is based on a number of facial coding schemes including the Facial Action Coding Scheme (FACS) (Ekman, 1977), BabyFACS and ChimpFACS (Reissland et al., 2016). The coding scheme was developed by assessment of healthy fetuses who were low risk for any complications and were healthy newborns. It was developed for human fetuses in utero using 4D ultrasound technology to capture live fine-grained fetal facial movements. The coding scheme was developed with fetuses aged between 23 to 37 weeks gestational age. The FOMS provides a reliable identification of facial muscles and movements and is considered an objective coding system (Reissland et al., 2016). Unlike KANET and the FENS, the FOMS relies fine-grained facial movements and facial touches, opposed to gross body movements. Facial movements are considered especially important during pregnancy, with many suggesting that these movements directly reflect the brain and CNS development (Antsaklis et al., 2013; Kurjak et al., 2007). Throughout all gestational weeks fetal mouth movements taken altogether are the most common, with the range of mouth movements shown by the fetus indicating maturity in the developing brain (AboEllail & Hata, 2017). However, when analysing individual mouth movements, lip parting occurs more frequently than lower lip depressor for example. When assessing facial movements overall that are identified

by the FOMS, a brow lower is more common than a mouth stretch for example (Reissland et al., 2016). In addition, individual facial movement frequency changes dependent upon gestational age. For example, lip corner depressor increases throughout the gestational ages of 24-, 28-, 32- and 36-weeks in contrast to lips parting which decreases from 28-, 32- and 36-weeks gestational age. Examining all facial movements of the FOMS, lips parting has the highest average frequency from 24 through to 36 weeks gestational age, with a dimpler having the lowest frequency across these gestational ages (Reissland et al., 2016). Research indicates that the changes of frequency of different facial movements is likely to reflect the changes occurring within the brain and CNS development across the gestational weeks (AboEllail & Hata, 2017; Morokuma et al., 2004).

The coding of mouth movements differs from the FENS. The FENS focuses on mouth movements generally, whereas the FOMS outlines 11 different mouth movements that are coded separately. Whilst the FENS focuses on chest, body, isolated limb movements and heart rate generally and in response to stimulation, the FOMS focuses on the facial movements and self-touch movement to the head with more specificity and in-depth coding. Upper face (e.g., brow movements), lower face (e.g., nasolabial crease), mouth area (e.g., lip stretch), additional movements such as yawning and tongue show, eye blinks and facial self-touches form the coding scheme of the FOMS.

Previous research assessing fetal facial movements have described such movements in the most simplistic terms, for example ‘smile’. However, the FOMS took a more fine-grained approach breaking down what was previously referred to as a ‘smile’

(AboEllail & Hata, 2017) to a ‘lip pull’ or ‘lip stretch’ which involves different facial muscles and removes emotional attribution, relating to the anatomy of facial muscles (Reissland et al., 2016). Due to the anatomical nature of such facial movement coding scheme, it allows for more objectivity (Reissland, et al., 2015). It is argued that the FOMS is a more sensitive measure in comparison to assessment tools such as the KANET as it focuses on more fine-grained movements opposed to overall body movements and therefore is thought to have better clinical potential (Reissland, et al., 2015).

Mouth movements

Outlined below are images of each individual fetal mouth movement as defined by the FOMS, see figures 3.1 to 3.11. The images on the left show the neutral face and the image on the right show a specific mouth movement of that same fetus for comparison. There are eleven different mouth movements. All images were taken from the research of this thesis. Coding fetal facial movements using the FOMS requires frame by frame analysis of fine-grained movements using the Observer XT software. Before coding can begin, it is important to identify the neutral fetal face as this allows relative judgements to be made on whether a specific mouth movement has occurred or not, with each fetal neutral face differing from another (Reissland & Kisilevsky, 2016).

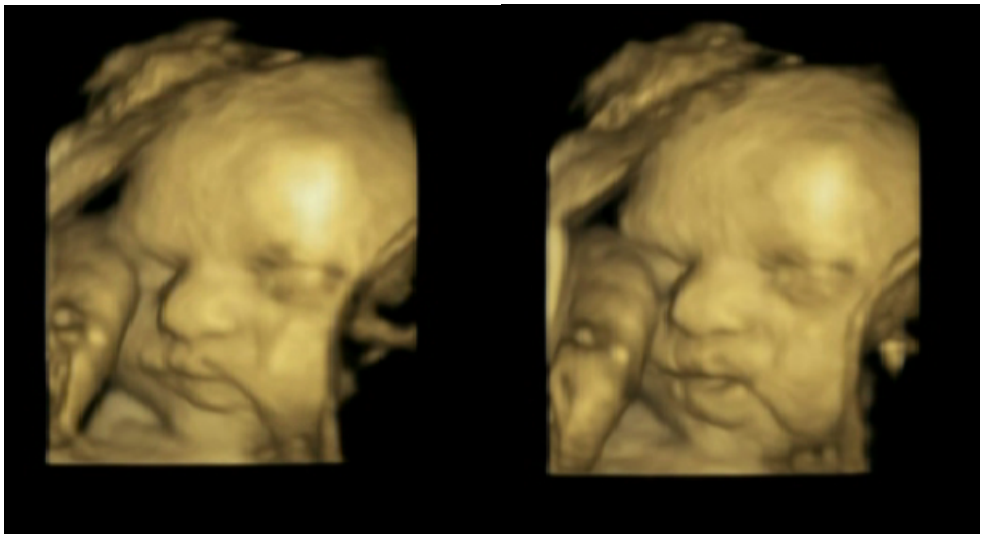

Figure 3.1. Lips parting (FM25). The lips can be seen parting, prior to the jaw dropping. The degree to what is considered as lips parting is not specified.

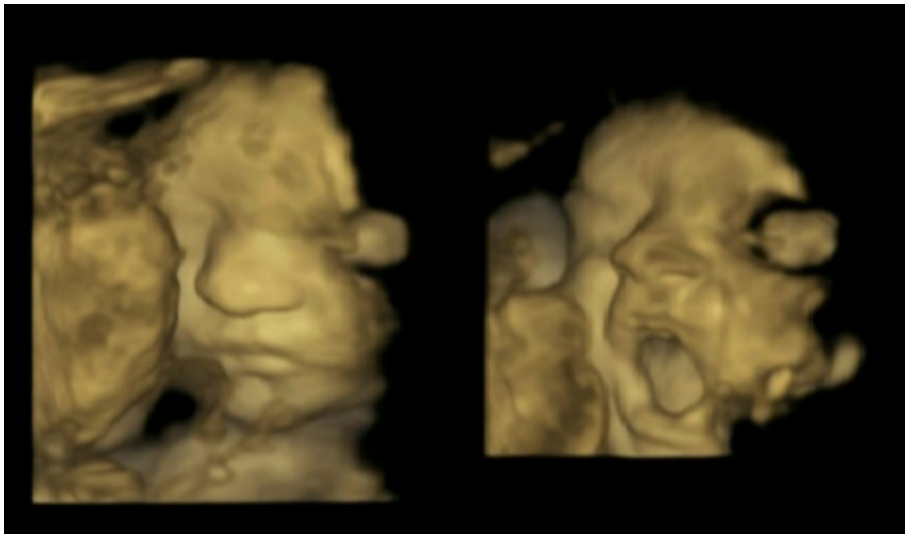

Figure 3.2. Mouth stretch (FM27). Mouth stretches differ to lips parting, as the jaw can be seen to drop. Here the mouth is open with the cheeks appearing stretched.

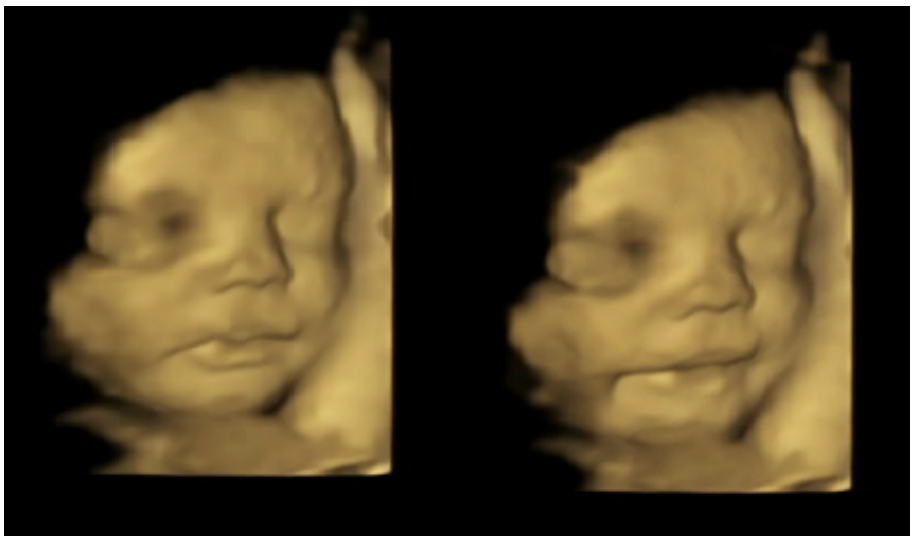

Figure 3.3. Lip stretch (FM20). The lips are stretched and elongated, here the lips also appear to be thinner.

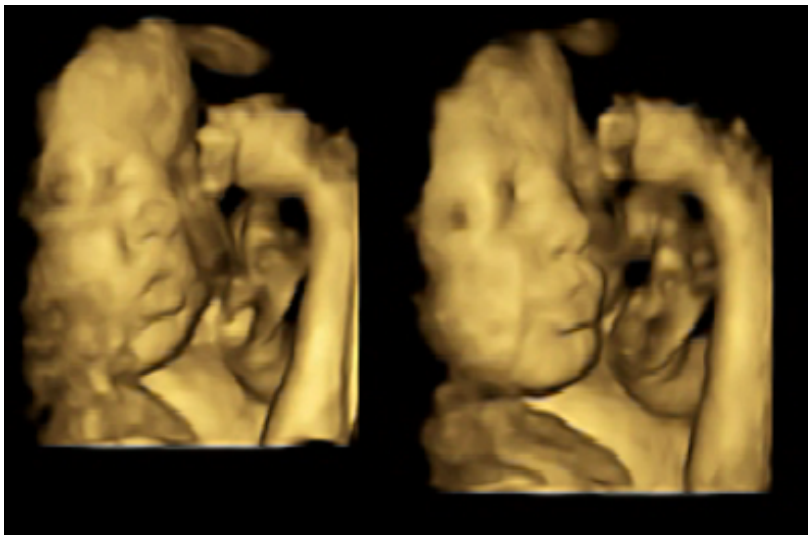

Figure 3.4. Lip pucker (FM18). This movement is where the lips become pursed and appear to be protruding forward. The skin on the lips will become creased and there is often bulging on the chin area. With increasing gestation, the lips often appear fuller, therefore it is important that this movement is not over coded.

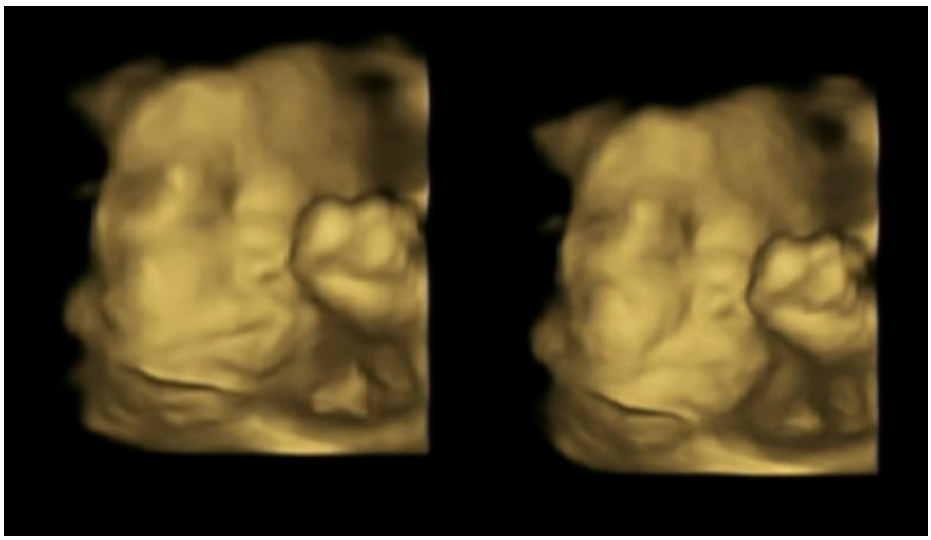

Figure 3.5. Lip pressor (FM24). This motion is characterised as the lips becoming narrower as they press down on one another and appear to look tighter.

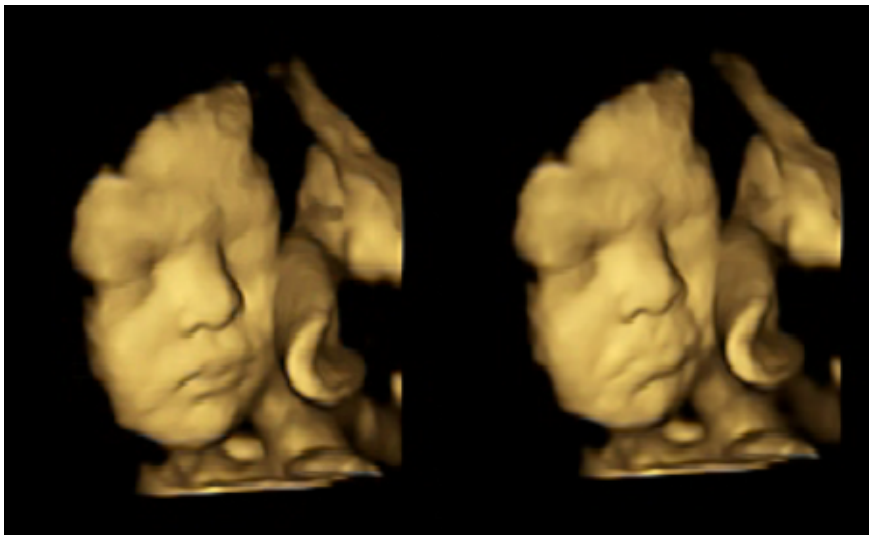

Figure 3.6. Lower lip depressor (FM16). A lower lip depressor is distinguishable from a lip corner depressor as the bottom lip as a whole will be pulled down opposed to just the corners of the lips.

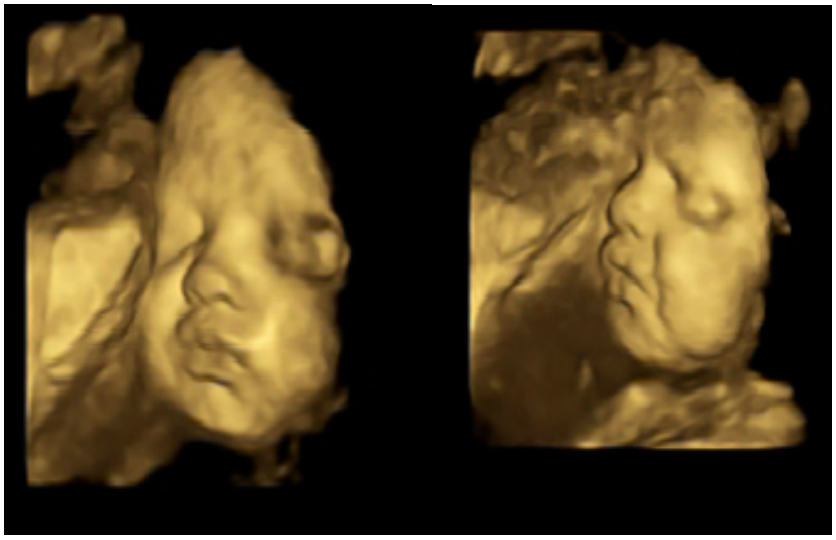

Figure 3.7. Lip corner depressor (FM15). Corners of the lips appear to be pulled down.

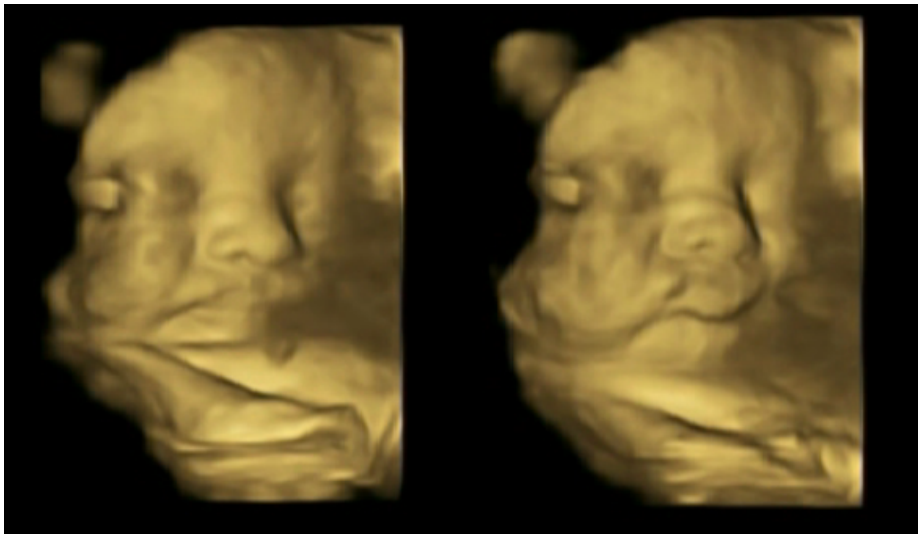

Figure 3.8. Upper lip raiser (FM10). The top portion of the lip is lifted up toward the nose, often on an angle.

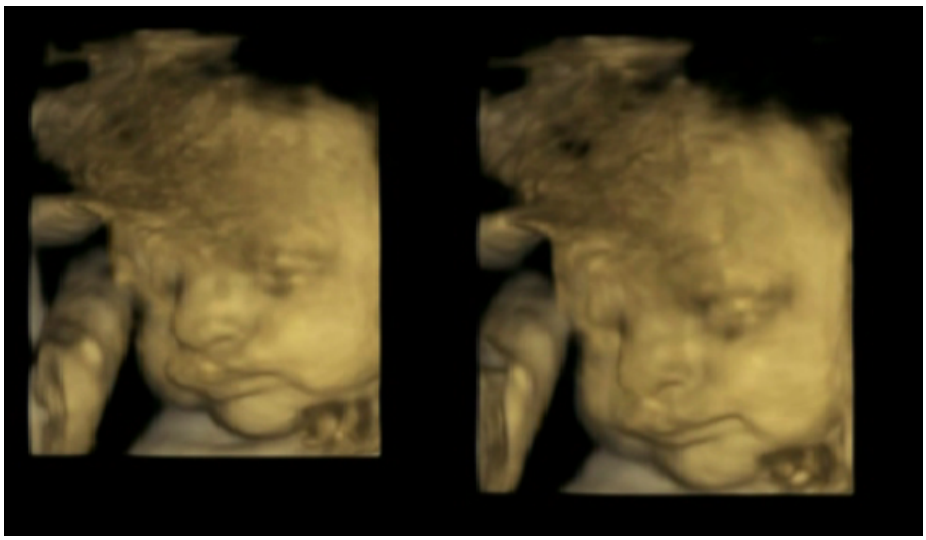

Figure 3.9. Lip pull (FM12). Similar to a lip stretch and can often be confused, however, in this movement the corners of the lips appear in a more upward movement, similar to what postnatally we would consider a smile. A good indicator to distinguish between lip stretch and lip pull is the bulging that appears in the check area as the lips are pulled upward in the direction toward the eyes.

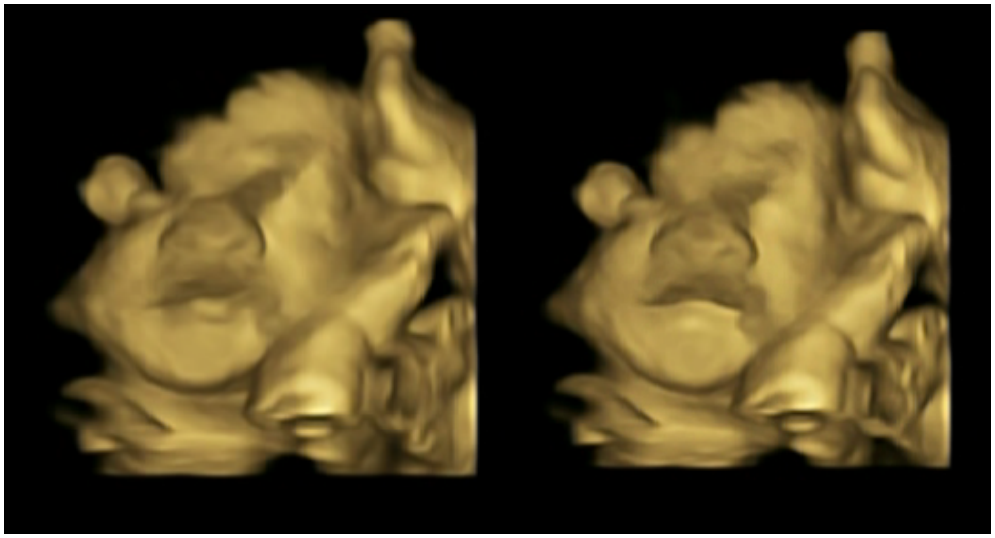

Figure 3.10. Lip suck (FM28). A lip suck is where the bottom, top or both lips are pulled in toward the mouth, the skin on the chin or top area of mouth will appear stretched.

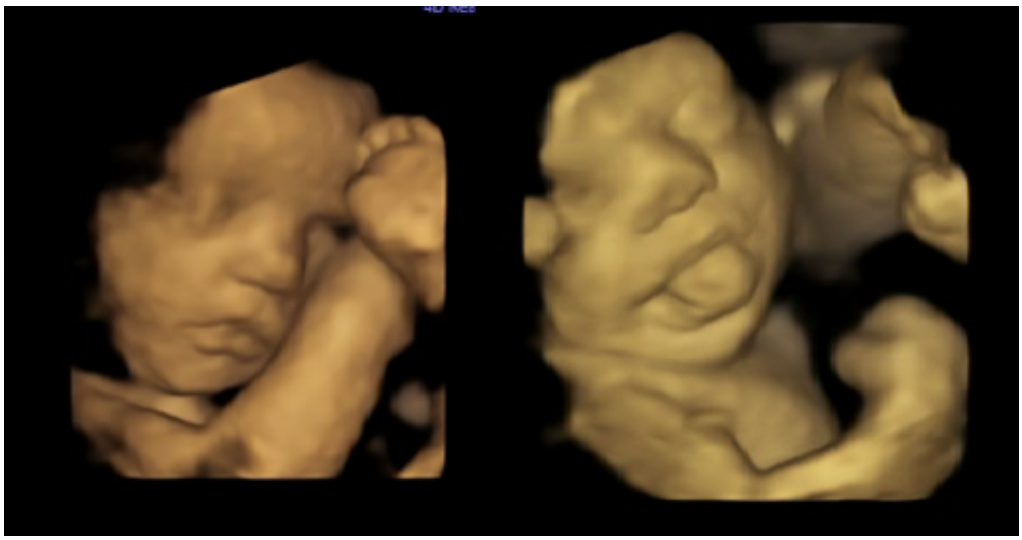

Figure 3.11.Tongue show. The tongue protrudes out of the mouth.

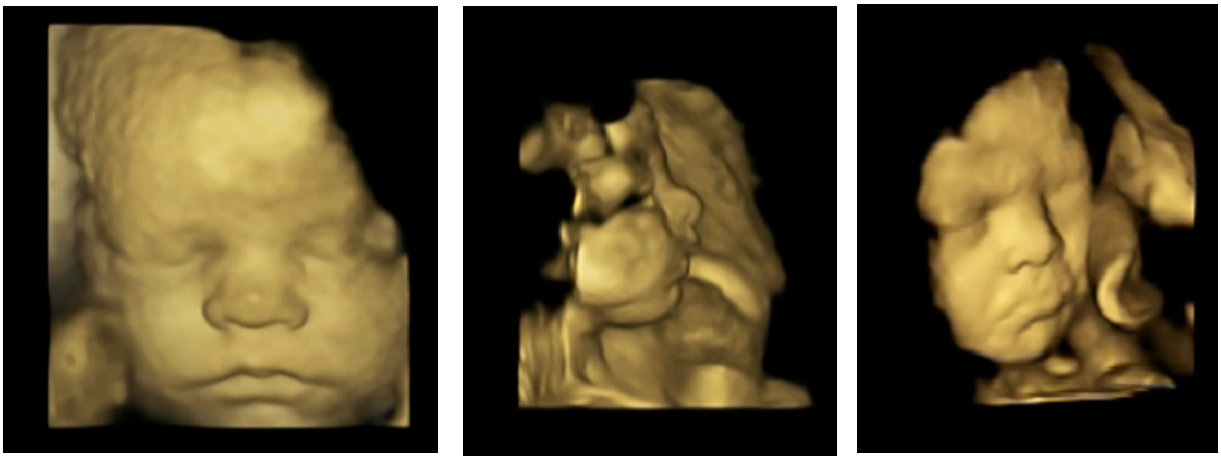

Figure 3.12. Acceptable images for coding.

In order for a fetal 4D scan to be considered acceptable for coding, at least half of the lips (both upper and lower lip) needs to be in clear view. Generally, if half of the lip and cheek area can be seen with good quality pixels, it is considered codable. Examples of acceptable scan images are shown in figure 3.12.

All 4D ultrasound scans lasted approximately 20 minutes, however during this time the mouth and lip area were not always visible. Therefore, stop and start codes are important in order to make a judgment about the relative frequency of movements. Coding can only be carried out when the mouth is visible. Figure 3.13 highlights examples of when coding has to be stopped due to clarity of the images. Should obstruction occur either by cord, placenta, limb or poor-quality image, coding is suspended until a clear view appears again. Coding is also stopped when the sonographer pauses the screen in order for the woman to move position for comfort, to try and get a clearer view of the fetus or when a picture was being taken for the mother to take home. Similarly, coding was also stopped when only 2D images were viewed, which was in order for the sonographer to refocus on the face to get a clear picture in 4D.

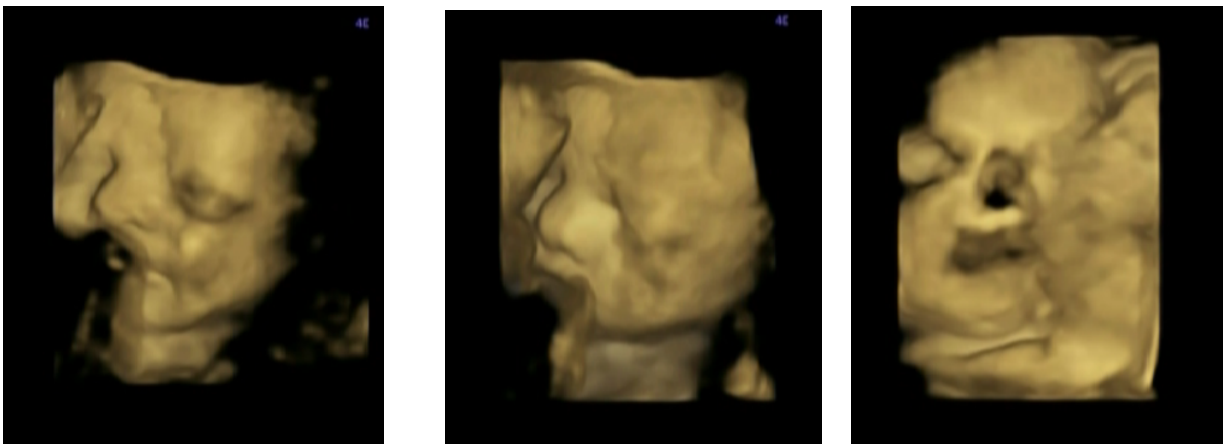

Figure 3.13. Unacceptable images for coding.

Occasionally, limbs could be seen on the fetal face, such as the hands. Coding was only stopped when the limb was covering the mouth area. However, these movements were not common enough in the current data set to be reliably coded for touch or hand position. In previous research, coding of self-touches has been conducted, however due to the quality, frequency and zoomed in approach to the fetal face, it

was not possible with the current data set. Examples of when the limbs were in front of the fetal face are shown in figure 3.14.

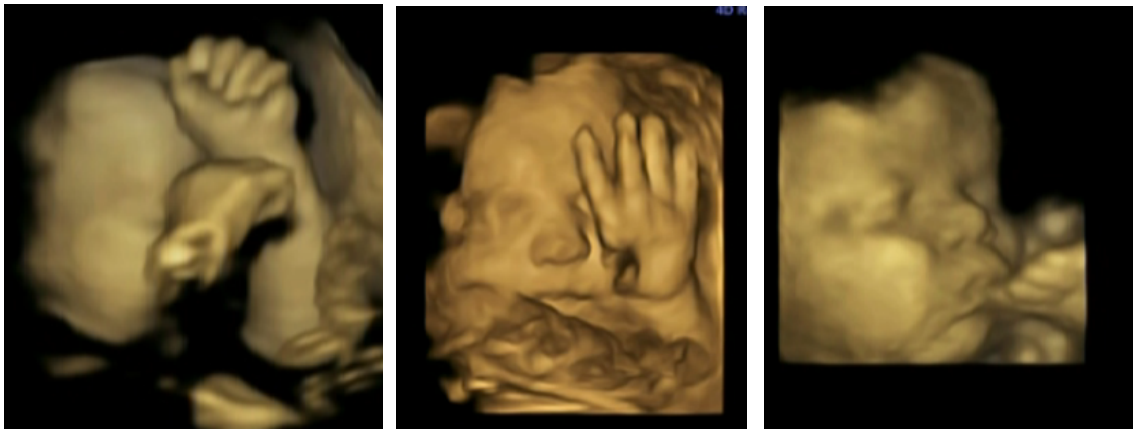

Figure 3.14. Fetal limbs, including the arms, hand and foot.

Overview of research using the FOMS

The FOMS has been used in a number of studies, some utilising the whole movement system, and others just focusing on the mouth movements. The FOMS has been used to assess fetuses according to several different conditions. For example, where the mother or fetus has a medical condition (Reissland, Makhmud, & Froggatt, 2019; Reissland, et al., 2020a), to assess the effect of light, sound or face-like stimulation (Reissland, Wood, Einbeck, & Lane, 2020b) and linked to exposures, such as prenatal cigarette exposure (Reissland, Francis, Kumarendran, & Mason, 2015). Prior to the development of the FOMS, research was conducted to assess the development of facial movements (Reissland, Francis, & Mason, 2012, 2013; Reissland, Francis, Mason, & Lincoln, 2011). From 24 to 35 weeks gestational age, unrelated mouth movements in the earlier gestations changed to recognisable gestalts at the later

gestational weeks, such as the ‘cry face gestalt’ and the ‘laughter gestalt’. Co-occurrence of 3 or more movements to create the cry gestalt increased from 0% to 42% and for laughter gestalt there was an increase from 0% to 35% (Reissland et al., 2011). Research using the FOMS has been carried out where all of the facial movements are coded. For example, findings indicate that there is a significant increase in complexity of facial movements from 24 to 36 weeks gestational age, with a recognisable ‘pain/distress’ gestalt observable (Reissland et al., 2013). Assessing mouth openings alone, research indicates that yawning can be distinguished from other mouth opening movements (Reissland et al., 2012). When analysing the timings of mouth movements and facial touches, there was an 8% increase in mouth opening before fetal self-touch to the face, increasing by 8% per gestational week, with a decrease in reactive mouth opening by 3% per additional gestational week (Reissland, Francis, Aydin, Mason, & Schaal, 2014).

Following a postnatal diagnosis of Prada Willi Syndrome (PWS), differences in fetal scans were noted. Despite a healthy and uncomplicated 20-week medical anomaly scan, a male fetus was recruited as part of a research study assessing fetal movements at 32- and 36-weeks gestational age in relation to reactions to light and sound stimulation of both male and female fetuses. This was the first study to outline a fetal behavioural profile of an infant with PWS, with findings indicating that there were significantly fewer mouth movements in comparison to a control group, this study focused on mouth movement alone (Reissland et al., 2019). This study highlights the potential medical benefit of conducting 4D ultrasound scans and coding the mouth movements using the FOMS.

Focusing on maternal medical conditions, Hyperemesis Gravidarum (HG) is a severe form of nausea and sickness during pregnancy which leads to a reduction in body weight of more than 5%, a debilitating condition which reduces nutritional intake and leads to dehydration (Fejzo et al., 2016). 4D ultrasound scans took place at 32- and 36-weeks gestational age, and mothers experiencing HG, their fetuses had an increased level of mouth movement in comparison to non-diagnosed women, again this study focused on the mouth movement of the FOMS (Reissland, et al., 2020a). This increase in movement at 32 weeks for the HG groups, then lead to a decline of movement levels below that of the non-diagnosed mothers' fetuses at 36 weeks gestational age.

The most relevant study to this thesis, is the pilot study conducted by Reissland et al. (2015) assessing fetal mouth movements and facial touch in relation to maternal cigarette smoking. Results indicated that, when controlling for maternal stress and depression, fetuses exposed to cigarette smoke had an increase in fetal mouth movements from 30 weeks gestational age, with differences across the groups widening with an increase in gestational weeks (Reissland, et al., 2015). Additionally, stress had a significant effect on mouth movements, for every ten unit increase in stress score there was a 9% increase in fetal mouth movements.

Fetal behaviour

With the advancement of 3D and 4D ultrasound techniques, it has led to a progression in perinatal medicine and research, as we are able to assess the anatomy and activity of the fetus in utero. This is an important development as it has been suggested that

by analysing the patterns of behaviour displayed by the fetus can provide an insight into the brain development and function of the CNS (Antsaklis et al., 2013).

Behaviour occurs as a direct impact of the CNS function (Hata, Kanenishi, Akiyama, Tanaka, & Kimura, 2005; Morokuma et al., 2007; Nijhuis, 2016). Identifying and observing fetal movements and behaviour is important as it is thought to correspond to both the CNS functioning and brain development (Koyanagi et al., 1993; Morokuma et al., 2013). It seems that abnormal fetal behaviour reflects abnormal CNS function and normal behavioural patterns reflect intact CNS functioning (Koyanagi et al., 1993). Fetal movement can be described as fetal behavioural patterns that are used as a proxy for understanding the development of the CNS prenatally and indicate potential brain development impairment (Andonotopo, Stanojevic, Kurjak, Azumendi, & Carrera, 2004; Lebit & Vladareanu, 2011).

Using a brief ultrasound examination, researchers have been able to identify normal and abnormal development of the fetus. The brief ultrasound examination includes five different measures including movement of extremities (one or more periods of movement of the limbs), breathing movements, periods of eye movement and no eye movement, rapid and slow eye movement patterns and concurrence of no eye movement coupled with mouthing movements. Of 29 fetuses prospectively examined, 96.6% were accurately identified as having normal or typical CNS function, with five retrospective ultrasound examinations of fetuses later known to have CNS abnormalities, the brief ultrasound examination was able to accurately identify 80% of fetuses with abnormal CNS function (Morokuma et al., 2007).

Conclusion

It has been well established that through the advance of ultrasound technology, it is possible to identify fetal behavioural patterns, both focusing on the whole body and by using a fine-grained method of analysis focusing on the fetal face. Studies have demonstrated that assessing mouth movements alone is sensitive enough to identify differences in fetuses dependent upon a number of conditions and exposures. However, what is not clear is the association between fetal behavioural patterns prenatally and the postnatal outcomes. Few studies have demonstrated links between overall fetal body movement and postnatal outcomes (Stroud, McCallum, & Salisbury, 2018), but to date such research has not been conducted with the use of the FOMS. Given the increasing number of studies using the FOMS, and in particular focusing on the mouth movements alone, it is important to assess what the postnatal implications between these differences in fetal behavioural profiles mean. Chapter 7, the pre-to-postnatal study, will attempt to address this issue by examining the relationship between fetal mouth movements identified by the FOMS and scores on the NBAS assessment.

Chapter 4

The effect of pregnant women's smoking status and e-cigarette use on fetal mouth movements

This research study is published in accordance with the guidance outlined for the journal Acta Paediatrica. Formatting, references, table and figure numbers have been changed to allow for consistency throughout the thesis.

The individual mouth movement analysis presented in this chapter was not submitted for review.

Abstract

Aim: To assess whether fetal mouth movement frequency changes across gestation and whether there are differences between cigarette and e-cigarette exposure conditions in comparison to a non-exposed group of fetuses.

Method: Pregnant women underwent 4-dimensional (4D) fetal ultrasound scans at 32-weeks (106 scans) and 36-weeks gestational age (87 scans) at James Cook University Hospital, UK. The 4D scans were coded using 11 mouth movements outlined in the Fetal Observable Movement System (FOMS). Measures of maternal smoking status, stress, depression, anxiety, attachment and time of scan were also collected. The pregnant women were part of one of four exposure groups: non-smokers, light smokers (<10 per day), heavy smokers (11-20 per day), or e-cigarette users.

Results: There were no significant differences in relative frequency or clusters of mouth movement between the exposure groups at 32- and 36-weeks gestational age.

Fetal mouth movements declined from 32 to 36 weeks gestation for non-exposed and e-cigarette exposed fetuses.

Conclusion: Due to variability in fetal behaviour, examining mouth movements alone may not be the most appropriate method for assessing group differences. However, in line with other research, mouth movement frequency did decline between 32- and 36-weeks gestational age. A combination of fetal behavioural assessments is needed to assess the effects of both cigarette and e-cigarette exposure on fetal neurobehavioural development.

Introduction

Rates of smoking at time of delivery (SATOD) remain relativity high in England (9.8%) with areas within the North East of England surpassing this rate (NHS Tees valley CCG 15.7%) above the national aim of 6% (NHS Digital, 2020). Pregnancy outcomes, including preterm birth, miscarriages and perinatal death, along with infant behavioural outcomes are known to be significantly associated to prenatal exposure to cigarettes and e-cigarettes (Cnattingius, 2004; Froggatt, Covey, & Reissland, 2020a; Froggatt, Reissland, & Covey, 2020b)[2]. Over the past couple of decades, research has focused on fetal neurobehaviour to provide insight into how cigarette exposure can affect the behaviour of the fetus (Habek, 2007; Reissland, Francis, Kumarendran, & Mason, 2015; Stroud, McCallum, & Salisbury, 2018).

Studies assessing fetal behaviour have examined a range of outcome measures including electrocardiograms (ECG), actocardiograms, 2-dimensional (2D) and 4-

[2] These papers are reported in Chapters 5 and 6 of this thesis.

dimensional (4D) imaging in order to assess facial movements, self-touches, brisk (strong, vigorous and purposeful) and sluggish (slow, idle and without purpose) body movements, isolated movements, breathing and heart rate variability (e.g., Habek, 2007; Peterfi, Kellenyi, Peterfi, & Szilagyi, 2019; Reissland, et al., 2015; Stroud et al., 2018). Research analysing the effects of smoking have reported increases in fetal heart rate whilst the mother smokes and in the short term thereafter; in contrast to maternal heart rate that remained stable across this time (Péterfi, Kellényi, Péterfi, & Szilágyi, 2019). The authors concluded that the increase in fetal heart rate in comparison to maternal heart rate demonstrated fetal distress as a result of current cigarette smoke exposure. However, generally, maternal smoking leads to decreases in fetal heart rate reactivity, in comparison to non-exposed fetuses (Oncken, Kranzler, O'Malley, Gendreau, & Campbell, 2002; Zeskind & Gingras, 2006).

As well as assessing fetal heart rate, researchers have examined the effects of maternal smoking on specific types of fetal movements. Studies assessing gross body movements have indicated that in comparison to non-smokers, fetuses exposed to cigarettes (regardless of number of cigarettes smoked per day) demonstrate an increase in body and isolated movements when assessed via 2D ultrasound (Stroud et al., 2018). The authors argued that differences in central nervous system (CNS) maturation led to different patterns of fetal movement, with an increase in isolated movements associated with an inability of the fetus to access the full range of co-ordinated patterns of movement. In contrast, when assessing quality and quantity of global fetal movements, spontaneous isolated head, arm and leg movements and fetal heart rate reactivity, comparing non-exposed, light exposed (<10 per day) and heavy exposed (11-20 cigarettes per day), the only significantly different group was the

heavy exposed fetuses. These fetuses demonstrated a decrease in movements that were sluggish in comparison to the other two groups where movement was brisk (Habek, 2007).

Additionally, research has been carried out using 4D ultrasound imaging focused on fine-grained mouth movements (Reissland, et al., 2015) using the Fetal Observable Movement System (FOMS) (Reissland, Francis, & Buttanshaw, 2016). An advantage of using a coding scheme focusing on fine-grained movements is that it may be more sensitive in differentiating fetuses in comparison to assessing gross body movement (Reissland, et al., 2015). One study based on a small sample by Reissland et al. (2015) indicated that fetuses exposed to maternal smoking (N= 4) had an overall higher rate of mouth movements and self-touches in comparison to non-exposed fetuses (N=16) (Reissland, et al., 2015). They suggested that the fetal CNS was affected as a consequence of maternal smoking during pregnancy resulting in differences in mouth movements between the exposure groups (Reissland, et al., 2015). As outlined above, the evidence is contradictory for the effects of maternal smoking on fetal movements, possibly owing to the differences in methodology (i.e., number of cigarettes smoked, 2D and 4D ultrasound scans, gross body movements and facial movements). To date, there have been no direct replications of such findings to provide further support. Hence, in the present study, the same methodology will be used for examining fetal facial movements as outlined by Reissland et al. (2015).

Mouth movements can provide an indication of the CNS development in the fetus, with the potential to identify normal and abnormal development in utero (Reissland &

Kisilevsky, 2016). In attempts to begin to examine this, research has identified that when the mother experiences extreme sickness and lack of nutrition in her pregnancy, these fetuses had significantly higher rates of mouth movements as identified by the FOMS at 32 weeks gestation in comparison to non-affected fetuses (Reissland et al., 2020a). Similarly for genetic disorders such as Prader-Willi Syndrome, a postnatally diagnosed fetus displayed significantly fewer mouth movements in comparison to a control group of healthy fetuses (Reissland, Makhmud, & Froggatt, 2019). Due to these fetuses displaying different patterns of behaviour in comparison to healthy controls, it could be argued that maternal health status and fetal genetic disorders can affect the development and function of the CNS differently. This may also explain the contradicting findings with the CNS being differently affected dependent upon amount of cigarette exposure the fetus is exposed too.

When assessing overall fetal activity (N=65) of a cross sectional sample between 24 to 37 weeks gestational age, fetuses exposed to maternal smoking (N=21) showed a higher rate of movement (frequency of head, limb and trunk movements) at 24 weeks which decreased below that of those non-exposed fetuses by 37 weeks (Stroud, Bublitz, Crespo, Lester, & Salisbury, 2020). This is also true for complex body movements, defined as head, trunk or limb movements occurring simultaneously (Stroud et al., 2020). A similar pattern of behaviour was also found in Reissland et al. (2015), with both frequency of mouth movements and self-touches declining from 24 to 36 weeks gestational age, at a rate of 1.5% per additional gestational week for smoke exposed fetuses and 3% for non-exposed fetuses. The decline in movement across gestation is thought to be reflective of CNS maturation, as movements become more co-ordinated and refined (Grant-Beuttler et al., 2011). Furthermore, the

research by Reissland et al. (2015) indcated that stress had an impact on fetal movement patterns, with each additional unit increase in stress score leading to a 1% increase in mouth movements and 2.8% increase in self-touch. Further, as depression scores increased, the level of fetal mouth movements decreased (Reissland, et al., 2015). Given these well documented effects of maternal mental health, the current study will also assess stress, depression and anxiety in relation to fetal mouth movement frequency and clusters of mouth movements.

New to this study is the effects of e-cigarettes on fetal behaviour, specifically mouth movements. The effects could be very different from smoking cigarettes especially in light of previous research attributing the effects of smoking on fetal activity to carbon monoxide (CO) exposure due to placenta insufficiency as a result of a reduction in oxygen (Habek, 2007; Zeskind & Gingras, 2006). However, this line of argument is omitted from Reissland et al. (2015) and Stroud et al. (2018) with very little discussion on specifically why cigarette exposed fetuses may have an increase in movement, instead drawing on supporting evidence derived from neonatal studies indicating increases in arousal and activity (e.g., Law et al., 2003). A meta-analysis examining a number of risk factors associated with reduced fetal movements identified that, based on five studies involving 29,557 participants, smoking during pregnancy leads to a reduction in the oxygen carrying capacity of blood and thus leading to higher CO levels, potentially resulting in a reduction of fetal movements (Carroll, Gallagher, & Smith, 2019). Although in this meta-analysis, the studies assessed maternal self-reporting of reduced fetal movement, the authors of the paper suggested that the reduction in movements were a result of cigarette exposure. Indeed, assessing a range of factors associated with reduced fetal movement found

that smoking in general was a risk factor (Carroll et al., 2019). Therefore, due to the known effects of CO exposure on fetal movements and the results outlined by Habek (2007), we anticipate a difference between light and heavy cigarette exposed fetuses, as amount of CO exposure may impact fetal behaviour, and thus the CNS, differently.

Nicotine on the other hand which is the primary toxic ingredient in e-cigarettes is a known psychomotor stimulant, which has the effect of increasing attention, alertness and behavioural excitement in human adults (Hsia, Mischel, & Brody, 2020; Singer, Min, Lang, & Minnes, 2016). Animal studies have found that when exposed to nicotine alone, there is an increase in spontaneous locomotion activity in rats, which is thought to be the result of the nicotine directly affecting the nicotine acetylcholine receptors (nAChRs) in the brain (Javadi-Paydar, Kerr, Harvey, Cole, & Taffe, 2019; Wang, Wan, Huang, & Clarke, 2020). Therefore, given the stimulating effects of nicotine, we predict that there will be an increase in frequency of fetal mouth movements when exposed to e-cigarettes. Although cigarettes also contain nicotine, the addition of CO may suppress the effects of nicotine in a cigarette, leading to a different behavioural profile in comparison to e-cigarette exposed fetuses.

The current study builds upon Reissland et al. (2015) pilot study in order to assess a larger sample of fetuses, including two groups of cigarette exposed (light and heavy) and one group of e-cigarette exposed fetuses to compare to a control group of non-exposed fetuses. The first hypothesis is that we expect variations in fetal mouth movement profiles across the four exposure groups. We anticipate that there will be differences between the non-exposed fetuses and both cigarette exposure groups, in addition a difference between light and heavy cigarette exposed fetuses. Due to the

stimulating effects of nicotine, we expect e-cigarette exposed fetuses to display higher levels of mouth movements in comparison to all other groups. For our second hypothesis, with increasing fetal age, the CNS development becomes more coordinated and precise movements can be observed, hence we also expect that mouth movement frequencies will differ at 32- and 36-weeks gestation.

Method

Participants

The fetal scans for this research were undertaken at James Cook University Hospital, Middlesbrough and the Friarage Hospital, Northallerton, UK. 123 pregnant women were recruited to participate in the study assessing the impact of smoking status on fetal mouth movements. Potential participants who met the inclusion criteria were identified by the hospital sonographers at their 20-week anomaly scan. The inclusion criteria consisted of currently not taking any medication or recreational drugs for a medical or mental health condition, not diagnosed with a medical problem that may affect the fetus, low risk pregnancy, BMI between 18-25 and aged between 18-40 years old.

Pregnant women provided informed consent prior to participating in the research. Ethical approval was granted by Durham University and the NHS ethics committee (REC reference, 11/NE/0361).

Table 4.1. Number of scans analysed per smoking condition.

Smoking status	**Recruited**	**Scans coded at 32 weeks**	**Scans coded at 36 weeks**
Non-smokers	54	46	34
Light cigarette smokers (<10 per day)	38	32[3]	27
Heavy cigarette smokers (11-20 per day)	15	13	12
E-cigarette users[4]	16	15	14
Total	123	106	87

The number of women recruited in each smoking status group and scans coded at 32 and 36 weeks are shown in Table 4.1. Although we were able to recruit 123 women into the study, not all scans could be coded and analysed due to a variety of reasons. At 32 weeks, some scans were not analysed due to the fetal mouth areas not visible (N=16) or due to technical difficulties with the recording of the scan (N=1). At 36 weeks, additional to the factors mentioned above (N=25), some women dropped out of the research (N=9) or had already given birth (N=2). A priori power calculations indicated a required sample of 196, therefore the present sample size was not quite at the desired threshold. However, based on the data at 32 weeks, the smallest effect size the achieved sample was powered to detect (80%) was d=.646, and d=.720 at 36 weeks.

[3] The number of scans analysed at 32 weeks differs by 1 participant between this paper and the pre-registration report, as further examination identified one of the scans was not of good enough quality.

Mothers attended a 30-minute 4D ultrasound appointment with an NHS qualified sonographer at James Cook University Hospital or the Friarage Hospital. The scan lasted approximately 15-20 minutes and time of day the scan took place was recorded. During this appointment all mothers regardless of exposure group were asked to do a smokerlyser breath test using the Bedfont smokerlyser piCObaby^{TM} to obtain a CO reading for both mother and fetus. This was used to assess level of CO at the time of the scan. If using an e-cigarette, Milligrams of nicotine were identified via maternal self-report, ranging from 3-16mg (M=7.76mg, S. D. =4.762).

Due to the known associations between maternal psychological state and fetal movement (Kinsella & Monk, 2009; Reissland, et al., 2015; Reissland, Froggatt, Reames, & Girkin, 2018), measures of stress (Perceived Stress Scale (PSS) (Cohen, Kamarck, & Mermelstein, 1983)), anxiety, depression (Hospital Anxiety and Depression Scale (HADS) (Zigmond & Snaith, 1983)) and attachment (Antenatal Attachment Scale (Condon & Corkindale, 1998)) were obtained. Additionally, mothers completed a smoking questionnaire indicating the number of cigarettes smoked per day, whether they had quit smoking and whether they use nicotine replacement therapy or e-cigarettes. The scans took place at 32- and 36-weeks gestational age.

There is limited observational research assessing the effects of time of day on fetal behaviour and activity, with research indicating that fetal heart rate variability is not affected (Lange, Van Leeuwen, Geue, Hatzmann, & Grönemeyer, 2005). Most research assessing the effects of time of day on fetal activity focuses on maternal perceptions of movements. According to such research, there is an increase in

awareness of fetal movements from afternoon (12-6pm) to evening (6-8pm) and night-time (8-midnight) (Raynes-Greenow, Gordon, Li, & Hyett, 2013). With an increase thought to be important due to an association between lack of evening fetal movements and rate of still birth (Bradford & Maude, 2018). However, there are a number of factors that may influence the perception of increased awareness of fetal movements in the evening such as maternal positioning and relaxation (Minors & Waterhouse, 1979), hence the importance of including an objective measure of fetal movement in relation to time of day in the present study.

The 4D ultrasound scans were coded frame by frame offline using the Observer XT. The method for coding was the Fetal Observable Movement System (FOMS) which assesses fetal facial muscles to identify a variety of different mouth movements (Reissland et al., 2016). The only facial movements coded were mouth movements, as was the case in Reissland et al. (2015). Reliability of coding was assessed on approximately 10% of the scans by an independent coder, blind to the study conditions. Based on 20 scans, mean Cohen's Kappa the mean was .86, and ranged between .75-.98. Mean re-test reliability was .97 and ranged between .92-1, indicating high reliability.

Data analysis

A pre-registration plan was submitted to the Open Science Framework (OSF) (https://mfr.osf.io/render?url=https://osf.io/xn768/?direct%26mode=render%26action=download%26mode=render) outlining our hypotheses, a priori predictions and data analysis plan. We hypothesised that there will be differences in the frequency fetal mouth movements across the four exposure groups. We also expected that there will

be a difference in frequency of fetal mouth movements between the 32- and 36-weeks gestational data.

Three different outcome measures were assessed. The total relative frequency of fetal mouth movements per minute, individual mouth movements and clusters of movements. There are 11 different mouth movements that were coded using the FOMS including; lip corner depressor, lip pressor, lip pucker, lip pull, lip stretch, lip suck, lower lip depressor, upper lip raiser, lips parting, mouth stretch and tongue show. Each of these relative frequencies of mouth movements were assessed in relation to exposure group. Cluster of mouth movements refers to bursts of individual mouth movements that occur immediately one after another (see method chapter).

As stated in the OSF plan, we planned to run a correlation between the 32- and 36-week gestational age data, and if the data were correlated, only one ANOVA would be conducted on the 32-week data due to the larger sample. If there was not a significant correlation, two separate ANOVA tests would be conducted, one referring to movement at 32 weeks and one at 36 weeks gestational age to assess the first hypothesis. Should any potential confounding factors (stress, depression, anxiety, attachment and time of scan[5]) be significantly associated to the outcome measure, then an ANCOVA would be carried out. We outlined that a mixed model ANOVA would be conducted to assess our second hypothesis.

[5] Time of day the scan took place was not reported as a potential covariate in the OSF plan, however, due to this data being collected and the literature indicating a possible association, it was added at the analysis stage of conducting this research.

As the data did not meet the assumptions of ANOVA, including non-normal data and homoscedasticity, non-parametric tests were used, including the Kruskal-Wallis test and Wilcoxon paired tests. As the data did not meet the assumptions for an ANCOVA, the correlations will be reported. Significantly correlated variables were included into a regression analysis, with a subsequent Kruskal-Wallis test using the residuals. To correct for multiple comparisons the Benjamini-Hochberg false discovery rate procedure was applied (Benjamini & Hochberg, 1995).

Results

There are differences in the means across the exposure groups, see table 4.2. E-cigarette exposed fetuses had the highest frequency of mouth movements at 32 weeks (M=8.581), with heavily smoke exposed fetuses having the lowest rate of mouth movement (M=1.977), with similar levels of movement for both non-exposed (M=4.662) and light exposed fetuses (M=3.781). However, at 32 weeks there are variations in the standard deviations, with a particularly large standard deviation for the e-cigarette exposed fetuses (S.D.=10.074). At 36 weeks the means are similar between the non-exposed and light exposed fetuses, with the heavily exposed fetuses displaying greater mouth movements at this time point and the largest variation (M=4.291, S.D.= 4.762).

Table 4.2. Means and standard deviation of total relative frequency of mouth movement per minute, stress, depression, anxiety, attachment and maternal CO.

	Not exposed M(S.D.)	**Light exposed (<10 per day)** M(S.D.)	**Heavy exposed (11-20 per day)** M(S.D.)	**E-cigarette exposed (3-16mg)** M(S.D.)
		32 weeks gestational age		
Mouth movements	4.662 (4.149) N = 46	3.781 (4.221) N = 32	1.977 (.882) N = 13	8.581 (10.074) N = 15
Stress	9.37 (6.097)	13.06 (6.816)	14.92 (8.986)	16.60 (6.822)
Depression	2.83 (2.341)	5.06 (3.110)	5.85 (4.356)	4.07 (3.305)
Anxiety	4.49 (2.841)	5.59 (3.271)	7.31 (4.385)	6.33 (3.266)
Attachment	83.02 (6.140)	81.16 (6.427)	82.80 (7.857)	83.67 (3.551)
Maternal CO	.984 (.146)	2.400 (.934)	3.436 (1.056)	.960 (.175)
		36 weeks gestational age		
Mouth movements	2.671 (2.082) N = 34	2.834 (2.584) N = 27	4.291 (4.762) N = 12	3.327 (2.339) N = 14
Stress	8.76 (5.836)	12.19 (5.967)	13.75 (8.635)	12.64 (6.295)
Depression	3.35 (2.806)	4.92 (2.756)	5.42 (4.502)	3.00 (1.958)

Anxiety	4.41 (3.276)	5.46 (3.361)	7.50 (4.602)	4.62 (2.902)
Attachment	85.41 (5.088)	83.58 (6.947)	84.63 (9.380)	89.42 (2.811)
Maternal CO	.945 (.197)	2.334 (1.139)	3.018 (1.027)	.763 (.300)

Table 4.3. Correlations between relative frequency and potential covariates.

		Time of day	Stress	Anxiety	Depression	Attachment	Maternal CO
Relative frequency 32 weeks	**Correlation**	**-.213**	-.028	-.100	-.033	.069	-.183
	Significance	**.032***	.780	.307	.738	.514	.060
Relative frequency 36 weeks	**Correlation**	-.024	.090	.174	**.258**	-.107	.070
	Significance	.833	.411	.111	**.017***	.369	.534
Clusters 32 weeks	**Correlation**	-.095	.056	.033	-.065	.008	-.150
	Significance	.348	.571	.743	.510	.940	.129
Clusters 36 weeks	**Correlation**	.012	.064	**.216**	**.312**	-.061	.066
	Significance	.912	.556	**.047***	**.004***	.608	.559

* *Significant correlation.*

At 32 weeks gestation, there were no significant correlations between frequency of fetal mouth movement and stress, depression, anxiety, maternal CO or attachment (see table 4.3). However, there was a significant correlation with time of day the 4D scan took place. Fetuses displayed a higher level of mouth movement frequency earlier in the day, compared to later in the day. There were no significant correlations for clusters of mouth movement at 32 weeks. At 36 weeks gestation, there were significant correlations between frequency of fetal mouth movements and depression. As level of depression increases, so does mouth movements, a finding inconsistent with results reported by Reissland et al. (2015). Heavy smokers have the highest scores of depression (M=5.42, S.D.=4.502), followed by light smokers (M=4.92, S.D.=2.756), with non-smokers (M=3.35, S.D.=2.806) and e-cigarette users having similar levels (M=3, S.D.=1.958). There were also significant correlations between clusters of mouth movement and depression and anxiety at 36 weeks. Similar to levels of depression, heavy smokers scored the highest on measures of anxiety (M=7.50, S.D.=4.602), followed by light smokers (M=5.46, S.D.=3.361), with e-cigarette users (M=4.62, S.D.=2.902) and non-smokers (M=4.41, S.D.=3.276) experiencing similar levels. However, an ANCOVA to include these variables could not be conducted due to the data not meeting the required assumptions (e.g., normal distribution of data).

There was no significant correlation between 32- and 36-weeks data (frequency of mouth movement, r=-.092, p=.422; cluster of mouth movement, r=-.100, p=.384) and due to data not meeting the assumptions of an ANOVA, separate Kruskal-Wallis tests were conducted.

32-weeks gestation

A Kruskal-Wallis test was conducted to compare the fetal mouth movements of the four smoking groups (non-smokers, light smokers (<10), heavy smokers (11-20) and e-cigarette users), based on 106 4D ultrasound scans.

The Kruskal-Wallis test indicates a significant overall effect of exposure group when assessing frequency of mouth movements, $X^2(3)= .8125$, p= .043, d=.296. Adjusted pairwise comparisons using the Benjamini-Hochberg procedure indicates significant differences between heavy exposed fetuses (11-20 cigarettes per day; M=1.977, S.D.= 0.882) and e-cigarette exposed fetuses (M=8.581, S.D.= 10.074), p=.041, d= 0.890 (see table 4.4). In addition, there were no significant differences in total number of clusters of movements between the four smoking groups $X^2(2)= 3.884$, p= .274, d=.199.

As an ANCOVA could not be conducted, a regression analysis was performed with variables that were significantly correlated, and the residuals were used in a subsequent Kruskal-Wallis test. For relative frequency of mouth movements, when time of day was considered, there were no significant differences between the four groups $X^2(3)= 7.388$, p= .060, d= .433.

Pooling together results from both cigarette exposure groups fetuses[6], there is a significant effect when assessing frequency of mouth movement, $X^2(2)= 6.947$, p= .031, d= .401. Adjusting using the Benjamini-Hochberg procedure, there are no

[6] Although this analysis was not planned in the OSF plan, it was later decided to include a pooled cigarette exposure group analysis to examine whether once light and heavy smokers were combined, as is the case for Reissland et al. (2015) whether findings would be similar to those reported in the pilot study.

significant pairwise comparisons for non-exposed compared to both cigarette exposed (p=.166) or e-cigarette exposed (p=.25) or between cigarette and e-cigarette exposed fetuses (p=.083). Accounting for time of day the scan took place, there is no significant difference between the three groups ($X^2(2)= 4.462$, p= .107, d= .319). Assessing clusters of mouth movement combining the cigarette groups, there is no significant differences between the three groups, $X^2(2)= 4.425$, p= .109, d= .312.

Table 4.4. Pairwise comparisons

Group	Significance	Adjusted sig (Benjamini-Hochberg)	Effect size and variance
Non v. <10	.202	.166	d = 0.210 CI = -.663, .241 V = 0.053
Non v. 11-20	**.038***	.083	d = 0.724 CI = -1.353, -0.094 V = 0.103
Non v. e-cigarettes	.289	.250	d = 0.642 CI = 0.048, 1.236 V =0.091
<10 v. 11-20	.278	.208	d = 0.499 CI = -0.153, 1.152 V= 0.110
<10 v. e-cigarettes	.052	.125	d = 0.724 CI = -1.355, -0.094 V= 0.103
11-20 v. e-cigarettes	**.011***	**.041***	d = 0.890 CI= -1.668, -0.112 V= 0.157

** Significant correlation.*

36-weeks gestation

Kruskal-Wallis tests were used to compare the four smoking groups (non-smokers, light smokers (<10), heavy smokers (11-20) and e-cigarette users) at 36 weeks gestational age based on 86 4D ultrasound scans.

The Kruskal-Wallis test for 36 weeks gestational age based on frequency of mouth movement was not significant, $X^2(3)= 2.402$, p= .493, d= .154. Taking into account the significant correlation between relative frequency of mouth movements and depression, a regression was conducted and a subsequent Kruskal-Wallis based on the residuals, indicating that there are no significant differences between the four smoking groups, $X^2(3)= 2.066$, p= .559, d= .210. Examining clusters of movement, there are no significant differences between the four smoking groups $X^2(3)= 1.686$, p= .640, d= .245. Similarly, when accounting for anxiety and depression by running the analysis with the residuals, there are no significant differences $X^2(3)= 3.766$, p= .288, d= .197. Depression and anxiety are positively related to fetal mouth movement at 36 weeks gestation and the higher levels in the heavier smoking group, which could explain why their movements at this time point are not significantly lower, as seen at 32 weeks gestational age.

When pooling results from both cigarette exposure groups, there are no significant differences when assessing frequency of mouth movement, $X^2(2)= 1.023$, p= .600, d= .312. There is no significant differences between the three groups when accounting for depression, $X^2(2)= 1.644$, p= .440, d= .552. Assessing clusters of mouth movements, there are also no significant differences between the three groups, $X^2(2)= 1.454$, p= .483, d= .163. Similarly, when accounting for anxiety and depression, there are no significant differences between the three groups, $X^2(2)= 5.122$, p= .077, d= .393.

Wilcoxon tests on paired data

There are 79 sets of paired 32- and 36-week data. A Wilcoxon test was conducted to assess whether there were any significant differences in the relative frequency of fetal mouth movements per minute shown at 32- and 36-weeks gestational age. Fetuses displayed a greater number of mouth movements per minute at 32 weeks gestation (M=4.856, S.D.=5.893) compared to 36 weeks gestation (M=3.087, S.D.=2.872), Z= -2.360, p = .018, r=-.265, a finding which is supported by Reissland et al. (2015).

To assess the differences between the two scan time points within each group, Wilcoxon tests were conducted for the non-exposed group (Z=-2.225, p=.026, r=-.250, N=32), light exposed group (Z=-.971, p = .331, r=-.109, N=24), heavily exposed group (Z=-.866, p=.386, r=-.097, N=10) and the e-cigarette exposed group (Z=-1.852, p=.064, r=-.208, N=13). Results indicate significant differences for the non-exposed group with fetuses displaying a great number of mouth movements at 32 weeks gestation (M=5.061, S.D.=4.561) compared to 36 weeks gestation (M=2.797, S.D.=2.082). Borderline differences were observed for the e-cigarette exposed fetuses, with a higher number of mouth movements at 32 weeks (M=9.030, S.D.=10.671) in comparison to 36 weeks gestation (M=3.416, S.D.=2.410).

Time of day the scans took place did not significantly differ between the four exposure groups at either 32 weeks ($X^2(3)=1.280$, p=.734, d=.262) or 36 weeks gestational age ($X^2(3)=3.349$, p=.341, d=.131). Time of day the scan took place was not significantly different between 32- and 36-weeks gestational age, Z= -.147, p= .883, d=.033. Nor for the individual exposure groups; non-exposed Z= -.143, p= .886,

d= .032, light exposed Z= -.592, p= .554, d= .004, heavy exposed Z= -.771, p= .441, d = .008 and e-cigarette exposed Z= -.830, p= .407, d= .009 .

Assessing maternal mental health scores across the two time points, there were no significant differences for stress (Z=-1.790, p=.073, r=-.201, N= 79), depression (Z= -.620, p=.535, r=-.069, N= 79) or anxiety (Z= -.937, p=.349, r=.-105, N=79). However, there were significant differences for attachment between the two time points (Z= -5.401, p <.001, r= .607, N=79), with attachment increasing over time (32 weeks M= 81.99, S.D. =6.214; 36 weeks M=85.73, S.D.= 5.924).

Individual mouth movements

The most frequent mouth movement across exposure groups and gestation is lips parting, with lip corner depressor being the least frequent mouth movement. Results are shown in table 4.5.

Table 4.5. Means, standard deviations and significance values for individual mouth movements and total clusters of movement at 32- and 36-weeks gestational age, comparing exposure groups.

	32 Weeks Mean Standard deviation				**36 Weeks Mean Standard deviation**				**32 weeks significance**	**36 weeks significance**
	Non-exposed	*Light exposed*	*Heavy exposed*	*E-cigarette exposed*	*Non-exposed*	*Light exposed*	*Heavy exposed*	*E-cigarette exposed*		
Lip corner depressor	0.26 0.820	0.24 0.502	0.31 0.630	0.20 0.561	0.11 0.530	.0.08 0.277	0.08 0.277	0.07 0.267	.817	.655
Lip pressor	0.94 1.420	0.70 0.984	1.15 2.193	1.33 1.988	0.46 0.741	0.20 0.408	0.77 1.691	0.79 1.578	.768	.855
Lip pucker	1.28 1.930	0.85 1.121	0.92 1.935	2.27 3.474	0.89 1.255	0.32 0.748	1.23 2.204	0.64 0.745	.720	.568
Lip pull	3.83 4.720	3.42 3.708	3.46 3.230	4.07 5.063	2.57 3.137	3.08 3.741	2.92 4.051	3.21 2.778	.946	.114
Lip stretch	1.57 2.30	1.61 2.061	1.54 2.602	2.27 2.492	1.71 1.296	1.68 1.57	4.08 4.752	3.07 3.269	.459	.823
Lip suck	1.70 2.570	0.97 1.571	0.85 1.345	1.73 2.712	1.46 1.961	0.60 0.913	1.92 3.639	1.29 1.267	.528	.490
Lower lip depressor	0.36 0.735	0.30 0.951	0.46 0.519	0.27 0.458	0.37 0.690	0.24 0.663	0.46 1.391	0.43 0.938	.157	.199
Upper lip raiser	1.09 1.626	0.64 1.194	1.15 1.345	1.00 1.195	0.57 0.698	0.68 1.435	0.46 0.660	1.14 1.292	.353	.652
Lips parting	14.87 16.712	14.21 13.235	7.08 5.708	17.40 15.968	9.00 10.137	7.08 6.164	9.54 9.052	9.21 9.947	.326	.349
Mouth stretch	3.57 6.436	2.52 3.751	1.00 1.225	4.73 5.548	2.06 3.325	0.88 1.453	2.69 3.660	2.50 3.917	.254	.927

Tongue show	0.83 1.785	0.73 2.081	0.31 1.109	1.73 2.789	0.66 1.187	0.36 0.757	0.62 1.387	0.71 1.383	.179	.160
Relative frequency of clusters	0.003 0.003	0.003 0.002	0.002 0.003	0.005 0.004	0.005 0.005	0.006 0.011	0.012 0.017	0.008 0.007	.274	.640

Discussion

We expected different fetal mouth movement profiles across the four exposure groups, with movements overall declining from 32 to 36 weeks gestational age. Initially, the findings of this study suggest that there are overall differences in fetal mouth movements at 32 weeks gestation, as indicated by a significant difference in the pairwise comparison between heavy smoke exposed and e-cigarette exposed fetuses. Heavily exposed fetuses displayed significantly reduced frequency of mouth movements in comparison to e-cigarette exposed fetuses. However, when accounting for the time of day the scan took place, the overall result is borderline, with a medium effect size, and thus no further group differences were explored. No significant differences were found at 36-weeks gestational age, between individual mouth movements or clusters of movements, in line with previous research (Cowperthwaite, Hains, & Kisilevsky, 2007). In contrast to previously published research including Stroud et al. (2018) and Habek (2007), our research does not support the hypothesis that fetal mouth movement frequency and clusters of movement differ between the exposure groups. The findings support the hypothesis that total relative frequency of fetal mouth movements per minute differ between 32- and 36-weeks gestational age, with the overall rate declining. Specifically, the declining rates of mouth movement are evident for the non-exposed and borderline for e-cigarette exposed fetuses.

The aim of the research was to extend with a larger sample and differentiated exposure groups, the pilot study by Reissland et al. (2015). In contrast to Reissland et al. (2015) where non-exposed fetuses displayed a lower rate of mouth movement in comparison to smoke exposed fetuses, despite using the same method of coding

mouth movements as outlined by the FOMS. In this study we found that there were significant differences between the exposure groups, with pairwise comparisons indicating this difference was between the heavy exposed and e-cigarette exposed fetuses, with a borderline result between non-exposed and heavy exposed. Though, in contrast to Reissland et al. heavy exposed fetuses had reduced rates of mouth movements compared to non-exposed fetuses. However, once accounting for time of day the scan took place, this overall effect became borderline with a medium effect size. Time of day the scan took place was not considered in Reissland et al. (2015) as all scans took place early morning, but this may explain the difference in results. In contrast to prior research (Bradford & Maude, 2018; Raynes-Greenow et al., 2013), in the present study there is a negative correlation between frequency of fetal mouth movements and time of day the scan took place at 32 weeks gestational age. At present, it is unknown how fetal mouth movements map onto general movements the mother may perceive, therefore it is currently challenging to compare our results to that of other studies.

It is possible that CO and nicotine exposure may have differing effects on fetal behaviour. Research indicates that CO exposure decreases fetal activity (Oncken et al., 2002; Zeskind & Gingras, 2006) and in contrast, we anticipated an increase in fetal mouth movement profiles in the e-cigarette exposed group. This is because nicotine is a known psychomotor stimulant with animal research indicating an increase in spontaneous behaviour (Hsia et al., 2020; Javadi-Paydar et al., 2019; Singer et al., 2016; Wang et al., 2020). Although in our study, once accounting for time of day for the scan, there was no significant difference between e-cigarette

exposed fetuses and the other three groups. It is possible that mouth movements alone are not sensitive enough to highlight the subtle differences between exposure groups.

It is important to note here the larger differences in the standard deviations between the heavily exposed and e-cigarette exposed fetuses. There is greater variability in the e-cigarette exposed group in comparison to the small variation in the heavily exposed group. One reason for the variability in the standard deviation for the e-cigarette exposure group most likely relates to the amount of nicotine consumed by the e-cigarette user, which is not controlled and hence fetal exposure to nicotine cannot be classified by the number of times it is used a day as it is for number of cigarettes smoked per day. Milligrams of nicotine in the e-cigarettes was self-reported in this study and it is difficult for the mother to control the amount she uses it in comparison to the number of cigarettes smoked per day. Future research should aim to obtain an objective measure of nicotine, such as a cotinine sample to provide an accurate measure of both cigarette and e-cigarette use (Park & Choi, 2019). Furthermore, the findings may be associated to the relatively small and uneven sample sizes across the groups and thus needs to be viewed with caution.

However, it is possible that coding only mouth movements using the FOMS might not be sensitive enough for assessing subtle differences in fetal facial movement profiles of CO and nicotine exposed fetuses. Whilst it is evident from a range of studies that prenatal cigarette exposure impacts fetal behaviour and postnatal behaviour (Froggatt, et al., 2020b; Reissland, et al., 2015; Stroud et al., 2020), this was not shown in the relative frequency of mouth movements observed in the current study. Hence, we conclude that coding fetal mouth movements using the FOMS alone

cannot differentiate between exposure groups. Other facial movements may also need to be coded which were not accounted for in either the present study or the pilot study and additionally a combination of assessment measures may be required (Reissland & Kisilevsky, 2016).

The results support the hypothesis that overall, the rate of mouth movement per minute does significantly differ between 32-and 36-weeks gestational age. This is in line with Reissland et al. (2015) whereby movement decreases as a function of gestational age (Grigore et al., 2018). Other research has also found a decline in fetal movements from 26 to 36 weeks gestational age. It is thought that this is an indication of the developing neural systems and maturation process with movements becoming more precise and co-ordinated, possibly reflecting the function and development of the CNS (Grant-Beuttler et al., 2011). In the current study we only observed a significant decline in mouth movement frequency for non-exposed and borderline results for e-cigarette exposed fetuses. This might be an indication that exposure of nicotine and CO via cigarette smoking delays the normal decrease of mouth movement frequency, thus impacting CNS development (Reissland, et al., 2015).

A range of studies have indicated that maternal mental health has an impact on fetal behaviour. For example, eye blink rate increases by 20% for each additional increase in anxiety score, with a 21% decrease for an increase in depression score (Reissland et al., 2018). Additionally, as stress scores increase, there is an increase in fetal mouth movements (Reissland, et al., 2015). We found significant correlations at 36 weeks between frequency of mouth movement and depression, and clusters of movement and depression and anxiety, with heavy smokers scoring the highest on both

measures. It could be the case that higher levels of depression and anxiety offset the effects of CO, therefore leading to this group no longer having a lower level of frequency of mouth movement. The effects of stress may explain the higher levels of mouth movements for smoke exposed fetuses in the pilot study by Reissland et al. (2015).

Although the current study involved an adequate sample size overall, fetuses were unevenly distributed in the three exposed and non-exposed groups which may be a contributing factor to the results and a limitation. There are a number of unmeasured sources of potential variance. For example, apart from maternal metal health status there are a number of additional factors associated with changes in fetal behaviour, including caffeine intake (Mulder, Tegaldo, Bruschettini, & Visser, 2010) and maternal fasting for example (Abd-El-Aal, Shahin, & Hamed, 2009), which should be assessed. Additionally, future research, whilst also focusing on fetal mouth movements, should assess other facial movements, self-touches (Reissland, Francis, et al., 2015) and overall fetal activity such as the Fetal Neurobehavioral Assessment System (FENS) (Salisbury, Fallone, & Lester, 2005) or Kurjak's Antenatal Neurodevelopmental Scoring Test (KANET) (Kurjak et al., 2008).

In conclusion because of the variability in fetal mouth movements observed in the present study, we argue that examining frequency of mouth movements alone may not be the most appropriate method for assessing group differences. Rather we suggest that a combination of fetal behavioural assessments is needed to demonstrate how smoking status impacts fetal neurobehavioural development. The finding that

mouth movements per minute decline as a function of gestation is in line with other research.

Chapter 5

Infant neurobehavioural consequences of prenatal cigarette exposure: A systematic review and meta-analysis

This research study is published in accordance with the guidance outlined for the journal Acta Paediatrica. Formatting, references, table and figure numbers have been changed to allow for consistency throughout the thesis.

Abstract

Aim: Prenatal exposure to cigarettes leads to alterations in brain development during pregnancy. This has an impact on postnatal psychological and behavioural processes, affecting an infant's neurobehavioural profile with little known about which aspects are affected. The evidence was synthesized to assess the effects of prenatal cigarette smoke exposure on neurobehavioural outcomes within the first year of life. Method: Six databases were searched (Web of science core collections, MEDLINE, Psychinfo, CINAHL, EBSCOhost ebook collection, Opengrey) in November 2018. Eligible studies had to include a measure of prenatal cigarette exposure and a neurobehavioural assessment <1 year of age. Results: In the first year of life specific areas of neurobehavioural functioning are related to prenatal cigarette exposure with eight out of 10 areas of neurobehaviour having significant medium (negative affect, attention, excitability, irritability, and orientation) or small (muscle tone, regulation, and temperament) pooled effect sizes. Only lethargy and stress did not show any significant pooled effects. Conclusions: Prenatal cigarette exposure affects a significant range of behaviours during the first year of life.

Introduction

Prenatal exposure to cigarette smoke has lasting postnatal effects including significant increased risk of cognitive impairment and learning difficulties (Ernst, Moolchan, & Robinson, 2001; Slotkin, 1998; Wakschlag, Pickett, Cook, Benowitz, & Leventhal, 2002). Research suggests toxins in cigarettes are causing these effects, namely carbon monoxide and nicotine. Carbon monoxide crosses the placenta binding to haemoglobin leading to a reduction in blood flow, ultimately impacting brain development and growth (Ekblad, Korkeila, & Lehtonen, 2015). Similarly, nicotine readily crosses the syncytium, a thin layer of tissue separating maternal and fetal blood (Dempsey & Benowitz, 2001). Although the fetal brain is protected from a range of neurotoxins, it is specifically sensitive to nicotine which targets specific neurotransmitters, leading to cell abnormalities and impaired fetal brain development by affecting synaptic activity (Dempsey & Benowitz, 2001). Since nicotine affects brain development, it has the potential to affect neurobehaviour (Dwyer, McQuown, & Leslie, 2009) including levels of excitability, negative affect, social orientation, and regulation in infants (Hernández-Martínez, Val, Subias, & Sans, 2012). However, there are a number of potential confounding factors that may influence human infant neurobehaviour, therefore animal studies can provide insights into how nicotine affects such behaviour. For example, where environmental factors are controlled, rats exposed to nicotine show increased motor activity as well as deficits in cognition, including attentional problems (Ernst et al., 2001).

Neurobehaviour is defined as a bidirectional relationship between biological and behavioural systems, in which behavioural output is moderated by neural feedback (Lester & Tronic, 2004). It is an interaction between biological and psychosocial

factors that influence human behaviour (Lester & Tronic, 2004). This definition was originally proposed in order to characterise neurobehaviour in late childhood. However, it also applies to infant assessments of neurobehavioural factors such as the availability and fluctuation of sleep and awake states, muscle tone assessed by items such as pulling the infant to sit, irritability and neurological reflexes such as the Babinski and glabella response (Lester & Tronic, 2004; Xu, Yolton, & Khoury, 2011). Specific measures assessing infant neurobehavioural development include habituation, muscle tone, attention, and stress (Barros, Mitsuhiro, Chalem, Laranjeira, & Guinsburg, 2011).

Measures of infant behavioural development are often not mentioned in information leaflets on prenatal tobacco exposure which is distributed to parents; rather parents are mostly informed about fetal and infant health risks of smoking (NHS., 2016). Whilst informed of such risks, smoking during their pregnancy may continue due to previous experiences by themselves or others of healthy uncomplicated pregnancies (Haslam & Draper, 2001). However, assessing neurobehavioural outcomes within the first year of life is essential in understanding later childhood difficulties, information which parents should be informed of. Indeed, research indicates that early neurobehavioural functioning may be predictive of later childhood developmental deficits (Liu et al., 2010), particularly for infants who have been exposed prenatally to cigarettes (Huizink & Mulder, 2006). There is a growing body of evidence that has assessed the neurobehavioural consequences of prenatal cigarette exposure on infant development during the first year of life (Hernandez-Martinez et al., 2017; Stroud et al., 2014). Although reviews have been carried out assessing prenatal exposure on developmental outcomes (Cornelius & Day, 2009; Olds et al., 1997) the current

review is the first meta-analysis assessing neurobehavioural outcomes within the first year of life. The emphasis is on the first year of life as insults during the critical period of development may have lasting impact, particularly for behaviour and cognition (Stettler, 2007). During prenatal and early infant development, the brain is rapidly changing in regards to structure and function, with toxins, such as metabolites of cigarettes, altering the programming for healthy behavioural development (Anderson & Thomason, 2013). For example, research highlights that scores on neurobehavioural assessments during infancy had the ability to predict childhood developmental outcomes (Sucharew, Khoury, Xu, Succop, & Yolton, 2012). Moreover, by employing meta-analytic methods to synthesize the results of the existing studies we can explore which subcategories of neurobehavioural development are most affected.

Method and materials

The methodological reporting of this review follows the PRISMA guidelines.

Literature search

In this meta-analysis, our aim is to identify which subcategories of neurobehaviour are impacted by prenatal cigarette exposure within the first year of life. A literature search of six databases was conducted (Web of Science Core Collections, MEDLINE, Psychinfo, CINAHL, EBSCOhost ebook collection and Opengrey) in November 2018. Search terms are listed in Table 5.1. Although the review focuses on tobacco exposure, nicotine was included as a term to make the search more exhaustive (Yolton et al., 2009).

Table 5.1. Web of Science Core Collections search strategy

Search terms

Web of Science: Core Collections (k=1190) 1950-2018	
Initial search	Maternal smoking pregnancy Prenatal nicotine exposure Prenatal tobacco exposure Prenatal cigarette exposure Prenatal smoke exposure Fetal nicotine exposure Fetal tobacco exposure Fetal cigarette exposure
Searched within *(separately for each phrase)*	Affect (k=208) Attention (k=130) Behaviour (k=127) Cogni* (k=158) Emotion (k=62) Excitability (k=0) Irritab* (k=4) Lethargy (k=1) Motor* (k=46) Muscle (k=7) Neurobehaviour* (k=30) Neurodevelopment* (k=53) Orientation (k=5) Regulation (k=33) Social (k=198) Stress (k=20) Temperament (k=8)
Applicable once duplicates removed: 809	

Note. Published articles are restricted from 1950 to 2018, with unpublished research having no time limits. The language was set to English. No methodological limits were applied.

Study selection

Studies were included if they reported both a measure of prenatal exposure to cigarettes and postnatal neurobehavioural measurements at ≤1-year post birth. A number of exclusions were in place, including animal studies, reviews (systematic, literature and meta-analyses), children >1 year of age, studies with no record of maternal prenatal cigarette use, studies focusing on medical, health or birth outcomes

and studies using nicotine replacement therapy. The database searches were combined, and duplicate records were removed. The studies were screened by the primary author to assess whether they met the inclusion criteria. Full-text articles were reviewed for further analysis of study inclusion criteria. The reference lists of these papers were screened for any additional articles.

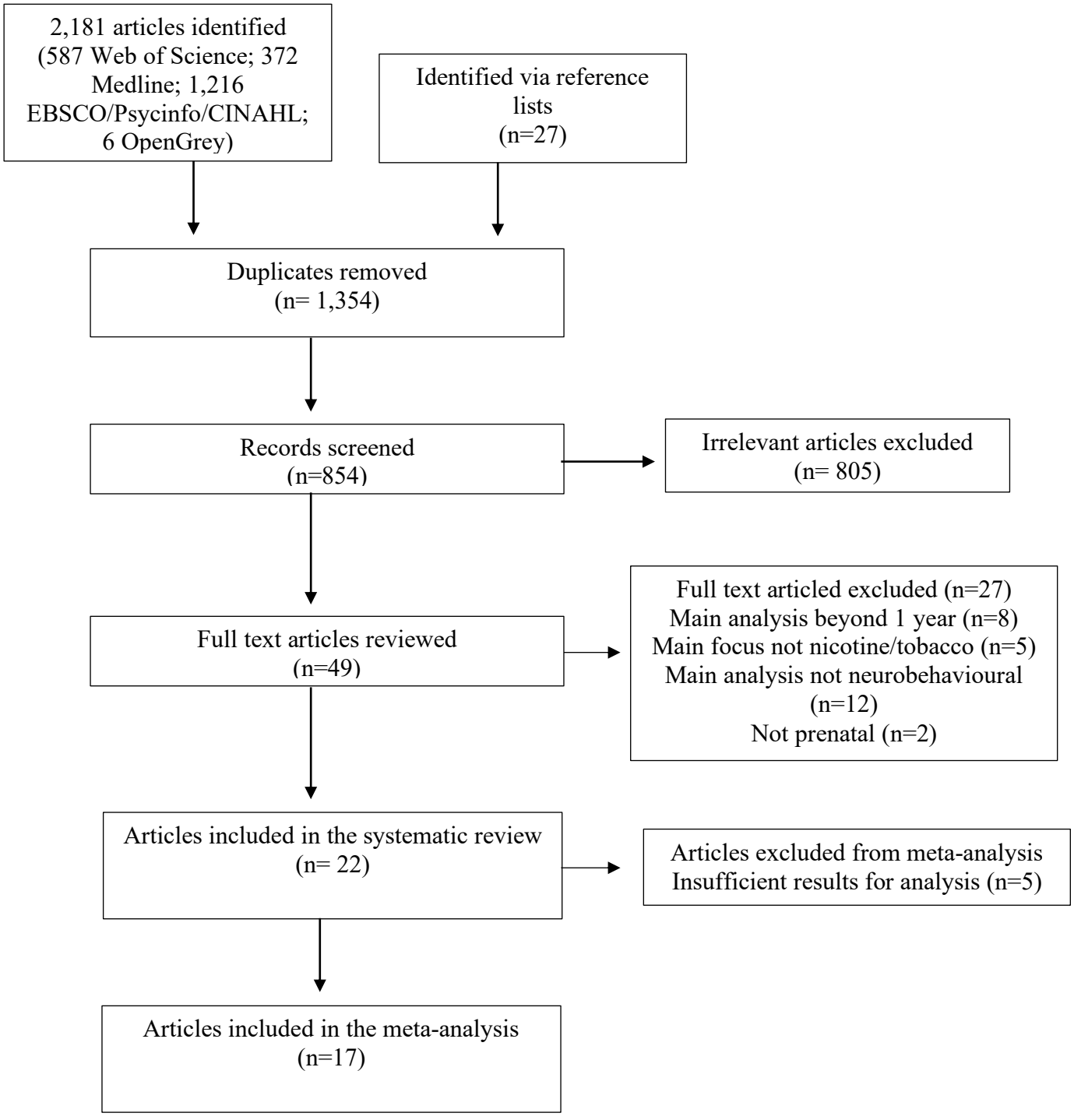

Figure 5.1. PRISMA flow diagram of studies

Data extraction and assessment of methodological quality

A pre-defined extraction sheet was used to record study characteristics. Extracted information included (a) main outcome measure, (b) participant characteristics (number of infants, infant age, number prenatally exposed and number not exposed), (c) tobacco measurement, (d) controls and (e) results. Where an effect size (Cohen's d) was not provided, it was calculated from the available data using the Campbell Collaboration effect size calculator (https://campbellcollaboration.org/effect-size-calculato.html). Where possible effect sizes were based on analyses in which potentially confounding variables such as preterm birth, gestational age at birth, maternal demographics, and substance use (e.g., alcohol) (Field et al., 2004; Lipper, Lee, Gartner, & Grellong, 1981), had been taken into consideration. Risk of bias for individual studies was calculated using the ROBINS-I tool (Sterne, 2016) (supplementary material, table 5.5).

Data analysis

Studies that were eligible for the review were grouped according to 10 different subcategories of outcome measures: negative affect, attention, excitability, irritability, lethargy, muscle-tone, orientation, regulation, stress, and difficult temperament. To be included in the meta-analysis, the assessment measures had to be similar across the subcategory. For subcategories to be included within the analysis, two or more studies were required (Valentine, Pigott, & Rothstein, 2010). The fail-safe N method was used to identify any publication bias by providing an estimate of the number of missing studies that would need to be published with an effect size of d=0 for the pooled effect size to not be significant (Rosenthal, 1978).

Table 5.2. Assessment measures.

Assessment measure	No. studies using assessment	Details
NICU Neurobehavioural Scale (NNNS)	4	This assessment was designed to capture the vulnerabilities of high-risk infants exposed to toxic substances and for new-borns between 30-46 weeks gestational age. Raw data were used to create summary scores based on 13 dimensions including; attention, arousal, excitability, hypertonicity, hypotonicity, lethargy, regulation, handling, stress and reflexes (Yolton et al., 2009).
Neonatal Behaviour Assessment Scale (NBAS)	3	Assesses early regulatory behaviour (Espy et al., 2011). State changes are provoked and the infants' habituation, self-consoling abilities and reflexes. It includes 28 behavioural items and 18 reflexes. Items given a score include motor abilities, habituation, orientation, reflexes and regulation (Mansi et al., 2007).
Carey Infant Temperament Scale	1	The scale assesses three areas of temperament; positive mood, receptivity to novelty and regularity (Pickett et al., 2008).
Infant Behaviour Questionnaire-Revised	2	This is a parental report questionnaire for infants between 3-12 months of age. There are three main subcategories of this scale including: extroversion, negative affect, orientating and regulation (Gartstein and Rothbart, 2003).
Graham-Rosenblith Behavioural Examination	1	This is a standardised assessment which involves observation and manipulation of the infant to assess reflexes, muscle and responses to stimulation. Additionally, measures of irritability and signs of neurological damage are assessed (Stroud et al., 2009a).

Laboratory Assessment Battery (Lab-TAB)	2	Designed to assess early infant temperament (Mundy, 2009).
Finnegan Withdrawal Scale	1	Evaluation of the Central Nervous System function and respiratory functions (Godding et al., 2004).
Neurological Scores	1	Assesses a range of abilities including muscle tone, reflexes e.g. sucking, stepping reactions and alertness e.g. eye opening (Godding et al., 2004).
Neonatal Temperament Assessment (NTA)	1	The assessment assesses early regulatory behaviours (Espy et al., 2011).

Table 5.3. Studies included within the analysis.

Reference/Country	No. Infants	Infant age	Assessment	Subcategory	Effect size (Cohen's d)	Covariates controlled for in the analysis	Overall bias
Barros et al., 2011 (10) *Brazil*	388 infants (365 not exposed, 23 exposed)	24-72 hours old	NICU Network Neurobehavioural Scale	*Attention* *Excitability* *Lethargy* *Stress*	-1.3 -0.636 -1.142 -0.587	Anaesthesia at birth, type of delivery, gender, age of new-born at assessment, time since last feed and duration of assessment.	Low
Espy et al., 2011 (54) *USA*	304 infants (161 not exposed; 143 exposed	2 days old	Neonatal Temperament Assessment	*Attention* *Irritability*	-0.465 -0.192	Mothers' IQ estimate. Marital status, maternal age, education, income, maternal age, alcohol intake, new-born gender, race, SHS exposure, medication use, gravida, parity, weight gain, maternal health, delivery health, BSI summary index, CAARS:S Attention Deficit/Hyperactivity Disorder index and BIA IQ estimate.	Low
Godding et al., 2004 (55) *Belgium*	33 infants (16 not exposed, 17 exposed)	up to 5 days old	Neurologic scores & Finnegan Withdrawal Scores	*Muscle tone*	-0.3785	Term of pregnancy and feeding method.	Low

Hernandez-Martinez et al., 2012 (32) *Spain*	265 infants (203 not exposed, 62 exposed)	48-72 hours old	Neonatal Behavioural Assessment Scale	*Negative Affect* *Excitability* *Orientation* *Regulation*	-0.02 -0.44 -0.35 -0.351	Socioeconomic status, birthweight and gestational age. Maternal age, socioeconomic status, new-born gender, birthweight, gestational age, Apgar scores, parity, delivery type, trait anxiety.	Low
King et al., 2017 (56) *USA*	48 infants (24 not exposed, 24 exposed)	3-5 months	Response to bell ring, brain response	*Orientation*	-0.8471	Maternal education, gestation at birth, age at assessment, birthweight, ethnicity.	Moderate
Law et al., 2003 (30) *USA*	56 infants (29 not exposed, 27 exposed)	between 36 -41 weeks gestation age	NICU Network Neurobehavioural Scale	*Excitability* *Muscle* *Stress*	-0.829 -0.711 -1.510	Parity, 5-minute Apgar scores and birthweight. Maternal age, gravida, education, employment, socioeconomic status, alcohol use, gestational age, Apgar score at 1 minute.	Low
Mansi et al., 2007 (33) *Italy*	50 infants (25 not exposed, 25 exposed)	56-72 hours old	Neonatal Behavioural Assessment Scale	*Attention* *Irritability* *Muscle* *Orientation* *Regulation*	-1.358 -1.949 -1.010 -1.115 -0.599	Gender, gestational age, postnatal age, birthweight, Apgar scores, Bilirubin.	Low
Mundy, 2009a (33)	71 infants	6 months	Laboratory Temperament	*Difficult Temperament*	-0.556	*None noted.*	Moderate

UK	(47 not exposed, 24 exposed)		Assessment Battery & Infant Behaviour Questionnaire				
Mundy, 2009b (33) *UK*	71 infants (47 not exposed, 24 exposed)	6 months	Laboratory Temperament Assessment Battery & Infant Behaviour Questionnaire	*Negative Affect* *Difficult Temperament*	-0.409 -0.399	*None noted.*	
Pickett et al., 2008 (37) *UK*	15,943 infants (11,747 not exposed, 4196 exposed)	9 months	Carey Infant Temperament Scale	*Negative Affect* *Orientation Regulation* *Difficult Temperament*	-0.759 -0.070 -0.114 -0.134	*None noted.*	Moderate
Saxton, 1978 (35) *UK*	32 infants (17 not exposed, 15 exposed)	4-6 days old	Neonatal Behavioural Assessment Scale	*Orientation Regulation*	-0.8471 -0.782	*None noted.*	Moderate
Schuetze et al., 2007 (28)	115 infants	2-4 weeks	Infant Behaviour Questionnaire	*Negative Affect*	-0.806	Mothers' age, education, socioeconomic status, parity,	Low

USA	(46 not exposed, 69 exposed)	old and again at 7 months old				number of prenatal visits, substance use, infant birth weight, head circumference and birth length.	
Shisler et al., 2009 (29) *USA*	258 infants (77 not exposed, 181 exposed)	2 & 9 months old	Focused attention assessment & behavioural reactivity	*Attention*	-0.238	Mothers age, education, prenatal alcohol and marijuana, partner status, birthweight, gestational age, gender, head circumference at birth.	Low
Stroud et al., 2009b (34) *USA*	56 infants (28 not exposed, 28 exposed)	17 days old	NICU Network Neurobehavioural Scale	*Excitability* *Regulation*	-0.665 -0.565	Maternal SHS exposure, infant SHS exposure, feeding, maternal depression, socioeconomic status, maternal age and depression.	Low
Stroud et al., 2009a (46) *USA*	962 infants (366 not exposed, 596 exposed)	< 3 days old	Graham-Rosenblith Behavioural Examination	*Irritability* *Muscle*	-0.125 -0.308	Maternal age, race, socioeconomic status, birthweight and infant age at assessment. Gravida, parity, Apgar at 1 minute and Apgar at 5 minutes.	Low
Wiebe et al., 2014 (57) *USA*	218 infants (91 not exposed, 127 exposed)	6 months old	A battery of assessments including attention, regulation and inhibition	*Orientation*	-0.236	Propensity scores – alcohol in first month of pregnancy, maternal age, education, IQ, hyperactivity. Parental stress and infant exposure.	Moderate

Yolton et al., 2009 (22) *USA*	251 infants (218 not exposed, 33 exposed)	5 weeks old	NICU Network Neurobehavioural Scale	*Attention* *Lethargy* *Regulation* *Stress*	-0.134 -0.147 -0.067 -0.002	Birthweight, age at assessment and infant gender. Maternal age, income, employment, education, marital status, parity, marijuana and alcohol use, maternal blood lead in pregnancy and weight change since birth and maternal depression.	Low

Results

Selection of studies

The search resulted in 2,208 studies. After removal of duplicates 854 studies were reviewed in terms of title and abstract, resulting in 49 eligible studies which were subjected to a full-text review. These articles were reviewed in-depth, checking for a measure of prenatal smoke exposure and a postnatal neurobehavioural measure and 27 articles were removed leaving 22 articles that based on our selection criteria could be included in the review (see Figure 5.1). Five of these articles reported insufficient data leaving 17 articles included in the meta-analysis. Authors of the five studies reporting insufficient results were contacted, where possible, to obtain further details. However, this was unsuccessful. See Figure 5.1 for flow diagram of study selection and Table 5.3 for details of the studies included in the analysis.

Study characteristics

The 17 studies included in the meta-analysis analysed 19,162 infants. There were 5,672 infants exposed to cigarettes prenatally and 13,490 who had no prenatal cigarette exposure. Studies came from eight different countries; USA (n=9), UK (n=4), Spain (n=1), Italy (n=1), Brazil (n=1) and Belgium (n=1). To assess level of maternal or infant smoke exposure, studies used either a questionnaire method (n=7), biological measures such as cotinine levels via saliva (n=2) or a combination of the two methods (n=8). Nine different assessment scales were used to measure a range of neurobehaviours. Details of the assessments are in Table 5.2.

Neurobehavioural subcategory analysis

See Figure 5.2 for forest plot of results and Table 5.4 for subcategory analysis.

Negative Affect

Negative affect in infancy is determined by establishing level of sadness, fear, soothability, and activity level (Gartstein & Rothbart, 2003). Four studies were included in the analysis of negative affect. 16,394 infants (12,043 not exposed and 4,351 exposed) between 48 hours and 9 months old were assessed on one of four measures: NBAS, Lab-TAB, Carey Infant Temperament Scale, Infant Behaviour Questionnaire -Revised. Individual study effect sizes ranged between -0.806 (Schuetze & Eiden, 2007) and -0.02 (Hernández-Martínez et al., 2012). Due to heterogeneity within the sample ($Q=28.222$, $p<.001$, $I^2=89.37\%$), the random effect size model is reported. The combined effect size for negative affect is significant ($d=-0.502$; 95% CI = -.886 to -.1191; $z=-2.568$, $p=.010$; fail-safe N=809). Infants prenatally exposed to smoking showed heightened negative affect.

Attention

Infant attentional abilities are assessed by the degree of energy the infant displays when engaging with the assessment and the level of facilitation required from the examiner to gain the infants attention (Shisler et al., 2016). Five studies were included in the assessment of the attention subcategory, assessing 1,251 infants (846 not exposed to nicotine and 405 exposed to nicotine), between 24 hours to 9 months old. Three different assessment scales were used: NBAS, NICU Neurobehavioural Scale, NTA. Individual study effect sizes range between -1.358 (Manis et al., 2007) and -0.134 (Yolton et al., 2009) and there is evidence of heterogeneity within the

sample (Q=32.451, p<.001, I^2=87.67%). Therefore, the random effects model is reported. The combined effect size for attention is significant (d= -0.635; 95% CI= -1.031 to -0.238; z=-3.129, p=.001; fail-safe N=98). Those exposed to cigarettes showed significantly poorer levels of attention.

Excitability

Excitability is assessed by measuring peak excitement and rapidity of build-up, which is a reflection of how much stimulation the baby can handle before entering the crying state, indicating higher levels of arousal (Law et al., 2003; Tronick & Lester, 2013). A total of 765 infants (625 not exposed and 140 exposed) between 24 hours and 17 days old, were included in the four studies analysed for excitability using two different assessment scales (NICU Neurobehavioural Scale and the NBAS). Individual study effect sizes ranged between -0.829 (Law et al., 2003) and -0.44 (Carmen Hernandez-Martinez, Arija Val, Escribano Subias, & Canals Sans, 2012). The data is homogeneous (Q=1.873, p=.599, I^2=60.13%) and therefore the fixed effect size model is reported. The combined effect size for excitability is significant (d= -0.5697; 95% CI = -0.772 to -0.367; z=-5.529, p<.001; fail-safe N=44). Infants prenatally exposed to cigarettes demonstrated significantly higher levels of excitability.

Irritability

Irritability is assessed through examining the amount of fussing and crying throughout neurobehavioural assessments. Three studies were included in the analysis for irritability with 1,316 (552 not exposed and 764 exposed) infants between 56 hours and 3 days old. The NICU Neurobehavioural Scale, Graham-Rosenblith

Behavioural Examination and NTA were used. Individual study effects between -1.949 (Mansi et al., 2007) and -0.125 (Stroud, Paster, Goodwin, et al., 2009). The random effect size model was used because of heterogeneity within the data (Q=27.185, p<.001, I^2=92.64%). The combined effect size for irritability was significant (d=-0.600; 95% CI= -1.148 to -.0519; z=-2.145, p=.031; fail-safe N=29). Infants prenatally exposed to cigarettes were significantly more irritable.

Lethargy

Lethargy examines the energy resources of the infants and is identified by items on the assessments such as general tone and reaction to the defensive movement by establishing level of movement (Tronick & Lester, 2013). Two studies were included in the analysis for lethargy with 639 infants (583 not exposed and 56 exposed) ranging between 24 hours and 5 weeks in age, tested with the NICU Neurobehavioural Scale. Individual study effect sizes ranged from -1.142 (Barros et al., 2011) to -0.147 (Yolton et al., 2009). The data is heterogeneous (Q=15.847 p<.00, I^2=93.68%), therefore the random effect size model is reported. The combined effect size for lethargy is not significant (d=-0.628; 95% CI= -1.680 to 0.346, z=-1.262, p=.206). Prenatal exposure to smoking is not significantly related to the lethargy levels of infants tested.

Muscle-tone

Muscle tone weakness is identified by assessing how smooth or jerky the infant's movements are and how much of the time the infant displays 90° arcs. Additionally, items such as pulling the infant to sit is used as an indication of muscle tone (Tronick & Lester, 2013). Muscle tone weakness is identified in the infant when the majority

of movements are jerky, restricted and when there is significant head lag when the infant is pulled to a seated position (Brazelton & Nugent, 1995). Four studies were included in the analysis for muscle tone with a total of 1,101 infants (436 not exposed and 665 exposed), between 56 hours and 5 days old assessed with one of four measures (NICU Neurobehavioural Scale, Graham-Rosenblith Behavioural Examination, NBAS, Neurological Scores). Individual studies had an effect size ranging between -1.010 (Mansi et al., 2007) and -0.308 (Stroud, Paster, Goodwin, et al., 2009). The data were homogeneous (Q=6.908, p=.074, I^2=56.57%), therefore the fixed effect size model is reported. The combined effect size is significant (d=-0.361; 95% CI = -0.484 to -0.239; z=-5.796, p<.001; fail-safe N=28). Infants prenatally exposed to smoking had significantly more muscle-tone weakness.

Orientation

Orientation items assess the infant ability to follow and engage with animate and inanimate object such as following a face or rattle for example (Tronick & Lester, 2013). 16,556 infants (12,107 not exposed and 4,449 exposed) between 48 hours to 9 months old, based on six studies, were included in the subcategory analysis for orientation. The assessments used were the NBAS and Carey Infant Temperament Scale. The range of effect sizes across individual studies were -1.115 (Mansi et al., 2007) and -0.070 (Pickett et al., 2008). Due to heterogeneity (Q=26.969, p=.001, I^2=81.46%) of the sample, the random effects model is reported. The combined effect size for orientation is significant (d=-0.464; 95% CI= -0.757 to -0.171; z=-3.104, p<.001; fail-safe N=98). Infants prenatally exposed to smoking demonstrated significantly worse levels of orientation.

Regulation

Regulation is assessed by the infants' abilities to self-sooth, for example whether they need support settling down following a period of crying (Tronick & Lester, 2013). 16,597 infants (12,238 not exposed and 4,359 exposed), between 48 hours to 9 months old, were analysed in the subcategory for regulation, based on six studies using three different assessment measures (NICU Neurobehavioural Scale, NBAS, Carey Infant Temperament Scale). Individual study effect sizes ranging between -0.782 (Saxton, 1978) and -0.067 (Yolton et al., 2009). This was a heterogeneous sample (Q=11.250, p=.046, I^2=55.55%) and therefore the random effects model is reported. The combined effect size for orientation abilities was significant (d=-0.261 (95% CI=-0.4411 to -0.082; z= -2.864, p=.004; fail-safe N=82). Infants prenatally exposed to smoking showed significantly more problems in their ability to regulate their behaviour.

Stress

Infant stress is a reflection of the autonomic nervous system and as such is determined by whether colour changes occur, number of startles and whether tremors can be seen throughout the assessment (Tronick & Lester, 2013). A total of 695 infants (612 not exposed and 83 exposed), between 24 hours and 5 weeks old, were tested using a single assessment measure, the NICU Network Neurobehavioural Scale across three studies. Individual study effect sizes varied between -1.510 (Law et al., 2003) and -0.002 (Yolton et al., 2009). Due to heterogeneity in the sample (Q=23.793, p<.001, I^2=91.59%) the random effect size model was used. The combined effect size for stress was not significant (d=-0.661; 95% CI= -1.459 to

0.137; z=-1.623, p=.104). Infants prenatally exposed to smoking did not show significantly higher stress compared with non-exposed infants.

Difficult Temperament

Difficultness of the infant i.e., fussiness, irritability and negative affect throughout the assessment is used to determine the infants temperament (Schuetze & Eiden, 2007). 192 infants (116 not exposed and 73 exposed), between 56 and 6 months old were assessed in three studies using the Lab-TAB and the Carey Infant Temperament Scale for temperament. Individual studies reported effect sizes between -0.556 (Mundy, 2009) and -0.134 (Pickett, Wood, Adamson, & D'Souza, 2008). Because of the heterogeneity within the sample (Q=6.596, p=.036, I^2=69.68%) the random effects model was used. The combined effect size for temperament was significant (d= -0.314; 95% CI = -.596 to -.032; z=-2.183, p=.029; fail-safe N=14). Infants prenatally exposed to cigarette smoke demonstrated higher levels of difficult temperament in comparison to infants not prenatally exposed to smoke.

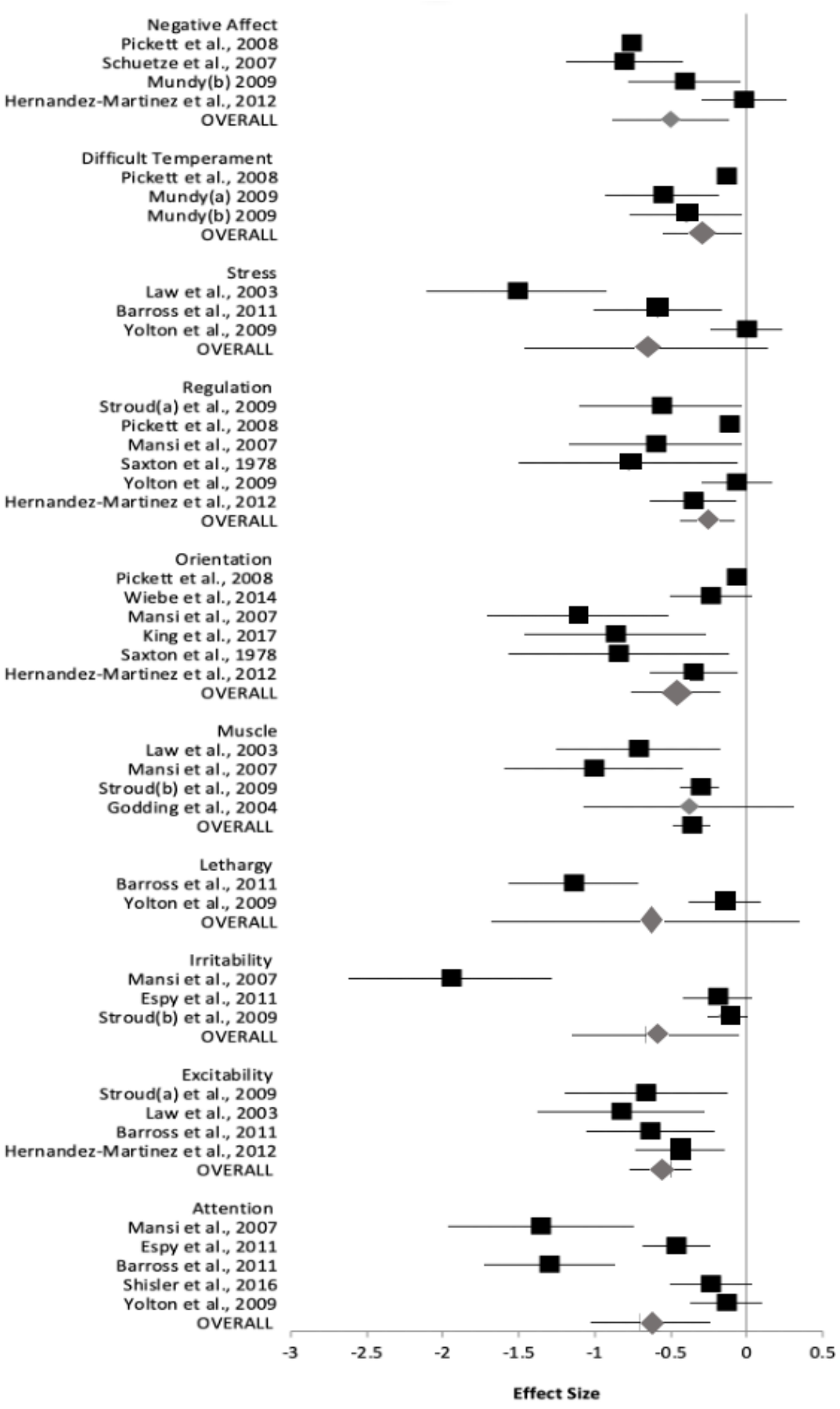

Figure 5.2. Forest plot of analysis. Diamonds represent the overall effect sizes, with the squares representing individual studies. Size of the diamonds and squares represent the size of the effect.

Table 5.4. Subcategory analysis.

Subcategory	No. of studies	Assessment measures	Cohen's d	95% CI	*Z*	*P* value (*Z*)	*Q*	*P* value (*Q*)
Negative Affect*	4	*NBAS, Lab-TAB, Carey Infant Temperament Scale, Infant Behaviour Questionnaire -Revised*	-0.5027	-0.8863, -0.1191	-2.5685	.0102	28.2227	<.001
Attention*	5	*NBAS, NICU Neurobehavioural Scale, NTA*	-0.6352	-1.0318, -0.2386	-3.1292	.001	32.4514	<.001
Excitability*	4	*NICU Neurobehavioural Scale, NBAS*	-0.5697	-0.7726, -0.3678	-5.5296	<.001	1.8737	.599
Irritability*	3	*NICU Neurobehavioural Scale, Graham-Rosenblith Behavioural Examination, NTA*	-0.6003	-1.1486, -.0519	-2.1456	.0319	27.185	<.001
Lethargy	2	*NICU Neurobehavioural Scale*	-0.6280	-1.680, 0.3469	-1.2625	.2068	15.8478	.001
Muscle*	4	*NICU Neurobehavioural Scale, Graham-Rosenblith Behavioural Examination, NBAS, Neurological Scores*	-0.3619	-0.4842, -0.2395	-5.7964	<.001	6.9088	.0749

Orientation*	6	*NBAS, Carey Infant Temperament Scale*	-0.4645	-0.7577, 0.1713	-3.1047	.001	26.9692	.009
Regulation*	6	*NICU Neurobehavioural Scale, NBAS, Carey Infant Temperament Scale*	-0.2619	-0.4411, -0.0827	-2.864	.004	11.2507	.0465
Stress	3	*NICU Neurobehavioural Scale*	-0.6613	-1.4598, 0.1373	-1.6231	.1046	23.7939	<.001
Difficult Temperament*	3	*Lab-TAB, Carey Infant Temperament Scale*	-0.3144	-.5966, -.0322	-2.1834	.0290	6.567	.0369

Note: If the Q statistic was significant ($p<.05$) the random effects model was used to compute the pooled effect size. If the Q statistic was not significant ($p>.05$) the fixed effects model was used to compute the pooled effect size. *Significant $p<.05$

Discussion

The aim of this systematic review and meta-analysis was to establish which areas of neurobehavior are most strongly related to prenatal cigarette exposure in infants up to one year of age. Overall, the results support the claim that prenatal exposure to smoking is associated with a range of neurobehavioural consequences in infants within the first year of life. Eight of the 10 subcategories that were analysed in the meta-analysis indicate that prenatal smoking is significantly associated with poorer neurobehavioural functioning in infancy. Measures of negative affect, attention, excitability, irritability, and orientation demonstrated medium significant effects, with regulation, difficult temperament and muscle tone weakness indicating smaller significant effects. Stress and lethargy tests, however, did not result in any significant pooled effects.

We argue that the neurobehavioural deficits evident in infants of mothers who smoke cigarettes reflect early behavioural dysregulation associated with prenatal exposure to cigarettes. The metabolites of cigarette smoke, carbon monoxide and nicotine interfere with the normal placental functioning acting as a vasoconstrictor, with uterine blood flow being restricted to roughly 38% (Bush et al., 2000; Ekblad et al., 2015; Suzuki, Minei, & Johnson, 1980). Carbon monoxide is likely to lead to fetal hypoxia depriving the developing brain of oxygen and nutrients required for typical brain development. Such effects can be seen in prenatally exposed newborns whose cerebral oxygen saturation level is lower in comparison to infants not exposed (Verhagen et al., 2011). This interpretation is supported by studies using animal models (Cohen et al., 2005; Slotkin, 2008). Similarly, studies highlight the

widespread effects of nicotine affecting a range of neurotransmitters, brain regions and systems which disrupt brain development. Specifically, the neurotransmitter nicotine acetylcholine plays a role in supporting the development of infant regulatory behaviours, such as temperament (Slotkin, 2008; Stroud, et al., 2009). Differences in neurobehaviour of infants prenatally exposed to cigarettes are based on changes in brain functioning as a result of carbon monoxide and nicotine exposure (Ekblad et al., 2015).

Research indicates that mother-infant relationships are under more stress, i.e., less responsiveness and emotional interactions, if the infant displays neurobehavioural deficits in areas such as affect, with infants demonstrating reduced eye contact and/or reduced smiling during parent-infant interaction (Papoušek & von Hofacker, 1998). This type of unresponsiveness by the infant leads to a negative feedback loop during mother-infant interactions. As this review indicates, maternal smoking during pregnancy is related to deficits in infant neurobehavioural functioning; for example, infants prenatally exposed to cigarettes are likely to be more irritable compared to non-exposed infants. A more irritable child will affect quality of parenting behaviours which have negative effects on the infant including less stimulation, less responsiveness and less physical contact (van den Bloom & Hoeksma, 1994). Because of these negative parenting engagements, the infant's neurobehavioural development is further dysregulated due to reduced interactions (Mansi et al., 2007). As a result, an infant who lacks stimulation and physical contact is more likely to show delays in their motor development (Gutman & Feinstein, 2010). This delay in turn will be an additional strain on the already stressed mother-infant relationship.

Long term attentional and behavioural problems can be reflective of these early deficits in neurobehavioural functioning of an infant (Stroud, et al., 2009).

Limitations

The relationship between neurobehavioural developmental factors and prenatal cigarette exposure is complex, often associated with a number of covariates such as preterm birth, gestational age at birth, maternal demographics and substance use (e.g., alcohol) (Field et al., 2004; Lipper et al., 1981). As shown in Table 2 these types of variables were controlled for in the effect size analysis in the majority of studies. Nevertheless, other covariates such as maternal psychological factors were not considered in many of the studies reviewed, despite the known effects on neurobehaviour. For example, maternal antenatal stress and anxiety is positively related to infant outcomes including behavioural and cognitive development such as regulation difficulties, irritability, and poorer attention (Van den Bergh, Mulder, Mennes, & Glover, 2005). Given that these factors were not controlled for in all the studies analysing the effect of cigarette exposure, it was difficult to determine in our current review the extent to which these factors may have influenced the test results.

Due to such confounding variables, it is possible that studies claiming to find a relationship between prenatal smoke exposure and subsequent infant neurobehaviour are measuring an indirect relationship rather than a true causal effect (Brion et al., 2010; Grimes & Schulz, 2002). As a consequence of the epidemiological nature of this research, not all potential confounds can be controlled for and it is difficult to carry out a true experimental design as cigarette exposure cannot be randomly assigned, thus highlighting a methodological limitation (D'Onofrio et al., 2008).

However, by synthesizing the available evidence across multiple populations and study designs, this meta-analysis strengthens the case for a true causal effect between cigarette exposure and infant neurobehaviour (Brion et al., 2010; Grimes & Schulz, 2002).

It is notable however that by studying infants up to one year of age (the range of ages of infants studied is shown in Table 2) we cannot rule out the possibility that in the older infants the effects of their mothers' smoking on neurobehavioural outcomes was due to postnatal rather than prenatal exposure (Xu et al., 2011). Furthermore, the amount of cigarette exposure and at what time point exposure occurred (including postnatal exposure) differed between studies. In the early stage of development, there is naturally a lot of variation and disorganisation in the neurobehavioural profile of infants since the brain is not fully developed at birth (Gerhardt, 2014), and environmental factors influence brain development (Cirulli, Berry, & Alleva, 2003). Therefore, we have to consider whether the differences seen in infant neurobehavioural development are short-term or long-term factors and whether the negative consequences can be reduced or potentially eliminated through neurobehavioural interventions.

Conclusions

The results from the meta-analysis indicate that exposure to prenatal cigarette smoking is associated with negative neurobehavioural outcomes in infants up to one year of age. Research indicates that not all women believe that smoking has negative behavioural consequences for their infant (Goszczyńska, Knol-Michałowska, & Petrykowska, 2016). Thus, examining neurobehavioural differences in smoke

exposed and non-exposed fetuses and infants is essential in order to convince pregnant women to abstain from cigarette consumption during their pregnancy and after birth. For example, smoking during pregnancy may result in irritable infants which cry more than infants with a calm temperament (Pickett et al., 2008). The current review and analysis provides further support of the negative effects prenatal smoke exposure has on infant neurobehaviour within the first year of life.

Supplementary material

Table 5.5. ROBINS-I: tool for risk of bias.

Study	Bias due to confounding	Bias in selection of participants into the study	Bias in classification of interventions	Bias due to deviations from intended interventions	Bias due to missing data	Bias in measurement of outcome	Bias in selection of the reported result	Overall bias
Barross et al., (2011)	Low	Low	Low	Low	Low	Low	Low	Low
Espy et al., (2011)	Low	Low	Low	Low	Low	Low	Low	Low
Godding et al., (2004)	Low	Low	Low	Low	Low	Low	Low	Low
Hernandez-Martinez et al., (2012)	Low	Low	Low	Low	Low	Low	Low	Low
King et al., (2017)	Low	Low	Low	Low	Moderate	Low	Low	Moderate
Law et al., (2003)	Low	Low	Low	Low	Low	Low	Low	Low
Mansi et al., (2007)	Low	Low	Low	Low	Low	Low	Low	Low
Mundy (2009)a	High	Low	Low	Low	Low	Low	Low	Moderate
Mundy (2009)b	High	Low	Low	Low	Low	Low	Low	Moderate
Pickett et al., (2008)	High	Low	Low	Low	Low	Low	Low	Moderate
Saxton (1978)	High	Low	Low	Low	Low	Low	Low	Moderate

Chapter 6

The effects of prenatal cigarette and e-cigarette exposure on infant neurobehaviour: A comparison to a control group

This research study is published in accordance with the guidance outlined for the journal EClinicalMedicine. Formatting, references, table and figure numbers have been changed to allow for consistency throughout the thesis.

Abstract

Background: Infant neurobehaviour provides an insight into the development of the central nervous system during infancy, with behavioural abnormalities highlighting a cause for concern. Research has demonstrated that prenatal exposure to cigarettes leads to deficits within neurobehavioural development, along with negative birth outcomes detrimental to subsequent development. With the growing use of e-cigarettes amongst pregnant women, this study explores how prenatal e-cigarette exposure compares to prenatal cigarette exposure.

Methods: Eighty-three infants were involved in the study, either exposed prenatally to cigarettes or e-cigarettes or not exposed to either. Differences were assessed between these three groups for birth outcomes and scores on the Neonatal Behavioural Assessment Scale (NBAS) at one month of age.

Findings: Both cigarette and e-cigarette exposed infants had a significantly greater number of abnormal reflexes (p=.001; p = .002). For both self-regulation and motor maturity, cigarette exposed infants performed significantly worse (p= .010; p= .002), with e-cigarette exposed infants having decreased motor maturity (p= .036) abilities

and marginally decreased for self-regulation (p= .057). Birth outcomes, namely birthweight, gestation and head circumference, did not differ for e-cigarette exposed infants compared with infants who were not prenatally exposed to nicotine. Cigarette exposed infants had a significantly lower birthweight (p= .021) and reduced head circumference (p= .008) in comparison to non-exposed infants.

Interpretation: To our knowledge, this is the first research study assessing a neurological outcome as a result of e-cigarette exposure. Findings of this have potentially important implications for public health policies regarding the safety and use of e-cigarettes throughout pregnancy.

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Added value of the study

This is the first study to assess any neurobehavioural responses of an infant as a result of prenatal e-cigarette exposure. The range of detrimental outcomes of prenatal cigarette exposure are well established. With public health initiatives focused on a reduction of cigarette smoking during pregnancy to 6% by 2022, despite lack of evidence regarding safety for the developing infant, e-cigarettes are used as a harm reduction method. The findings indicate that whilst birth outcomes do not appear to be affected by e-cigarette exposure, these infants do have a greater number of abnormal primitive reflexes and marginally decreased self-regulation abilities similar to prenatally cigarette exposed infants, in comparison to non-exposed infants.

Implications of the evidence

Further research is required to test the effects of e-cigarette use during pregnancy, alongside other forms of nicotine replacement therapy to fully explore the impact of nicotine on the infant. This study adds to the current debate regarding e-cigarette use as a method of harm reduction with possible implications for public health policy.

Introduction

Reducing smoking during pregnancy is a key public health priority due to a range of detrimental birth outcomes, including intrauterine growth restriction, low birth weight (<2500g), small for gestational age, preterm delivery (<37 weeks) and reduced head circumference (Inoue et al., 2017; Ko et al., 2014). Accompanying the birth outcomes, such as low birth weight, are the neurobehavioural deficits that may occur as a result of prenatal cigarette exposure, including irritability, poor muscle tone, decreased self-regulation, increased negative affect and difficult temperament (Froggatt, Covey, & Reissland, 2020a). These neurobehavioural deficits have been shown to predict subsequent infant development including psychomotor, cognitive and emotional development (Canals, Hernández-Martínez, Esparó, & Fernández-Ballart, 2011). Low birth weight in infants of mothers who smoke indicates fetal growth restriction thought to be related to Carbon Monoxide (CO) exposure affecting the oxygen carrying capacity of the fetal blood (Merklinger-Gruchala, Jasienska, & Kapiszewska, 2017). Alternatives to cigarette smoking, such as nicotine replacement therapy (NRT) and e-cigarettes are therefore considered by some to be a harm reduction method and information provided in healthcare leaflets for pregnant women state that nicotine alone is relatively harmless (NHS, 2019). There is however

growing concern about the increasing use of e-cigarettes and the safety of nicotine exposure for the developing fetus (Smoking in Pregnancy Challenge Group, 2019). Therefore, assessing birth and infant outcomes in fetuses that have been exposed to e-cigarettes, will add to the debate regarding their use during pregnancy.

Although the use of e-cigarettes in pregnancy will not expose the fetus to CO, they will be exposed to nicotine which has been shown to have a negative impact on neurobehaviour. Nicotine has extensive effects on the central nervous system (CNS), with the deficits reflecting the biological and behavioural systems that are modulated through neural feedback (Ekblad, Korkeila, & Lehtonen, 2015; Hsieh et al., 2011; Law et al., 2003; Lester & Tronic, 2004). Later in childhood, exposure to nicotine has been associated to attention deficit hyperactivity disorder (ADHD) (Sourander et al., 2019). However, no research has currently been published to establish the impact of prenatal exposure to e-cigarettes may have on neurobehavioural outcomes of human infants. At present, animal studies have been the main focus emphasising the negative result of nicotine exposure on brain development, (Slotkin et al., 2005) with human infant research yet to be undertaken. Primate models on the effects of nicotine exposure demonstrate that nicotine is highly selective for various brain regions with cell signalling and cell damage occurring leading to disrupted brain development. Specifically, the cognitive impairments observed are likely to be a result of proliferation and maturation in the medial prefrontal cortex of the progenitor cells leading to a decrease of glutamatergic neurons (Aoyama et al., 2016). This has been shown in primates and rodents when exposed to levels of nicotine comparable to that of an adult smoker, with sufficient amount of nicotine reaching the fetal brain

eliciting neurodevelopmental changes, regardless of the gestational time point nicotine is administered (Alkam et al., 2013; Slotkin et al., 2005).

Due to the critical role of neurobehaviour in an infant's development and the lack of guidance regarding the effects of e-cigarette use during pregnancy, the present study aims to examine how prenatal exposure to e-cigarettes compares to cigarettes and to no exposure on birth outcomes (i.e., gestation at birth, birth weight and head circumference). Additionally, neurobehavioural outcomes in one-month old infants (i.e., measured using the Neonatal Behavioural Assessment Scale (NBAS) will be reported (Brazelton & Nugent, 1995). Based on current evidence it is hypothesised that there will be a significant difference in birth outcomes (i.e., shorter gestation, lower birth weight and smaller head circumference) in cigarette exposed compared with non-exposed infants, but no significant differences are expected between e-cigarette exposed infants and non-exposed infants because e-cigarette use in pregnancy is not expected to reduce the oxygen carrying capacity of fetal blood. Secondly, it is hypothesised, that due to the direct impact of nicotine on brain development, e-cigarette exposed infants will demonstrate a similar pattern of neurobehavioural deficits to cigarette exposed infants. This is the first study assessing the neurobehavioural outcomes of the new-born as a result of nicotine exposure via e-cigarette use.

Methods

The report is written in accordance with the STROBE guidelines (Vandenbrouckel et al., 2007). Ethical approval was granted by Durham University and mothers provided informed consent before any assessment was conducted.

This case-control study includes 83 white British infants who were assessed in their home at one time point at approximately one month of age (m=32.6 days, S.D.=5.33) using the NBAS (Brazelton & Nugent, 1995). These infants were part of a larger study assessing fetal and infant behavioural development in relation to nicotine exposure conducted in collaboration with The James Cook University Hospital, Middlesbrough, UK. Eligibility criteria for inclusion was the infant was born at term (>37 weeks), healthy and no NICU admission, no prenatal alcohol consumption and no prescription or recreational drug use. Women using alternative methods of NRT such as patches, gum or inhaler were not eligible for this study due to the interest in e-cigarettes as a harm reduction method.

The e-cigarette use and cigarette smoking behaviour of the mother was obtained at 32 weeks gestation due to the known effects of nicotine exposure on the fetal brain leading to behavioural differences in the early infancy period (Ekblad et al., 2015). Smoking status was self-reported with a CO breath test to confirm nicotine groupings (see Table 1). All mothers were assessed using the Bedfont Smokerlyser breath test, with scores >3 parts per million (ppm) for CO indicative of mothers who smoked. This measure was used to confirm maternal self-report of smoking status. For e-cigarette users, milligrams of nicotine stated on the product's packaging was self-reported. Two prenatal e-cigarette users reverted back to cigarette use following the

birth of their infant, but due to prenatal exposure, these infants remained in the prenatal e-cigarette exposure group. The demographic information for each group is shown in Table 6.1.

Table 6.1. Demographic information

Nicotine group	Mean CO reading (% of CO in maternal blood)	Number of infants	Gender Male/Female	Number of households with additional cigarette smokers	Mean years of maternal cigarette use prior to conception	Number of primiparous mothers	Highest educational qualification
Non-exposed	0·97	44	23/21	3	0·34	21	None: 0 GCSE: 9 College/A-levels: 9 Degree: 18 Masters: 8
Cigarette exposed (1-20 per day)	2·74	29	15/14	10	11·2	8	None: 9 GCSE: 14 College/A-levels: 4 Degree: 2 Masters: 0
E-cigarette exposed	0·95	10	1/9	2	4·2	7	None: 0 GCSE: 5

(3-16mg in the product)							College/A-levels: 5 Degree: 0 Masters: 0

Birth outcomes for each infant were received from the hospital or recorded at the one month follow up. Given the known association between maternal mental health to both fetal and infant outcomes (Federenko & Wadhwa, 2004), mothers completed a range of questionnaires assessing perceived stress (Cohen, Kamarck, & Mermelstein, 1983), depression and anxiety as measured by the Hospital Anxiety and Depression Scale (Zigmond & Snaith, 1983) at the 32 week ultrasound scan. A postnatal attachment questionnaire was completed at the one month follow up (Condon & Corkindale, 1998). Alongside maternal age and additional household smokers, these factors were controlled for in the analysis where appropriate.

For measures of orientation, motor maturity, range of states, regulation, and automatic stability, the NBAS scores infants on a Likert scale from 1-9 (Brazelton & Nugent, 1995) and recoded following the method outlined by Lester (1984; as cited in Brazelton & Nugent, 1995). The reflexes were tested for the number of abnormal reflexes (Lester, 1984). Seventeen reflexes were assessed as outlined by the NBAS including; Plantar, Babinski, ankle clonus, rooting, glabella, passive leg tone, passive arm tone, palmer grasp, placing, standing, stepping, crawling, incurvation, tonic deviation, nystagmus, TNR and Moro. These reflexes were rated at the time of the assessment between 0-3. For ankle clonus, nystagmus and TNR, scores of 3 are considered abnormal. For all other reflexes, a score of 2 is normal and scores of 0, 1 or 3 are considered abnormal. Normal reflexes are co-ordinated, strong, and modulated responses, anything other is considered abnormal such as weak reflexes or obligatory reflexes with little relaxation following the end of the reflex (Brazelton & Nugent, 1995). The NBAS was used as previous research has indicated assocations between maternal prenatal smoking and neurobehvaiour using this method

(Hernandez-Martinez et al., 2021; Mansi et al., 2007), which suggests it is senstive enough to capture potential subtle differences in early infancy, with good predicitive validity (Ohgi et al., 2003) and reliability (Başdaş et al., 2018) and validity (Lizarazp et al., 2012).

Data analysis

ANOVAs were conducted to assess group differences for birth outcomes (gestation, birthweight and head circumference) and NBAS outcomes (reflexes, regulation, motor maturity, orientation, range of states and automatic stability). Seven potential covariates (maternal age, infant sex, primiparity, additional household smokers, stress, depression and anxiety) were correlated with each outcome measure to assess suitability for inclusion in an ANCOVA. Covariates which significantly correlated with the outcomes were included in the ANCOVA.

We also correlated the self-reported mg of nicotine (for the e-cigarette group) and the number of years the mother smoked prior to conception (all exposure groups) with NBAS outcomes. However, given the data is not independent of exposure group, significant correlations could not be included in the ANCOVA.

Series means estimates were used for missing data. Bootstrap methods were employed due to the small sample and likely variation within the population, 1,000 resamplings were performed. Analysis was conducted using the Statistical Package for the Social Sciences version 26 (SPSS).

Role of the funding source

The funding source had no involvement in the study design, data collection, data analysis, interpretation, report writing or decision to submit the paper for publication.

Results

The aims of the study were to assess whether birth outcomes and neurobehavioural outcomes differed between prenatal non-exposed, cigarette exposed and e-cigarette exposed infants.

As shown in Table 6.2, there were significant differences in maternal age between the groups, $F(2,82)=8.263$, p=.001, $\eta^2=.171$. Mothers who did not smoke during pregnancy were significantly older in comparison to smokers (p=.004, d=.680) and e-cigarette users (p=.001, d=1.253). None of the other covariates were significantly different between the groups. The correlations between the covariates and the birth outcomes and NBAS measures are shown in Table 6.3. Only covariates that significantly correlated with the outcomes were included in the ANCOVA.

Regarding birth outcomes, no significant differences for gestation at birth between the three exposure groups were observed, $F(2,82) = 1.652$, p=.198, $\eta^2=.040$. Significant differences were observed for birthweight, $F(2,82) = 4.192$, p=·019, $\eta^2=.095$. Pairwise comparisons applying the Bonferroni correction confirmed that cigarette exposed infants had a significantly lower birthweight in comparison to non-exposed infants (p= .021, d=.656), but differences in birthweight for e-cigarette

exposed compared to non-exposed and cigarette infants was not significant (p=1, d=.030; p=.188, d=.893). None of the covariates were significantly correlated with birthweight (see Table 6.3). Therefore, no ANCOVA was conducted.

There were also significant differences between the exposure groups in head circumference, $F(2,82)=4.771$, p= .011, $\eta^2=.107$. Cigarette exposed infants had a significantly reduced head circumference in comparison to non-exposed infants (p=.008, d=.763), with e-cigarette exposed infants not differing to non-exposed infants (p=1, d=.242) or cigarette exposed infants (p=.525, d=.533). No covariate correlated with head circumference (see Table 6.3), therefore no ANCOVA was conducted.

Table 6.2. Means and standard deviations for birth outcomes, maternal characteristics and NBAS outcomes split by nicotine group.

	Mean Non-exposed [(a)]	Standard deviation	Mean Cigarette exposed[(b)]	Standard deviation	Mean E-cigarette exposed [(c)]	Standard deviation
Maternal age (years)[*,a-b, a-c]	28.84	4.86	25.52	4.911	22.60	5.52
Stress	10.64	6.36	13.14	6.84	15.40	4.37
Depression	2.86	2.59	5.21	3.29	4.50	2.71
Anxiety	4.55	3.02	6.41	3.67	5.50	2.75
Attachment	72.104	3.979	72.942	3.062	71.026	3.952
Gestation (weeks)	39.178	1.36	39.11	1.26	39.98	.77
Birthweight (grams)[*, a-b]	3451.92	596.69	3098.37	434.89	3477.11	257.91
Head circumference (cm)[*,a-b]	34.75	1.48	33.63	1.45	34.38	.89
Apgar 1 minute	8.833	.618	8.935	.428	8.841	.319
Apgar 5 minutes	9.435	.455	9.592	.473	9.178	.576
Labour length (minutes)	287.699	192.719	311.827	298.391	250.375	178.959
Reflexes[*,a-b, a-c]	2.11	1.72	4.59	2.18	5.60	2.503
Orientation	6.18	1.38	5.83	.94	5.63	1.60

Motor maturity[*,a-b]	5.97	.57	5.39	.82	5.48	.755
Range of states	3.70	.97	3.55	.95	3.80	1.01
Regulation[*,a-b]	4.88	1.22	4.20	.84	3.80	1.76
Automatic stability	6.97	1.18	7.08	1.08	7.21	.83

[*]*Significant main effect, p<.05*

[a-b] *significant posthoc between non-exposed and cigarette exposed.*

[a-c] *significant posthoc between non-exposed and e-cigarette exposed.*

[b-c] *significant posthoc between cigarette exposed and e-cigarette exposed.*

Significant differences were observed across the nicotine groups for reflexes $F(2,82) = 20.338$, $p<\cdot001$, $\eta^2=.338$, motor maturity, $F(2,82) = 6.769$, $p=.002$, $\eta^2=.145$, and regulation $F(2,82) = 4.877$, $p=.010$, $\eta^2=.110$. There were no significant differences observed for measures of orientation ($p=.340$, $\eta^2=.027$), range of states ($p=.725$, $\eta^2=.008$) and automatic stability ($p=.798$, $\eta^2=.006$). There were significant correlations between number of years smoked prior to conception and reflexes (r= .432, p=<.001), motor maturity (r=-.232, p=.035) and regulation (r=-.226, p=.758). In addition, there was a significant correlation between mg of nicotine in the e-cigarette exposure group and motor maturity (r= -.349, p=.001), however no other NBAS outcome measures were significantly associated with mg of nicotine.

Pairwise comparisons applying the Bonferroni correction for reflexes indicate significant differences between infants not exposed and exposed to cigarettes ($p=.001$, $d=1.263$) and e-cigarettes ($p=.002$, $d=1.625$). There were no significant differences found between cigarette exposed and e-cigarette exposed infants ($p=.236$, $d=.287$). Similarly, when adjusting for maternal depression (see Table 6.3), significant differences were observed across the three nicotine groups for reflexes $F(2,82) = 16.479$, $p<.001$, $\eta^2=.294$. Assessing the pairwise comparison for the NBAS outcomes accounting for maternal depression using the Bonferroni correction, significant differences were found between non-exposed and cigarette exposed ($p=.001$, $d=1.263$) and e-cigarette exposed infants ($p=.001$, $d=1.625$).

Similarly, for motor maturity, pairwise comparisons with the Bonferroni correction indicate significant differences between non-exposed and those exposed to cigarettes ($p=.002$, $d=.821$) and between non-exposed and e-cigarette exposed infants ($p=.036$,

d=.732). There were no significant differences between e-cigarette and cigarette exposed infants (p=.745, d=.103). When controlling for maternal age and maternal depression, this effect becomes marginal, $F(2,82) = 2.941$, p=.059, η^2=.070.

For regulation, pairwise comparisons with the Bonferroni correction indicate significant differences between non-exposed and those exposed to cigarettes (p=.010, d=.649). There were no significant differences between non-exposed and e-cigarette exposed infants (p=.057, d=.713) and between cigarette exposed and e-cigarette exposed infants (p=.454, d=.358). No covariates were significantly correlated to regulation (see Table 6.3), therefore ANCOVA was not conducted.

Table 6.3. Correlations (with p-values) between maternal and infant characteristics and birth outcomes and NBAS

	Maternal age	Stress 32[7]	Anxiety[8] 32	Depression[9] 32	Attachment Postnatal	Additional Smokers	Number of years smoked prior to conception [10]	Infant sex	Primiparity
Gestation	-.116 (.296)	-.071 (.526)	-.097 (.384)	-.109 (.327)	.120 (.320)	.080 (.473)	-.038 (.736)	.129 (.282)	.095 (.395)
Birthweight	-.089 (.423)	-.076 (.266)	-.123 (.266)	-.020 (.857)	-.188 (.117)	-.012 (.916)	-.292 (.007)*	.118 (.324)	.022 (.843)
Head circumference	-.102 (.360)	-.037 (.737)	-.093 (.405)	-.037 (.742)	-.052 (.667)	-.132 (.234)	-.292 (.007)*	.071 (.551)	-.010 (.927)
Reflex	-.204 (.064)	.118 (.288)	.147 (.184)	.263 (.016)*	-.114 (.345)	.157 (.157)	.432 (<.001)*	-.184 (.121)	-.175 (.113)
Motor maturity	.218	-.033	-.139	-.253	-.232	-.033	-.232	-.014	.125

[7] The Perceived Stress Scale was administered prenatally at the mother's 32-week hospital ultrasound appointment.
[8,3] The Hospital Anxiety and Depression Scale was administered prenatally at the mother's 32-week hospital ultrasound appointment.

[10] As this measure is not independent of the IV (exposure group), significant correlations could not be included in the ANCOVA.

	(.047)*	(.768)	(.209)	(.021)*	(.051)	(.770)	(.035)*	(.905)	(.254)
Regulation	.022	-.097	-.095	-.114	-.020	.001	-.226	.016	-.020
	(.844)	(.387)	(.394)	(.306)	(.868)	(.991)	(.042)*	(.891)	(.861)
Orientation	-.062	.017	-.032	-.139	.011	.004	-.179	.185	.150
	(.584)	(.880)	(.775)	(.217)	(.929)	(.971)	(.111)	(.126)	(.182)
Range states	-.083	-.053	.026	.056	-.090	-.079	-.075	-.010	.177
	(.457)	(.634)	(.813)	(.616)	(.868)	(.479)	(.500)	(.930)	(.110)
Automatic stability	-.116	.034	.024	-.034	-.231	-.008	.034	-.058	.163
	(.296)	(.763)	(.831)	(.760)	(.053)	(.940)	(.758)	(.626)	(.141)

*$p<.05$

Discussion

It was hypothesised that there would be a significant difference in birth outcomes (birthweight, gestation at birth and head circumference) between cigarette exposed and non-exposed infants, but no significant difference between e-cigarette exposed and non-exposed infants. Secondly, it was hypothesised that e-cigarette exposed infants will demonstrate similar neurobehavioural outcomes to cigarette exposed infants, compared to non-exposed infants. These hypotheses received partial support.

The results regarding the birth outcomes indicate that, in contrast to previous research (Pereira, Da Mata, Figueiredo, de Andrade, & Pereira, 2017; Shah & Bracken, 2000), there is no significant difference between cigarette exposed and non-exposed infants for gestation at birth. The majority of research assessing prenatal cigarette exposure and gestation at birth focuses on the greater risk of preterm delivery before <37 weeks gestation. However, in the present study, infants were only included if they were born at at least 37 weeks gestation, due to the associated complications with preterm delivery such as poorer physiological health and developmental immaturity (McGowan, Alderdice, Holmes, & Johnston, 2011). This could explain why we did not find a difference between cigarette and non-exposed groups. Nevertheless, as predicted there are significant differences regarding birthweight and head circumference between these two groups. For e-cigarette exposed infants, no significant differences were observed in comparison to non-exposed infants for gestation, birthweight or head circumference, in line with previous findings and our predictions (McDonnell, Bergin, & Regan, 2019). In this particular sample, there is no evidence suggesting birth outcomes are affected as a result of e-cigarette exposure.

Given that infants prenatally exposed to e-cigarettes did not experience the same birth outcomes as cigarette exposed, but were similar to non-exposed infants, it could indicate a likely culprit for these negative outcomes is CO exposure. It is well established that CO exposure is associated with low birth weight (Merklinger-Gruchala et al., 2017; Stieb, Chen, Eshoul, & Judek, 2012). This is due to CO binding to haemoglobin reducing blood flow and subsequently leading to growth restriction (Ekblad et al., 2015). Based on the current findings, when CO is removed, through use of an e-cigarette, low birth weight appears to be no longer concerning, however, further exploration on larger samples is needed to add further support.

In relation to NBAS outcomes, the results indicate that motor maturity, self-regulation, and reflexes are different across exposure groups. Interestingly, these measures were also correlated to number of years the mothers smoked prior to conception. The longer the mother smoked, the worse the infants' regulation and motor maturity, and these infants would also demonstrate a greater number of abnormal reflexes. Epigenetic research argues that smoking can have a cumulative effect, with the month prior to conception being a critical time point for early placental development, with altered development leading to changes in brain structure and function (Stephenson et al., 2018).

The findings indicated that both cigarette exposed and e-cigarette exposed infants demonstrate a decrease in motor maturity when compared to non-exposed infants. However, in contrast to previous literature (Froggatt et al., 2020a), when the maternal age and maternal depression were controlled for, the effect smoking has on motor maturity was no longer significant. The differences between the groups might partly

reside in the fact that the non-smokers in our sample were older and reported fewer depressive symptoms, although not significant, in comparison to the mothers using e-cigarettes or smoking. Interestingly, mg of nicotine for the e-cigarette exposed infants correlated with their motor maturity score, indicating that the higher the mg of nicotine, the lower they score on motor maturity.

In regard to self-regulation, cigarette exposed infants displayed decreased abilities in comparison to non-exposed infants, which is consistent with previous research (Froggatt et al., 2020a). Although the difference between non-exposed and e-cigarette exposed infants was not significant, this result was approaching significance with a large effect size. Measures of self-regulation include self-relaxation of the infant when held, how consolable the infant is following a period of crying, self-quieting abilities and hand-to-mouth movements (Brazelton & Nugent, 1995). Infants who demonstrate decreased self-regulation abilities are often more irritable and need external consoling. Regulation is important for subsequent infant psychomotor and emotional development. In addition, early regulation abilities predict development at 4 and 12 months and in turn predict intellectual development at 6 years of age (Canals et al., 2011). Because of potential long-term consequences associated with decreased self-regulation abilities, and due to the large effect size, this warrants further exploration.

The novel findings reported here demonstrate the negative effect e-cigarettes have on reflexes. When controlling for maternal depression, a large effect size was shown between non-exposed and e-cigarette exposed infants, with the latter demonstrating more abnormal reflexes. The results between non-exposed and cigarette exposed

infants are supported by previous research (Froggatt et al., 2020a). It is likely that these results are generalisable to the population, given the large effect size. Given that reflexes are related to both cigarettes and e-cigarette exposure, this suggests that nicotine consumption in pregnancy regardless of delivery method is a potential cause for concern.

Primitive reflexes have a developmental role allowing the infant to interact with their environment in a basic way, essential for newborn survival and preparing the infant for voluntary movements (Melillo, 2016; Sohn, Ahn, & Lee, 2011). These reflexes are automatic involuntary patterns of movement that are mediated by the brainstem (Modrell & Tadi, 2020). They support the development of natural movement patterns allowing the infant to reach early voluntary motor milestones such as grasping, rolling, and crawling (Melillo, 2016). They gradually reduce when the infant is between 4-6 months of age and occurs once the CNS matures with movements becoming voluntary, with retained reflexes a cause for concern. The CNS maturation leads to a transition of control of movements from brainstem responses, to cortically controlled responses (Gieysztor, Choińska, & Paprocka-Borowicz, 2018). As primitive reflexes are controlled by the CNS, mediated by the brainstem (Gieysztor et al., 2018) it is likely that exposure group differences are a result of the widespread effects of nicotine activating nicotinic acetylcholine receptors (nAChRs) across the CNS (Lv et al., 2008).

These results may have occurred due to exposure to nicotine prenatally. The fetal brain is susceptible to damage and the vulnerability is dependent upon whether a toxin can penetrate the fetal CNS (Rice & Barone Jr, 2000). The developing brain is

protected from a range of neurotoxins; however, nicotine readily crosses the syncytium, targeting specific neurotransmitters, causing an accumulation of nicotine in fetal tissue, ultimately resulting in impaired fetal brain development (Dempsey & Benowtiz, 2001). NAChRs are widespread throughout the CNS controlling cell replication and differentiation (Lv et al., 2008; Slotkin et al., 2005). Rodent studies indicate brain growth restriction, fetal hypoxia and brain development are negatively impacted by prenatal nicotine exposure as a result of nAChRs expression (Lv et al., 2008). However, a key concern of reflecting on rodent studies to provide an indication of the impact of nicotine is that in comparison to human infants, rodents have a longer period of postnatal CNS maturation, therefore comparison is difficult (Rice & Barone Jr, 2000). However, primate studies do not pose such problems, yet have found similar results. In primates, nicotine exposure leads to cell damage and cell signalling disruptions leading to changes within brain development (Slotkin et al., 2005). Whilst animal studies indicate the brain changes as a result of prenatal nicotine exposure, they are unable to provide evidence of 'real-life' application effects, such as neurobehavioural implications. Therefore, in order to provide evidence for policy change, research should focus on the impact on human infants.

A concern is that e-cigarettes are termed a harm reduction method for use in pregnancy (Smoking in Pregnancy Challenge Group, 2019). However, the present findings indicate that there could be harm associated with e-cigarette use and therefore the ultimate aim must be to stop smoking, without the use of e-cigarettes. Indeed, caution should probably be applied to all NRT products. Given the predictive nature of newborn assessments (Canals et al., 2011), in particular the NBAS, the

notion that nicotine by itself is relatively harmless, is a concept that needs to be further questioned and further investigated.

Further research is vital in order to establish the effects of nicotine on postnatal neurological outcomes, including a biological element. It is difficult to quantify how much of an e-cigarette is used on a daily basis and in this study self-report was relied on to measure mg of nicotine in the e-cigarette product. This is in comparison to daily self-reported use of cigarettes which may be easier to quantify. Therefore, a more objective measure of nicotine exposure, via cotinine, would aid further development of such research. Cotinine is a metabolite of nicotine and can be measured in both the smoker and those exposed to secondhand smoke (Aylward, 2018). Whilst measuring cotinine can provide further evidence to support the effects of nicotine on infant neurobehavioural outcomes, it is important to note that e-cigarettes contain a variety of other toxic compounds. For example, one study identified metals present in the e-liquid vapour such as cadmium, chromium, lead, manganese and nickel which could also be producing carcinogenic effects (Hess et al., 2017). Nonetheless, given that this research has demonstrated that nicotine exposure through e-cigarette use is associated with a significantly greater number of abnormal reflexes, future research needs to explore the risks associated with NRT, such as patches and inhalers for use in pregnancy.

An additional limitation of the research, as with all epidemiological research, is the potential impact of unmeasured possible confounding factors. For example, in this study, socioeconomic status (SES) was not assessed. And although research suggests that SES can influence child development through its effects on how parents interact

with their children, there is little evidence that SES is directly associated with infant outcomes (Hoff, Laursen, Tardif, & Bornstein, 2002; Law et al., 2003). Addtionally, research has suggested that highly educated mothers spend more time engaging with the infant and providing more cognitively stimulating activities for their infants in comparison to mothers with a lower educational level (McLoyd, 1990; Ryan & Corey, 2012; Padilla, Hines & Ryan, 2020). Due to the lack of stimulation and potential physical contact, the infant is likely to show delays in their neurodevelopment (Gutman & Feinstein, 2010). However, in this study maternal educational level was not associated to infant outcomes.

Additonally, infants who score high on measures of breastfeeding also score highly on neurobehvaioural measures (Radzyminski, 2005). This is particular important to measure given that mothers who smoke during pregnancy often have reduced rates of breastfeeding (Giglia, Binns & Alfonso, 2006). Research has indicated that infants born to smoking mothers have lower breastfeeding rates and lower scores on neonatal behavioural assessments (Bertini, Elia, Lori & Dani, 2019), thus it would be interesting to assess in a future study whether breastfeeding by smoking mothers will lead to an increased neurobehavioural score in their infants. Although as part of the wider study breastfeeding was assessed, this particular question was not assessed.

This is the first study assessing neurobehavioural outcomes associated with prenatal nicotine exposure through cigarettes or e-cigarettes at one month old. Overall, results indicate that birthweight, gestation and head circumference measurements do not differ between prenatal e-cigarette exposure and no exposure. Importantly, regardless of prenatal nicotine exposure (cigarettes or e-cigarettes), this research found a

significantly greater number of abnormal primitive reflexes, alongside marginally decreased self-regulation abilities compared with non-exposed infants. These findings have important implications for policy guidelines regarding the use and safety of e-cigarettes during pregnancy as a method of harm reduction.

Chapter 7

The association between prenatal mouth movement frequency and postnatal behaviour at one-month post birth

Abstract

Fetal neurobehaviour is a relatively new field of psychology, with a number of assessment measures being developed. The Fetal Observable Movement System (FOMS) is a fine-grained analysis tool focusing on fetal facial movements. Despite the increasing number of research articles using this method, it is currently unknown what the postnatal implications of such fetal mouth movements are. The frequency of fetal mouth movements were assessed at 32- (N=75) and 36- (N=67) weeks gestational age via 4D ultrasound scans and infants were followed up at one-month post-birth using the Neonatal Behavioural Assessment Scale (NBAS). Results indicated there is no significant relationship between frequency or clusters of fetal mouth movements at either gestational time point to neurobehaviour at one month. It may be that a variety of fetal assessment measures are needed in order to understand what prenatal movements mean for postnatal behaviour, focusing on the complexity of movements.

Introduction

Over the past 40 years ultrasound technology has progressed from basic 2-dimensional (2D) to 4-dimensional (4D) imaging. This advancement has led to changes in the field of perinatal medicine, not only to clearly assess fetal anatomy,

but also fetal neurobehaviour by recognising behavioural movement patterns (Birnholz, Stephens, & Faria, 1978). In light of the development of 4D-ultrasound, a number of assessment tools have been developed. The three main assessments are Kurjak's Antenatal Neurodevelopmental Test (KANET) (Kurjak et al., 2008), the Fetal Neurobehavioral Assessment System (FENS) (Salisbury, Fallone, & Lester, 2005), both which focus on overall gross body movements and the Fetal Observable Movement System (FOMS) (Reissland, Francis, & Buttanshaw, 2016), a fine-grained movement analysis system focusing on fetal facial movements. All three assessment measures have demonstrated differences in fetal behaviour dependent upon a range of maternal and fetal conditions (Neto & Kurjak, 2015; Reissland, Makhmud, & Froggatt, 2019; Reissland et al., 2020a; Stroud, Bublitz, Crespo, Lester, & Salisbury, 2020). However, relatively little research has attempted to understand the implications of what these fetal behavioural patterns mean for postnatal behaviour and development (DiPietro et al., 2010; Stroud, McCallum, & Salisbury, 2018).

Prenatal behaviour serves as a foundation for postnatal functioning (DiPietro et al., 2010; Glover, O'connor, & O'Donnell, 2010), with some early research relying on mothers counting fetal movements such as kicks and hiccups (Walters, 1965). Correlations have been shown between fetal activity, as perceived by the mothers, and measures of language, motor, adaptive and social development in childhood, at three, six and nine months of age (Walters, 1965). However, due to the drawbacks of the subjective nature of maternal perceptions of fetal movements (Hijazi & East, 2009), a better objective method was made possible with the development of ultrasound scanning. Some research suggests continuity of behavioural patterns from

pre to postnatal life, measured by eye blinks, mouth movements and hand-to-mouth motions (Kurjak et al., 2004).

One of the greatest challenges in perinatal medicine is the ability to detect and determine abnormalities prenatally, due to the uncertainty of the timing of when the impairment may have occurred: prenatally, during birth or postnatally. However, with the use of ultrasound and fetal behavioural assessment measures, clinicians are increasingly able to detect neurological impairments prenatally using measures such as KANET (Kurjak et al., 2017). KANET is a diagnostic tool that has the ability to identify neurological signs of impairment prenatally by assessing isolated limb movements, facial movements and hand to mouth movements, with the majority of such impairments being confirmed in the postnatal period (Kadić et al., 2016; Neto & Kurjak, 2015). In addition, a relationship between the FENS and the NICU Network Neurobehavioural Scale indicated that fetal activity, complex body movements, isolated movements and coupling index were associated with a range of infant measures including self-regulation, attention, handling, lethargy and quality of movement in the first month after birth (Stroud et al., 2018).

Research in the prenatal period has been conducted using the FOMS (Reissland et al., 2016; Reissland, Francis, Kumarendran, & Mason, 2015; Reissland et al., 2019; Reissland et al., 2020a), yet to date, the postnatal implications of this assessment measure are unknown. Using the FOMS, experimenters are able to identify differences in fetal behavioural profiles between fetuses exposed to toxins, maternal conditions and genetic disorders. For example, a recent study assessed the impact of Hyperemesis Gravidarum (HG) on fetal mouth movement profiles and found that the

fetuses of mothers experiencing HG compared with healthy mothers, demonstrate a higher rate of mouth movements (Reissland et al., 2020a). Additionally, following a retrospective assessment of a 4D ultrasound scan using the FOMS after a postnatal diagnosis of Prader Willi Syndrome, results indicated abnormality of fetal activity due to the significant lack of mouth movement in comparison to healthy fetuses, when exposed to sound and light stimulation (Reissland et al., 2019). Such research indicates that the FOMS can be used as a marker for potential genetic disorders. However, research using the FOMS is mixed with one study indicating that rate of mouth movements change dependent upon maternal stress and exposure to cigarettes (Reissland et al., 2015), but in a partial-replication study (Chapter 4) this finding was not supported. In the current study, we focused on mouth movements as much of the prenatal research using the FOMS focused on this (Reissland et al., 2015; Reissland et al., 2019; Reissland et al., 2020a), due to the suggestion that fetal behavioural differences can provide an insight into normal and abnormal development (Reissland & Kisilevsky, 2016). At present, the implications of fetal mouth movement differences are unknown.

The FOMS focuses on the fetal facial movements, as it is thought these movements can provide an indication of the development of the fetal brain (AboEllail & Hata, 2017; Grigore et al., 2018). Correlations between the development of fetal facial movements and the structure of the central nervous system (CNS) have been established (Morokuma et al., 2004). However, the implications of such fetal research are currently unknown. As the fetus develops, their movements become increasingly co-ordinated and precise, generally decreasing which indicates further precision with these movements becoming smooth opposed to jerky between 26-36 weeks

gestational age (Grant-Beuttler et al., 2011; Reissland & Francis, 2010). The frequency of movements decrease as a function of gestational age, for example, when assessing leg movements, these decline in the third trimester from 30-37 weeks' gestation (Almli, Ball, & Wheeler, 2001). Almli et al. (2001) argue that a decline in leg movements is linked to CNS maturation and behavioural development. They suggest that in the third trimester, the fetus undergoes vast changes both in terms of structure and function of the CNS, which can be observed by examining fetal spontaneous movements and behavioural states. The decline in frequency of fetal movements is an indication of the fetal maturation processes. Such claims are supported by research assessing the relative frequency of fetal mouth movements across the gestational age, with the rate declining by 3% for each additional gestational week (Reissland, Francis, Aydin, Mason, & Schaal, 2014). A similar finding was also shown in Chapter 4, demonstrating a reduction in mouth movement over time. Hence, we expect that at 36 weeks gestational age, due to the overall decline in movement becoming more coherent, that movements at this age are likely to be a stronger predictor, in comparison to the 32-week data, of infant neurobehaviour at one-month post birth.

As fetal mouth movements are thought to be reflective of the maturation processes of the CNS (Morokuma et al., 2004; Reissland, Francis, Aydin, Mason, & Exley, 2014), in order to provide support for this claim, in the current study, a neurobehavioural assessment was conducted at one month of age. Neurobehaviour is observable behaviour that is moderated by neural feedback (Lester & Tronic, 2004). The NBAS is an assessment measure that is widely used to assess neurological behavioural development of infants and is particularly useful when examining a range of

environmental toxins. For example, research has demonstrated that prenatal exposure to nicotine (cigarettes and e-cigarettes) leads to a greater number of abnormal reflexes and worse regulation [11](Froggatt, Reissland, & Covey, 2020b). Additionally, exposure to toxins, in particular polychlorinated biphenyls (PCBs) consumed via maternal fish consumption from contaminated lakes leads to poorer performance on a range of NBAS outcomes (Sagiv et al., 2008; Stewart, Reihman, Lonky, Darvil, & Pagano, 2000). Furthermore, infants who were small for gestational age demonstrated poorer outcomes on the NBAS (Figueras et al., 2009). The NBAS has been a useful tool for identifying children who have later behavioural problems, with poor motor maturity, reduced self-regulation and orientation significantly linked to later childhood behavioural problems (Ohgi, Takahashi, Nugent, Arisawa, & Akiyama, 2003). The NBAS is considered a gold standard approach to assessing infant neurobehaviour as the outcome measures reflect the brain maturation processes (Cruz-Martinez et al., 2009).

The current study aims to assess whether prenatal behaviour at either 32- or 36-weeks gestational age, as identified by fetal mouth movements, relates to postnatal neurobehaviour, in a sample of heterogenous women. If support for such claim is provided, it would indicate the importance and implications of examining fetal mouth movement profiles according to a range of maternal conditions and exposures. Mouth movements were the focus of the study, due to the number of studies using the FOMS but specifically examining the mouth movements (Reissland et al., 2015; Reissland, Francis, & Mason, 2012; Reissland et al., 2019; Reissland et al., 2020a), despite the unknown postnatal behavioural implications. Given that the NBAS is a well-

[11] See Chapter 6.

established neurobehavioural assessment measurement and the FOMS, claiming to assess fetal neurobehaviour, we anticipate a relationship between these two measures. Neurobehavioural assessments generally are thought to provide an indication of brain development, with research stating that scores on such measures relate to intracranial and cortical gray matter volume (Tolsa et al., 2004). The current study tests whether relative frequency or clusters of fetal mouth movements are an indication of neurobehaviour, by associating it to a known neurobehavioural postnatal assessment.

The importance of this research resides in the understanding that fetal activity, in particular mouth movement profiles, have the ability to differentiate fetuses according to a range of insults, and thus can provide an early indication of impairment (AboEllail & Hata, 2017; Grigore et al., 2018; Reissland et al., 2015; Reissland et al., 2019; Reissland et al., 2020a). Continuity and associations between pre-and-postnatal behaviours have been identified when assessed via the FENS and KANET. In spite of the increasing research using the FOMS, there is currently no evidence to indicate the implications of the changes in fetal mouth movements, and hence the present research aims to address this. We hypothesise that prenatal behaviour, as identified by mouth movements, will be associated to postnatal behaviour assessed by the NBAS, with stronger predictive validity at 36-weeks gestational age in comparison to 32-weeks' gestation.

Method

The 4D ultrasound scans were conducted at James Cook University Hospital, Middlesbrough and the Friarage Hospital, Northallerton, UK. Initially, 123 pregnant

women were recruited to participate in the research, with the following inclusion criteria; aged 18-40 years, BMI between 18-25, not taking any prescription or recreational drugs, no maternal medical conditions such as diabetes and a low-risk pregnancy.

Fetuses were scanned at 32 weeks (M=31.890 weeks, S.D.= .650) and 36 weeks (M= 35.679 weeks, S.D. = .519) gestational age. The scans lasted approximately 15-20 minutes and carried out by an NHS trained sonographer. The 4D ultrasound scans were recorded for offline analysis.

As shown in Table 7.1, the sample is heterogeneous with pregnant women varying in age, education, smoking status, levels of stress, anxiety, depression, and attachment scores[12]. All mothers provided informed consent prior to participating in the research. Ethical approval was granted by NHS ethics committee (REC reference, 11/NE/0361) and Durham University (reference 17/27).

[12] This information was collected as part of the prenatal study outlined in Chapter 4.

Table 7.1. Demographic information based on the sample at 32 weeks gestation.

Age	**Highest educational level**	**Smoking status**	**Stress**	**Depression**	**Anxiety**	**Attachment**	**Maternal CO**
M= 26.95 years S.D.= 5.555 *Min= 18 years Max= 39 years	None= 8 GCSE= 26 A-level/College=16 Degree= 16 Masters= 8 Missing= 1	Non-smoker=38 Smoker=27 E-cigarette user= 10	M=12.03 S.D.=6.522 Min= 0 Max= 31	M= 3.92 S.D.=3.075 Min= 0 Max= 16	M=5.40 S.D.=3.205 Min= 0 Max =17	M= 82.48 S.D.= 6.08 Min= 67 Max= 94	M= 1.60 S.D.= 1.07 Min= .79 Max= 5.27

***** *Minimum and maximum scores on each assessment measure of the included participants.*

Despite 123 pregnant women volunteering to participate in the research, not all scans were analysed at 32- and 36-weeks gestational age. This was due to the fetus in a poor position for facial analysis, technical recording issues and subsequent drop out. Postnatally, some families did not participate due to the infant being born <37 weeks, NICU admission or dropped out of the research. Paired data sets are assessed as the focus is on the relationship between the prenatal and postnatal behavioural measurements. Based on 32 weeks gestational age there are 75 pairs and 67 pairs based on the 36-week data. Based on this sample, we would need an effect size at 32 weeks of f^2= .107, and f^2= .120 based on the 36-week gestational age data.

The 4D ultrasound scans were coded offline using the Observer software for frame-by-frame analysis. Fetal mouth movements were assessed using the FOMS (Reissland et al., 2016). Both relative frequency of total mouth movements and movement clusters were assessed. Movement clusters refers to the number of times a burst of mouth movement occurs, where a number of individual movements either co-occur or occur immediately after one another. Reliability was conducted by an independent observer on 10% of the scans. Cohens Kappa was .86, ranging between .75-.98. Test-retest reliability was .97 ranging between .92-1, indicating very good reliability overall (Cohen, 1960).

Following the birth, mothers were invited to participate in the follow up phase of the research. At one month old (M=32.3 days, S.D.=4.88) infants were assessed on the NBAS (Brazelton & Nugent, 1995) in their own home. Six areas of the NBAS were assessed including orientation (e.g., following an animate of inanimate object), reflexes (e.g., Moro and Babinski reflexes), motor maturity (e.g., muscle tone when

pulled to a seated position), range of states (e.g., fluctuations between sleep and awake states), regulation (e.g., infants' ability to self-sooth), and automatic stability (colour changes when crying for example). The habituation component of the NBAS was not assessed, due to the timing issues associated with infant sleep prior to this assessment. Therefore, it was an unreliable measure and not included.

Data analysis

A pre-registration plan was submitted to the Open Science Framework (https://osf.io/9c58a). To assess our hypotheses that prenatal behaviour will be associated to postnatal behaviour, with stronger predictive validity at 36 weeks gestational age compared to 32 weeks gestion, a number of regression analyses were performed.

The FOMS outlines 11 different mouth movements including lip corner depressor, lip pressor, lip pucker, lip pull, lip stretch, lip suck, lower lip depressor, upper lip raiser, lips parting, mouth stretch and tongue show. Relative frequency of total mouth movements will be used as the first predictor at both 32- and 36-weeks gestational age. In addition, clusters of mouth movements will be used as an additional predictor, which is the number of bursts of movements occurring immediately one after another. The outcome measures are the different subsections of the NBAS including reflexes, motor maturity, self-regulation, orientation, range of states and automatic stability. High scores on measures of orientation, motor maturity, regulation and autonomic stability are indicative of an infant with optimal neurobehaviour. In contrast, for both number of abnormal reflexes and higher scores on range of states indicate less

optimal functioning and thus indicative of an infant displaying worse neurobehaviour (Brazelton & Nugent, 2011).

Results

Fetuses were on average were 32.1 weeks (S.D.= .58) at the 32-week scan and 35.65 weeks (S.D.= .52) at the 36-week scan. Based on the sample at 32 weeks' gestation, the mean gestation at birth was 39 weeks 3 days (S.D.=1.13), with a mean birthweight of 3350.15g (S.D.=524.76) and at the one month follow up infants were on average 32.3 days old (S.D.=4.88), with 35 males and 40 females.

Regression analyses were used to establish whether relative frequency of mouth movement per minute or clusters of mouth movements at both 32- and 36-weeks gestational age could predict a range of NBAS outcome measures at one-month post birth (See Table 7.2).

32-weeks gestational age and NBAS outcomes

None of the NBAS outcomes were predicted by relative frequency of mouth movement per minute at 32 weeks gestational age; reflex (p=.214), orientation (p=.939), motor maturity (p=.814), range of states (p=.160), regulation (p=.182) and automatic stability (p=.110).

Using clusters of movements at 32 weeks as a predictor, none of the NBAS outcomes were significantly predicted; reflexes (p=.086), orientation (p=.292), motor maturity (p=.680), range of states (p=.066), regulation (p=.162) and automatic stability

(p=.647). Borderline results are shown for both reflexes and range of states suggesting the greater number of movement clusters, the worse the infants' score on these two measures.

36-weeks gestational age and NBAS outcomes

Neither the relative frequency of mouth movement per minute nor cluster of movements at 36 weeks significantly predict any of the NBAS outcomes; reflexes (p=.339; p=.251), orientation (p=.172; p=.070), motor maturity (p=.468; p=.619), range of states (p=.815; p=.737), regulation (p=.860; p=.959) and automatic stability (p=.667; p=.922). The borderline result indicates that the greater number of clusters, the better the infant performs on measures of orientation.

Table 7.2. Regression results.

Predictor	Outcome measure	Significance	Unstandardised B coefficient	f^2 effect size
32 weeks relative frequency	Reflex	.214	.028	.021
	Orientation	.939	.004	.0001
	Motor maturity	.814	.006	.001
	Range of states	.160	.019	.027
	Regulation	.182	.026	.025
	Automatic stability	.110	.040	.037
32 weeks clusters	Reflex	.086	75.567	.048
	Orientation	.292	-95.023	.018
	Motor maturity	.680	-21.269	.003
	Range of states	.066	44.832	.055
	Regulation	.162	51.977	.031
	Automatic stability	.647	22.307	.003
36 weeks relative frequency	Reflex	.339	.047	.014
	Orientation	.172	.144	.029
	Motor maturity	.468	-.038	.009
	Range of states	.815	.008	.001
	Regulation	.860	.007	.0001
	Automatic stability	.667	-.024	.003
36 weeks clusters	Reflex	.251	14.217	.021
	Orientation	.070	48.337	.052
	Motor maturity	.619	-6.704	.004
	Range of states	.737	2.842	.002
	Regulation	.959	.536	.0001
	Automatic stability	.922	-1.404	.0001

Discussion

We expected that the relative frequency and clusters of fetal mouth movements at 32- and 36-weeks gestational age would predict a range of postnatal neurobehavioural outcomes at one-month post birth as assessed by the NBAS. In addition, the

predictive validity would be stronger at 36 weeks gestational age. Contrary to our hypotheses, we did not find support for either.

Previous research has demonstrated that prenatal overall body movement has the ability to predict postnatal behaviour (Stroud et al., 2018) and that general fetal activity is associated with infant reflexes and heart rate variability subsequently predicting motor activity (DiPietro et al., 2010). However, in the present study of fine grained fetal mouth movements, our findings do not support those claims. We hypothesized that mouth movements at 36 weeks would have a stronger predictive validity in comparison to the 32-week data for NBAS outcomes at one month. This was based on research suggesting that fetal movement declines over time becoming more coordinated and precise (Grant-Beuttler et al., 2011). However, although not using ultrasound imaging, research has indicated that when assessing fetal heart rate and motor activity at 24-, 32- and 36-weeks gestational age based on a sample of 385 infants, only the data from 32 weeks was predictive of later childhood temperament (DiPietro, Voegtline, Pater, & Costigan, 2018). Throughout development there are a number of key developmental shifts reflecting neural reorganization, with a pivotal shift occurring at 32 weeks (DiPietro et al., 2018). It could be possible that when analysing mouth movements alone, that earlier gestational time points are more important for predicting postnatal behaviour, prior to such a developmental shift. The precision and coordination of movements at later gestations may reduce the variability needed in order to be associated to postnatal behaviour.

Despite non-significant results for frequency of mouth movements, there was a trend toward significance for clusters of mouth movement at 32 weeks gestational age for

reflexes and a borderline result for range of states. The borderline results indicate that the greater the number of clusters of movements at 32 weeks, possibly indicating lack of control of facial muscles, the greater the fluctuations of behavioural states indicating an unavailability of the infant to the outside world (i.e., either passive or agitated and unsettled) and a greater number of abnormal reflexes. However, this is in contrast to the borderline results indicating that a greater number of clusters at 36 weeks gestation leads to an infant showing a better ability to orientate socially (i.e., following faces, voices and animate objects). It is a complex picture requiring additional research to further unpick the associations between clusters of movements, therefore complexity of mouth movements and the postnatal behavioural implications. The focus on clusters, opposed to frequency may be required, as this may indicate complexity, co-ordination and precision and therefore the maturational processes (Grant-Beuttler et al., 2011) which may be more suitable when reflecting on infant behaviour.

One reason why we might find a lack of support for such research is likely to be associated to the method of fetal analysis. We used the FOMS, which is a fine-grained coding method of analysing a range of fetal facial movements, with the focus on mouth movement. One reason we chose to focus on mouth movements alone was due to a number of studies using this method with results indicating differences between fetal conditions. For example, fetuses of mothers experiencing HG (Reissland et al., 2020a) and a decline in movements from differing gestational time points (Reissland et al., 2015). It is the facial movements that are thought to be an important marker for neurobehaviour. The facial movement of the fetus, and thus frequency of such movements, is likely to represent the brain development and

function during different time points of gestation (Grigore et al., 2018). Fetal neurobehaviour generally, is thought to be a reflection of the CNS (Kurjak et al., 2017). Spontaneous movements have the ability to provide an insight into neurological development (Reissland & Kisilevsky, 2016). This is supported by Stroud et al. (2018) with findings indicating that gross body movements of fetal activity, complexity of movements and isolated movements relate to a number of infant neurobehavioural outcomes including self-regulation, attention, handling, lethargy and quality of movement. However, despite the FOMS ability to accurately and reliably code individual facial muscles of the fetus, in particular mouth movements, it may not provide enough complexity to be related to postnatal neurobehaviour. The present research does indicate that clusters of mouth movements, albeit borderline results, may be a better indication of later infant behaviour. Therefore, future research should analyse all facial movements and facial self-touch as more complex coding may allow for associations to be made between the pre-to-postnatal period.

Whilst it has been suggested that identifying differences in fetal facial movements can provide an indication of normal and abnormal development (Reissland & Kisilevsky, 2016) and it has been the case prenatally in a range of studies (Reissland et al., 2019; Reissland et al., 2020a), for a sample of otherwise healthy mothers with a range of maternal mental health scores and smoking status, these prenatal movements are not associated to postnatal neurobehaviour. However, results from the current study indicate that it is likely that mouth movement profiles alone cannot be responsible for providing an insight into the neurological development of the fetus and in fact in order to understand the whole picture, future research should use a

combination of fetal behavioural tests. This has implications for future research indicating that the FOMS should be used as a whole assessment including upper facial movements, opposed to just focusing on mouth movement alone, as it has been the case for a number of studies.

This study poses a number of limitations. Firstly, we only focused on mouth movements. This was done as mouth movements are the most frequent facial movements to occur (Kurjak et al., 2005). However, the FOMS itself also provides codes for a range of other facial movements, including brow movements. Self-touch alongside other whole-body movements such as those identified in the FENS and KANET may help to better understand the pre to postnatal neurobehavioural relationship. Secondly, there is a large window in development not accounted for. We only assessed the fetus at 32- and 36-weeks gestational age and then the infant at one month old. There may have been late pregnancy, birth trauma or early postnatal environmental influences that may better shape development at one month opposed to focusing on mouth movement at 32 and 36 weeks alone. For example, maternity care providers believe that the birth experience itself does have an impact on infant behaviour (Power, Williams, & Brown, 2019). Additionally, research has suggested that childbirth experience is linked to subsequent infant fussing and crying up until 3 months post birth (St James-Roberts & Conroy, 2005), with the suggestion that this is associated to an infants overstimulated HPA-axis in labour as a result of higher levels of circulating cortisol (Douglas & Hill, 2013).

In order for the FOMS to be used in a clinical setting to provide an insight into neurological functioning, it must first be established what the real-life application of

this assessment tool is. Research has indicated that when using the FOMS there is a difference across smoking status prenatally (Reissland et al., 2015), although this finding was not replicated (Chapter 4), and differences postnatally using the NBAS (Froggatt et al., 2020b). However, generally assessing the trajectory of behaviour from pre-to-postnatal behaviour using these two assessment methods, regardless of smoking status, we do not find such an association. The null findings of this paper highlight the need to establish what such prenatal mouth movements mean, given there is a growing number of research studies using this method. It is likely that mouth movements alone are not sufficient enough to indicate prenatal CNS development. Although both assessment measures claim to assess the maturational processes of the brain and CNS for both the fetus and infant, we do not find an association between the two measures. This has implications for other research solely focusing on mouth movements as it is still currently unknown what the fetal differences mean for the developing infant.

Chapter 8

Risk Perception of Cigarette and E-cigarette use during Pregnancy: A Qualitative Postpartum Perspective

This research study is published in accordance with the guidance outlined for the journal Midwifery. Formatting, references, table and figure numbers have been changed to allow for consistency throughout the thesis.

Abstract

Aim: The aim of this exploratory qualitative analysis is to assess the perceptions of risks of cigarette and e-cigarette use during pregnancy.

Background: An important public health aim is a reduction of smoking at time of delivery (SATOD) from 10.6% to less than 6% by 2022 in the United Kingdom (UK). In order to successfully meet this target, we need to have a better understanding of the perceived risks associated with cigarette smoking. Additionally, the use of e-cigarettes is increasing in the general population, with pregnant women being supported to use such products if it helps them remain smoke free. However, in contrast to cigarette smoking, there is little definitive research assessing the safety of e-cigarette use during pregnancy, with most information disregarding the health of the growing fetus. E-cigarettes are of special interest, given they are an unlicensed product for use during pregnancy, yet women are being supported to use them as a method of harm reduction. A better understanding of perceived risks is essential.

Method: Fourteen interviews were conducted one month postpartum with women who smoked during pregnancy and continued to smoke after the birth. Thematic analysis was conducted.

Findings: Two themes emerged for cigarette smoking; health and justifications. Six themes were identified for e-cigarette use; the unknown, experience, comparison to cigarettes, the product, advice and healthier option. A range of subthemes are discussed.

Conclusion: Women provided a range of justifications for continuing to smoke during pregnancy. Women felt e-cigarettes were a riskier option than continuing to smoke.

Introduction

Smoking throughout pregnancy still remains one of the largest public health concerns across the United Kingdom (UK), with 10.6% of women smoking during pregnancy and in some regions, such as the North East, rates surpass 19% (Public Health England, 2020). In order to reduce the associated negative health effects and cost to the National Health Service (NHS), a public health interim aim for the UK is a reduction of smoking at time of delivery (SATOD) to less than 6% by the end of 2022, a 4.7% reduction within the next two years (Global and Public Health, 2017).

In efforts to reduce the high prevalence of SATOD, a number of regional initiatives have been employed. The babyClear© approach has been rolled out across the North East of England since 2013 and in line with the National Institute for Health and Care Excellence (NICE) guidance includes information regarding the risks associated with smoking during pregnancy for both the mother and fetus, such as placental abruption

and low birth weight (NICE, 2010). Midwives are trained on the delivery of the programme and use a breath test on every pregnant woman. Women who smoke are automatically referred to Stop Smoking Services (SSS) and undergo further intervention through an antenatal clinic with a midwife. The risk perception element of the babyClear© programme for pregnant smokers involves a visual demonstration of risks using a doll and disk representing the placenta designed to illustrate how toxins, such as Carbon Monoxide (CO), from a cigarette affect the developing fetus. To demonstrate the amount of CO is in the pregnant woman's body, mothers undergo a breath test. The device is linked to a computer programme whereby a fetal avatar changes colour from green, to amber to red depending on the levels of CO present in maternal and fetal blood (Fendall, Griffith, Iliff, & Radford, 2012). This type of visual risk education has been found to have a large impact on women's quitting attempts (Fergie, Coleman, Ussher, Cooper, & Campbell, 2019).

For pregnant women who continue to smoke during pregnancy, feelings of guilt can arise due to societal pressures to quit to protect their baby from harm (Ebert & Fahy, 2007; Walker, Graham, Palmer, Jagroop, & Tipene-Leach, 2019). In order to reduce these feelings, women provide a range of justifications, for example they might say that nothing happened to the baby in the first trimester, so it is ok to continue. Some women also argue that smoking provides little risk in comparison to other factors e.g., drinking alcohol and there are additional stressors which would cause more harm to the fetus; furthermore, they argue that quitting at a later stage in pregnancy would be pointless (Goszczyńska, Knol-Michałowska, & Petrykowska, 2016). When discussing smoking in a healthcare setting, women often feel ignored. They feel that in order to be successful in their quitting attempts, the healthcare professional should have an

understanding of their background and provide individualised advice (Ebert & Fahy, 2007; Walker et al., 2019). Given these findings, the current study explored maternal perception of risks related to cigarette use associated with themselves, the fetus and infant, in light of the risk education intervention offered within the North East of England.

As part of the smoking reduction initiative, women are referred to SSS where nicotine replacement therapy (NRT) is offered in cases where quitting without these methods had been unsuccessful (NICE, 2010). However, even when women are motivated to quit, uncertainty about the products and how to use it can hinder the success of NRT during pregnancy (McDaid et al., 2020). Furthermore, in the general population, adults find NRT unsatisfactory in their quitting attempts and in fact many claim that e-cigarettes provide beneficial long-term support and hence they have become popular within recent years (Tamimi, 2018). Therefore, SSS are e-cigarette friendly and advocate quitting attempts by whichever means are necessary, including the use of e-cigarettes during pregnancy.

Research from Action on Smoking and Health (ASH) (Action on Smoking and Health, 2019) reports a growing trend of e-cigarette use in the UK population, rising from 7000,000 in 2012 to 3.6 million in 2019. ASH is a public health registered charity in the UK who campaign to change policy in order to reduce harm associated with tobacco. Generally, there appears to be a division amongst healthcare organisations regarding the safety of such products. For example, Public Health England (PHE), ASH and the Royal College of Physicians support claims that e-cigarettes are 95% safer than traditional cigarettes. However, NICE and the World

Health Organisation (WHO) appear much more cautious in their approach and recommendations of such products (Farrimond & Abraham, 2018).

The safety of e-cigarette use during pregnancy is currently debated, with most information derived from animal studies or an extrapolation from general adult health information, disregarding the health of the growing fetus (Smoking in Pregnancy Challenge Group, 2019; Spindel & McEvoy, 2016). In fact, e-cigarettes are being recommended, by organisations such as PHE and ASH, as a method of harm reduction without peer reviewed research on the effects on the fetus and subsequently the infant. In 2019, studies indicated that in the general population, 27% of individuals approached could not say how harmful e-cigarettes were and 26% believed e-cigarettes to be more harmful than cigarettes. In contrast, when asked about licensed products of NRT, 35% were unsure about the risks but only 6% thought they were more harmful in comparison to cigarette smoking (Action on Smoking and Health, 2019).

With respect to pregnancy, it is impossible to estimate the number of pregnant women using e-cigarettes, as these women are recorded as 'non-smokers' in maternity notes, similar to those who have quit (Smoking in Pregnancy Challenge Group, 2019). Furthermore, there is little clarity regarding the effects of e-cigarette use during pregnancy, even for information provided to healthcare professionals. The Smoking in Pregnancy Challenge Group (2019) highlights that there is little evidence regarding the safety of e-cigarette use during pregnancy and draws on cases from the general adult population. It is recommended that a woman should use a licensed NRT product. However, if a woman chooses to use an e-cigarette then she should be

supported to do so if it helps her stay smoke free. Hence, pregnant women should not be discouraged from using an e-cigarette (Smoking in Pregnancy Challenge Group, 2019).

To be successful at meeting the aim of a reduction to 6% or less SATOD by 2022, an understanding of maternal risk perception of cigarette use is essential, particularly for pregnant women who live in a region where risk education is provided. Additionally, with the growing trend of e-cigarette use, and the support of these products being offered without acceptable levels of scientific evidence, it is essential to assess the perception of risk of e-cigarette use by a group of women who are targeted for smoking cessation support in the future. Undertaking a qualitative approach may aid the development of a maternal focused intervention for supporting smoking cessation in pregnancy in order to meet the public health target of <6% SATOD.

Method

Recruitment

Fourteen women volunteered to participate in a semi-structured interview. These women were recruited from a larger sample of pregnant women taking part in a study assessing fetal and newborn behavioural effects of nicotine exposure during pregnancy. The larger study used 4-dimensional ultrasound scans at 32- and 36-weeks gestational age to assess fetal mouth movements across four groups of women; non-smokers, light smokers, heavy smokers and e-cigarette users. At one-month post birth, a neurobehavioural assessment was conducted with the newborns, of which 29 were exposed to cigarettes prenatally. All cigarette smokers were invited to

participate in the interview, with 14 volunteering. All women were cigarette smokers throughout their pregnancy and continued to smoke following the birth of their baby. All infants were born healthy with no identified health conditions. Ethical approval was granted by the Durham University Ethics Committee (PSYCH-2018-05-08T11:27:21-flbm2).

Semi-structured interview

A semi-structured interview was conducted one month following the birth of their baby. Questions included reasons for smoking, risks associated with cigarette and e-cigarette use and perceived behavioural differences between infants exposed to cigarettes or e-cigarettes and those infants born to non-smokers/e-cigarettes users. Questions were based on a review of the literature and an unpublished master's dissertation project. For the purpose of this study, the focus is on the two questions relating to risks of cigarette smoking and risks of e-cigarette use, see table 8.1. Questions associated with risks were the focus for this study due to the high rates of SATOD in the area, despite risk-based educational interventions being part of routine antenatal care. Understanding perceived risk may help with the development of new smoking cessation interventions. Women were asked to elaborate their responses by providing reasons for their answers.

Table 8.1. Questions associated to risk

Do you believe there is any harm associated with smoking during pregnancy as
A risk to you?
A risk to the fetus?
A risk to the newborn?
Do you believe there is any harm associated with e-cigarette use during pregnancy as
A risk to you?
A risk to the fetus?
A risk to the newborn?

Analysis

Interviews were audio recorded, transcribed verbatim and imported into NVivo for data management. An inductive thematic analysis approach was used (Braun & Clarke, 2006). The six-stage process of thematic analysis was conducted in line with Braun and Clarke's method. Themes and subthemes were discussed and agreed with by the second author.

Results

Sample characteristics

Maternal characteristics were recorded for this interview study. Mean maternal age was 26.35 years (S.D.=5.22 years), with nine light smokers (<10 per day) and five heavy smokers (11-20 cigarettes per day). The highest level of education attainment was recorded, with variability; four women had no qualifications, seven women obtaining GCSE's, one woman received college education and two women receiving a degree. In relation to their infants, eight were male. The average gestation at birth was 39 weeks and one day (S.D.=1.38) and birthweight was 3166g (S.D.=382.43).

Only two women were first time mothers. As part of their routine antenatal care, women received a risk-based educational intervention through their midwife in an antenatal clinic appointment, using methods outlined by the babyClear© approach.

Two key topic areas were discussed in relation to risks during pregnancy and in the immediate postnatal period: cigarette smoking and e-cigarette use. Three questions were asked for each topic area: risk to self, risk to the fetus and risk to the newborn.

Cigarette smoking

Regarding cigarette smoking, when asked about risks of use to self, there was an equal division of responses, seven participants stating there was no risk and seven claiming there is a risk of cigarette smoking to themselves. In terms of risk to the fetus, two women claimed there was no risk, six stated there were risks and six said they were unsure about the risks. All women, irrespective of their view of risks, provided justifications for their smoking behaviour. Regarding risks to the newborn, eight women said there was no risk. However, of those eight, three proceeded to state that there was no risk as they took measures to ensure the baby was not exposed to smoke. Six women felt there was a risk to the newborn baby, again these women proceeded to outline steps they took to reduce the risks. Two key themes emerged from the thematic analysis: health and justification. A range of subthemes were created within each key theme.

Health

Two subthemes resulted from the discussions of the women regarding the health effects associated with smoking during pregnancy and the immediate postnatal period. These subthemes were general health and infant health outcomes.

General health

For women who felt there were risks, they discussed the generic health effects that can occur through smoking, highlighting they were aware of the health implications.

"Obviously you can get cancer and like lung cancer" (P4)

"You're just going to have loads of risks aren't you with smoking, with your health, cancer, so you're going to have risks whether you are pregnant or not pregnant aren't you" (P10)

"It's not really a healthy option is it. Everybody knows that" (P12)

Infant health outcomes

Women were also able to identify a number of negative effects on infant health associated with smoking during pregnancy.

"Yes, possible breathing problems" (P1)

"Still birth, early, so that's obviously like at the time I think still births and the small miscarriages too" (P3)

"On a night if she's going to sleep as well (be)cause you hear a lot of things of erm, SIDs (sudden infant death syndrome) is it called? If you breath on a child, like on a baby, it could cause cot death, so yeah I do believe" (P9)

"They're going to be small, more crying don't they, I don't know I haven't really thought about it much, I just carried on smoking didn't I" (P13)

Justifications for continued smoking

Six subthemes emerged within justifications for continued smoking. These were pregnancy experience, previous experience, other's experience, quantity of cigarettes, cigarettes do not harm and following advice.

Pregnancy experience

This subtheme relates to the experiences some women have had throughout their pregnancy that suggested to them there were risks associated with smoking during this time in their lives.

"I got told that was a bit disgusting when it come out was my placenta…I think it was black, quite mucky, my partner pulled a face, he said 'that's disgusting', I said why and he said that it was your smoking" (P2)

"I know it is (be)cause I could tell when I was like, especially pregnancy, I got more out of breath" (P3)

"The increase chance of blood clots and like there is anyway when pregnant and smoking like on my own and because that's what they thought as well at first when I'd gone into hospital, they thought it could have been a blood clot, but it wasn't" (P7)

Previous experience

Women drew upon their experiences and observations from their own previous pregnancies as well as their current pregnancy to justify their continuation of smoking.

"I haven't had any problems with both of them, they've been perfect, height wise and everything and weight" (P6)

"No, well I know there is risks but with me having three of them, there's been no complications, so probably a no, in my opinion anyway" (P9)

Others experience

In addition to their own experiences, women recalled experiences of friends and other family members who also smoked during their pregnancies and did not experience any adverse effects.

"Like my nanna and everyone said to me like 'oh they didn't tell us we couldn't do anything when we had ours, we could smoke and drink' and I know there wasn't much research back then, but I think it could be other things. I think there are a lot of things blamed on smoking, I've has three babies and smoked through all of them" (P7)

"I've got a lot of family members who smoked through them and I know it sounds stupid but like nothings ever happened to any of them kids" (P12)

Quantity of cigarettes

Women justified their behaviours by stating that they smoked less therefore posing less of a risk, and the amount of harm is dependent upon the number of cigarettes smoked throughout the pregnancy.

"I think there could be if you are sitting smoking one after the other, but that's what I convince myself, just couple off, she won't get much, she won't get that, I think you end up convincing yourself there won't, but if you sat and smoked all day long, then definitely" (P3)

"I think it depends how many, I think there's a lot of different factors with it, like me, I've always tried to cut down as much as I could, do you know what I mean, I've never just stopped. There's a big difference between someone smoking ten a day and someone smoking thirty a day" (P12)

Cigarettes won't harm

Within this subtheme, women expressed that their smoking behaviour was unlikely to have a negative impact on the infant.

"You sort of think that it won't harm them" (P4)

"Obviously there is risks like lung cancer and that but not that anything is going to happen to any of them just because I go and have a fag (cigarette)" (P7)

"For me I don't feel like there was any risk, erm I lessened it myself, I cut down myself, my intake of it because I know there is concerns there…it was cut down and because of pregnancy that was it… I know it's damaging to myself" (P11)

"If I thought it was a big risk I would have stopped" (P13)

Following advice

A way in which women justified their smoking behaviour in the newborn phase was to state that they follow the advice from healthcare professionals and did not smoke in the presence of the infant.

"I'll make sure I have my 5 minutes before I go grab her and you know what I mean, and I always sterilise my hands" (P3)

"I wouldn't hold him and smoke, I don't smoke around him anyway. I put something over the top, a coat, a cardigan something like that that's just going to keep that smell away from him as well. I wash my hands when I come back in so he's completely distant from that" (P11)

E-cigarette use

When asked about the risk associated with nicotine in e-cigarettes to themselves, four women thought there was no risk, six stated there was a risk and four women were unsure of the risks. Women were asked whether they thought the e-cigarettes posed a risk to the fetus. Only one woman thought that e-cigarettes posed no risk, whilst eight women felt there was a risk, and five women being unsure about the risks. Of the ten women asked whether e-cigarettes would be harmful to the newborn, six claimed it would not pose a risk and four stated there was a possible risk.

The unknown

The women argued that e-cigarettes were new products and that the long-term effects were unknown and therefore more research was required. From the discussions, two subthemes emerged.

Long term effects

This subtheme relates to the lack of knowledge regarding e-cigarettes and that the implications of the health effects are unknown.

"These people who are smoking e-cigarettes, how do they know the actual complications what's going to come in 30 years' time, where you know what you're getting with a cigarette, they've been out that long" (P12)

"You don't know how and what the effects are in the future" (P12)

"There's the unknown… there could be things in that e-cigarette that could affect the brain and anything" (P12)

Research

Women recognised the need for further research to be conducted on e-cigarettes in order to provide accurate advice for use during pregnancy.

"More research and to see if they were allowed to be used in pregnancy" (P2)

"They haven't had enough time to be tested properly and like to see the long-term effects" (P7)

"I don't think they've been looked into enough. I don't think there's been enough research on them, I think everyone's going to start falling down dead in about 15 years off them" (P10)

Experience

Women drew upon their own experiences and that of others to evidence potential risks associated with e-cigarette use.

Past experience

Prior to pregnancy, some women had tried using an e-cigarette and they discuss the negative effects from it.

"I've tried them in the past…I've felt worse on them…them oils were going in my mouth…they are in your mouth and then you're swallowing that actual oil" (P3)

"I didn't agree with it, it made me feel like my chest and throat was closing up and I just don't like them" (P7)

Others' experience

One woman described the experience of someone she knows regarding the negative health consequence of using an e-cigarette.

"I actually know someone who quit cigarettes with an e-cigarette, and they got popcorn lung and the doctor in the hospital told them that their lung collapsed and that was through the e-cigarettes" (P12)

Comparison to cigarettes

Many of the women discussed e-cigarettes in comparison to cigarettes.

"Supposed to be just as bad as cigarettes" (P5)

"For years they've been making fags (cigarettes), do you know what I mean cigarettes and they know what's in them and all of that, but I think these e-cigarettes they've only just randomly been made" (P7)

"Smoking that (e-cigarette) was more harsh on my throat than a cigarette, so it was a lot stronger" (P9)

"No if you weren't around the child…you would treat it the same as a cigarette, you would go outside and away from the child, again you don't know what's in it, it could be more harmful than a cigarette" (P9)

"I think it's the same with smoking, there's that slight risk there with yourself as well with the baby while you're pregnant" (P11)

" You can smoke a cigarette and you know what like obviously it can affect their lungs and stuff like that and the size, but you don't know what the other things it could do" (P12)

"They're worse than smoking a fag (cigarette)…the nicotine, the thing that goes in them… probably more harmful for him" (P14)

The product itself

Three subthemes emerged relating to the product itself.

The chemicals

A concern was expressed by the women that there is little information regarding what chemicals and toxins are in e-cigarettes.

"I thought they would be worse being the chemicals" (P3)

"You don't actually know what is in them, so you don't know what you are inhaling" (P9)

"The e-cigarettes as well because there's stuff in there is toxic, so there's always going to be a risk" (P11)

"We don't know much about them really do we, the e-cigarettes, we don't even know what's in them or what" (P13)

Physical product

Women also described the dangers of the product itself and reflected on stories they had heard.

"Just all the stories I've heard about them as well, like blowing up and killing people and stuff like that" (P9)

"They blow up, they pop in your face don't they, I've seen loads about them e-cigarettes, they're dangerous" (P14)

Quality

One woman stated that an e-cigarette might be ok for use, depending on the quality of the product, suggesting that some are better than others.

"I think obviously if you get a good one and you're alright, but if you're swallowing, it's probably worse"(P3)

Advice

A clear concern was related to the advice that women were offered from healthcare professionals regarding the safety of e-cigarettes in terms of use for during pregnancy.

"I got told that obviously you can use them and then I got told you can't, obviously I never touched them" (P2)

"Well they told me when I was doing the growth scans and stuff they could put us with the non-smoking, like to help me quit smoking (be)cause I said at the beginning I

couldn't smoke (be)cause I'd be sick and I was on the e-cigarette, but they're saying that there's no proof that it can't harm the baby yet" (P6)

"With e-cigarettes, I'd do the same thing, it's just one of them thing, just keep away from that sort of seeing it, smelling it, tasting it sort of thing" (P11)

Healthier option

Two women felt that e-cigarettes might be a healthier option due to less toxins in the product.

"Suppose it would be better than smoking normal cigarettes" (P13)

"It would probably be more healthy wouldn't it… it's not going to affect him much like with smoke (be)cause they haven't got the chemicals and stuff in them like the smoke have they, but like I don't smoke in the house anyway" (P13)

Discussion

The purpose of the study was to explore maternal perceptions of risks associated with both cigarette and e-cigarette use during pregnancy and the postpartum period. By exploring themes, which became apparent during the interviews, the voices of women are heard and can be used to inform future interventions. The present thematic analysis indicated that for assessment of cigarette smoking, two key themes emerged: justification and health. For e-cigarettes, six key themes emerged: the unknown, experience, comparison to cigarettes, the product, advice and healthier option.

With respect to cigarette smoking, it was evident that some of the women interviewed were aware of some of the health-related risks to both themselves and the infant and

were able to provide examples. However, these same women then provided justifications of their behaviour in light of such risks. Women continue to smoke throughout their pregnancy as they reduce the perception of risk by self-justifying (Goszczyńska et al., 2016). Despite advice from healthcare professionals, women who do not modify their behaviour instead adjust their beliefs of the risks associated to smoking during pregnancy. Rather than attempting to quit, they rationalised their behaviour, despite the potential devastating risks. Having an understanding of the risks associated with smoking during pregnancy does not motivate these women to initiate quitting attempts for the sake of the health of their unborn child (Goszczyńska et al., 2016). Given their awareness of risks, it is unlikely that risk education interventions are helpful as women often provide counterarguments to justify their behaviour (Goszczyńska et al., 2016).

Such behaviour can be explained by cognitive dissonance theory. Cognitive dissonance theory (Festinger, 1962) states that we want consistency between our attitudes, thoughts and behaviours which must align to create harmony. When there is a conflict in this system, dissonance occurs. In order to reduce this dissonance, individuals are likely to avoid certain situations in order to reduce the dissonance (Festinger, 1962). Women in the current study voiced the risks associated with smoking, however, they smoked throughout their pregnancy and continued to do so following the birth of their baby. It is likely that these women rationalised their behaviour in order to reduce any dissonance felt, therefore relieving any discomfort they were feeling regarding their smoking behaviour (Orcullo & San, 2016). Although dissonance can be reduced by changing behaviour, individuals instead opt to change their cognitions to align them with their behaviour. With regards to

smoking, women feel the risk is negligible in comparison to behaviours carried out by others during pregnancy such as drug and alcohol use (Harmon-Jones & Mills, 2019). Additionally, a paradigm within cognitive dissonance theory relates to the belief-disconfirmation, in that these women, particularly those who suggest that only women who are heavy smokers are causing the damage, are misinterpreting the information in order to satisfy their own behaviours and beliefs (Harmon-Jones & Mills, 2019). Women who have had previous healthy pregnancies are unlikely to change their thoughts and behaviours, due to their past uncomplicated 'risk free' experiences, with denial of smoking harm being the most common theme across such research (Orcullo & San, 2016).

This study suggests that in spite of identifying risks associated with cigarette smoking, women continue to smoke throughout pregnancy and in the immediate postpartum period by justifying their behaviours. In contrast to cigarette smoking, these women view e-cigarettes as riskier due to the unknown risks. Hence, these women do not view e-cigarettes as a safe alternative for harm reduction during pregnancy due to a number of reasons.

Six key themes emerged from the discussion regarding e-cigarette use during pregnancy and the immediate postnatal period. These themes related to the unknown risks, experience with e-cigarettes, the product itself, advice for using e-cigarettes, comparison to cigarettes and a suggestion they are a healthier option. Five of these themes had a negative evaluation toward e-cigarette use. The results indicate that women believed e-cigarettes carry significant risks during pregnancy. These women worried about the long-term effects, safety and that the harm of e-cigarettes were

equal to or worse than smoking cigarettes. There are many unknown risks, not just for pregnancy, but across the general population, with other research suggesting that a 'stick with the devil you know' concept often being adhered to (Vasconcelos & Gilbert, 2019).

The evidence regarding the safety of e-cigarette use during pregnancy remains unclear (Suter, Mastrobattista, Sachs, & Agaard, 2015), thus leading to mixed perceptions from the pregnant population regarding the use as a harm reduction method. Previous research suggests that women perceive e-cigarettes to be safer in pregnancy than cigarette smoking (e.g., Mark, Farquhar, Chisolm, Coleman-Cowger, & Terplan, 2015; Wagner, Camerota, & Propper, 2017). In contrast to these studies, the current thematic analysis of smoker's views of e-cigarette is rather negative. It is suggested that because of both the public and health professionals having a limited understanding of safety and long-term impact on the fetus, and child, many women are reluctant to use these products (Bowker et al., 2016). As evidence is contradictory (Schilling et al., 2019), the views expressed in the current study may reduce potential feelings of dissonance caused by cigarette smoking throughout their pregnancy, by emphasising the risk of an alternative 'harm reduction' method.

Adding to the safety concerns of e-cigarettes is the chemical make-up. Ingredients are variable, with the contents often not clearly labelled. Notably, some e-cigarettes contain ingredients that have been banned in cigarettes, such as ethylene glycol, a highly toxic substance (Hutzler et al., 2014). These concerns were expressed by the women in the present study, commenting that not knowing what is in the product leads to a perception of greater risk. Despite the dangerous chemicals in both

cigarettes (Talhout et al., 2011) and e-cigarettes (Hutzler et al., 2014), the perception of risk differs greatly in the sample of women interviewed. Women use cigarettes as a comparison to e-cigarettes when discussing the associated risks, with the suggestion that the unknown risk outweighs the known risk, therefore leading to a continuation of smoking. The concerns outlined by these women are reasonable due to the lack of scientific research and guidance. However, a recent study assessing the effects that prenatal cigarette and e-cigarette exposure has on infant behaviour indicates that birth outcomes were only affected in the cigarette exposed group. With behaviour at one month negatively affected for both cigarette and e-cigarette exposed infants (Froggatt, Reissland, & Covey, 2020b). Further research assessing risks of e-cigarette use during pregnancy will help women weigh up the balance of known and unknown risks.

Due to the lack of sufficient guidance on e-cigarette use during pregnancy, women opt to continue smoking cigarettes despite the known risks. This adds to the debate regarding the safety of e-cigarettes. It is evident from the statements that these women are receiving conflicting advice and therefore require access to guidance based on science; hence further research is warranted. The current research highlights the challenges that may be experienced within a midwifery department when supporting smoking cessation. Women in the current study, due to their previous experiences of healthy pregnancies, do not recognise the immediate risk to themselves or their offspring. There is a suggestion that the views regarding e-cigarettes are not shared between pregnant women and healthcare professionals, which indicates the need for further research.

The study reflects the views of women living in the North East of England where SATOD rate is high, 19.3% (Public Health England, 2020). These views are expressed in light of these women receiving risk based educational interventions and referral to stop smoking services. Therefore, the suggestion that educational interventions are effective (Fergie et al., 2019), does not appear to apply in this sample of women. Women in the present study place emphasis on their own and others' experience of previous uncomplicated pregnancies as a way of justifying their smoking behaviour. To combat these justifications, providing real life vignettes of women who have experienced the negative pregnancy outcomes as a result of smoking may aid behaviour change in these women. Smoking mothers may be able to relate to such examples supporting their quitting attempts. However, given the support the women in the study were already receiving, it may be possible that we are beginning to reach groups of women who are unwilling to change their smoking behaviour, regardless of the interventions offered. Additionally, the views regarding the use of e-cigarettes in this small cohort of women are in some cases contrary to the literature that suggests e-cigarettes are perceived as a less harmful than cigarettes. A possible reason for contradictory views across studies may be due to different samples of women assessed together; non-smokers, cigarette smokers, e-cigarette users, dual users (Mark et al., 2015). However, in the current study only cigarettes smokers were assessed, as these women are prime targets for smoking cessation interventions. Although only a relatively small group of women were interviewed, the sample size is similar to a number of other similar studies, suggesting 14 women is an appropriate sample size (Grant, Morgan, Gallagher, & Mannay, 2020; McDaid et al., 2020).

In summary, this exploratory analysis demonstrates that although women are aware of the health associated risks with cigarette smoking, they continue to smoke throughout pregnancy expressing a range of justifications. Healthcare professionals need to target these justifications opposed to providing risk information. Additionally, despite e-cigarettes being supported by healthcare professionals as a harm reduction method, women in the present sample were not convinced of the safety of these products and highlight a number of potential reasons. Women appear to favour the defined possible detrimental risks of cigarette smoking over the unknown effects e-cigarettes may pose. It is possible that risk education alone is not an effective intervention to support women quitting smoking. Furthermore, e-cigarettes require further research to understand the safety and effectiveness during pregnancy in order for women to make an informed choice regarding their smoking behaviour.

Chapter 9

General Discussion

The experimental studies presented in this thesis have investigated the effects of cigarette and e-cigarette use on fetal and infant behaviour, as well as the predictive nature of pre to postnatal behaviour, irrespective of nicotine exposure. In addition, maternal understanding of risks associated with cigarette and e-cigarette exposure were assessed. This final chapter provides an overview of the main findings associated with each study, the implications for theory and policy, methodological limitations, and future research.

Summary of findings

There were five central objectives of the research outlined in this thesis.

1) To conduct a partial replication of Reissland et al. (2015) pilot study assessing the impact of cigarette exposure on fetal behaviour, defined by relative frequency of mouth movements, with four main alterations. Expand the sample size from 20 to at least 100, to separate cigarette exposure groups into light (<10 cigarettes per day) and heavy smokers (11-20 cigarettes per day), include e-cigarette users and to focus on the later gestational ages at 32- and 36-weeks gestational age.
2) To conduct a meta-analysis assessing the impact of prenatal cigarette exposure on infant neurobehaviour up to one year of age.

3) To assess the broader prenatal smoking status, including non-smokers, cigarette smokers and e-cigarette users, and the impact on birth outcomes and infant neurobehaviour at one month post birth.
4) To explore the relationship between prenatal mouth movements and infant neurobehaviour, regardless of smoking status.
5) To understand maternal views of risks associated with cigarette and e-cigarette use during pregnancy.

The prenatal study, the effect of pregnant women's smoking status and e-cigarette use on fetal mouth movements (Chapter 4), addressed objective one. One hundred and twenty-three pregnant women were recruited for ultrasound scans at 32 weeks (scans analysed N= 106) and 36 weeks gestation (scans analysed N= 87). At 32 weeks gestational age, the results indicated that there were significant differences between the four exposure groups. Heavily exposed fetuses had a significantly different pattern of mouth movements (fewer movements) in comparison to e-cigarette exposed fetuses (more mouth movement), which could indicate that carbon monoxide (CO) and nicotine together could lead to a different effect in comparison to nicotine exposure alone. However, when controlling for time of day the ultrasound scan was performed results indicated only an overall borderline significant result. These suggestive results imply that nicotine and CO exposure might affect the brain in different ways (see Chapter 1), which may be age related as the borderline effect only occurred at 32 weeks gestational age. However, neither group was significantly different to non-exposed fetuses. These findings are not consistent with the results reported in the Reissland et al. pilot study (Reissland, Francis, Kumarendran, & Mason, 2015). It could be that once more refined exposure groups were assessed,

such subtle differences between groups could not be identified by assessing mouth movements alone. Similarly, the results are also inconsistent with other research identifying behavioural differences between cigarette exposure groups (e.g., Habek, 2007; Stroud, Bublitz, Crespo, Lester, & Salisbury, 2020). The specific reasons for the variations in results is not clear, however, it could be associated with the fine-grained method (Fetal Observable Movement System, FOMS) used and the type of behaviours coded; fetal mouth movements only. The majority of research assessing fetal behaviour in relation to cigarette exposure focuses on general body movements (isolated limb movements, head movements and trunk movements) that are known to reflect CNS development and can therefore provide an insight into neurobehaviour (Einspieler & Prechtl, 2005). Additionally, research has suggested that when fetal facial movements form 'expressions' or gestalts, particularly when observed in response to stimuli, this is likely to reflect brain function and provide an indication of fetal neurobehaviour (AboEllail & Hata, 2017; Grigore et al., 2018). However, in the current study, the sole focus was on mouth movement, with research indicating that at present it is unknown how fetal mouth movements indicate CNS and brain development (Salihagic-Kadic, Kurjak, Medić, Andonotopo, & Azumendi, 2005). Given the number of studies assessing fetal behaviour using the FOMS, in particular mouth movements (Reissland et al., 2015; Reissland, Makhmud, & Froggatt, 2019; Reissland et al., 2020a) it was important to establish how such movements related to infant behaviour (see Chapter 7). However, the results from the prenatal study would suggest that assessing behaviour using this method alone may not have been the most appropriate method for identifying subtle group differences.

Additionally, the study indicated that mouth movement frequency declined from 32 to 36 weeks gestational age. These results are in line with previous research examining fetal activity as a function of gestation (e.g., Grant-Beuttler et al., 2011; Grigore et al., 2018; Reissland et al., 2015). However, the decline in movements from 32 to 36 weeks gestational age were only found for the non-exposed and e-cigarette exposed groups, suggesting that exposure to both nicotine and CO combined (but not nicotine alone) delays the normal decline in movements and thus interferences with the maturational processes. It seems possible that this is due to the CNS and maturation processes refining, leading to precise and further co-ordination of movements (Grant-Beuttler et al., 2011; Grigore et al., 2018; Reissland et al., 2015).

Whilst no exposure group differences were established in the prenatal period, the meta-analysis (Chapter 5) provides clear support for postnatal behavioural differences as a result of prenatal cigarette exposure. The meta-analysis assessed 19,162 infants up to one year of age from 17 eligible studies across six countries, comparing infants who had been prenatally exposed to cigarettes with infants not prenatally exposed. The results of this meta-analysis confirm that prenatal cigarette exposure leads to a number of differences in postnatal neurobehavioural assessment measures (e.g., Hernandez-Martinez, Arija Val, Escribano Subias, & Canals Sans, 2012; Law et al., 2003; Mansi et al., 2007). The results indicated that there were significant medium effects for negative affect, attention, excitability, irritability and orientation, with small significant effects for muscle tone, regulation and temperament. Such results indicate that infants who were prenatally exposed to cigarettes performed worse on these measures in comparison to non-exposed infants. These convincing findings

support the idea that early behavioural dysregulation occurs as a result of prenatal exposure to cigarettes. Here a prominent question concerns whether this is also true for those exposed to e-cigarettes prenatally, and this may contribute to the understanding of whether it is nicotine or CO contributing to these negative behavioural effects postnatally. This has important implications for policy development as such research may guide the recommendations of whether e-cigarettes are safe to use during pregnancy or not when reflecting on the later infant behavioural outcomes.

The one-month follow up study assessing neurobehaviour (Chapter 6) on 83 infants, indicated that birth outcomes were worse (birthweight and head circumference) for those exposed to prenatal cigarette smoke in comparison to both non-exposed and e-cigarette exposed infants. However, the postnatal neurobehavioural outcomes, as measured using the NBAS, for both cigarette and e-cigarette exposed infants showed a greater number of abnormal reflexes in comparison to non-exposed infants. Infants exposed to cigarette smoke showed reduced self-regulation and motor maturity, and prenatal exposure to e-cigarettes resulted in decreased motor maturity and marginally decreased self-regulation abilities. One explanation for the results relates to the CO exposure experienced by the cigarette group leading to the reduction in birthweight and head circumference (Merklinger-Gruchala, Jasienska, & Kapiszewska, 2017), with the similar behavioural outcomes being linked to the nicotine exposure impacting on brain and CNS development (Dempsey & Benowitz, 2001). Despite these promising results, the data need to be interpreted with caution and replicated in a bigger sample including a greater number of e-cigarette users. Although there are still many unanswered questions regarding the use of e-cigarettes during pregnancy,

the current study is important in indicating the urgent need for research on the safety of e-cigarette use during pregnancy when considering the development of the fetus and infant, including the effects of e-cigarette use on breastfeeding infants (Wickstrom, 2007).

The hypothesis for the pre-to-postnatal study was developed based on research indicating that fetal behavioural patterns can provide an insight into neurodevelopment (Salihagic-Kadic et al., 2005). Given that the FOMS and NBAS both claim to assess neurobehaviour, it was anticipated that there would be an association between these measurements. However, the results from this research indicated that fetal mouth movement frequency and clusters do not appear to significantly predict measures of neurobehaviour at one month old, as assessed by the NBAS (Chapter 7). However, when analysing clusters of movements, there was a trend toward significance at 32 weeks gestational age for reflexes and range of states, with the greater number of movement clusters indicating the infant performing worse on these measures. Additionally, at 36 weeks gestation, a greater number of clusters indicated infants performed better on measures of social orientation, with a borderline significant result. Hence, further research is needed to understand how prenatal mouth movement clusters relate to postnatal behaviour. To date, this is the only study attempting to use very specific indicative movements, namely mouth movements, to address this issue and it is a key research question given the previous studies assessing mouth movement frequency alone (e.g., Reissland et al., 2019; Reissland et al., 2020a). Previous studies have attempted to assess continuity of fetal movements (DiPietro et al., 2002), with one recent publication focusing on the relationship between fetal behaviour and infant neurobehaviour (Stroud, McCallum, & Salisbury,

2018). However, the methods of observation were different to the research conducted as part of this thesis, including an actocardiotocograph which assesses fetal heart rate and measurement of gross body movement. Stroud et al. (2018) focused on general gross body movements with results indicating that fetal activity, isolated movements, complex body movements and coupling index was related to infant self-regulation, attention, handling, lethargy and quality of movement up to one month of age. In comparison to the research presented in this thesis, Stroud et al. (2018) may find an association between the two measures given that gross body movements are known to be reflective of CNS (Einspieler & Prechtl, 2005). Nevertheless, the question of what different prenatal mouth movement profiles mean postnatally and the implications of such differences is still a question yet to be answered and needs to be further explored. Mouth movements alone may not be the most appropriate measure for providing an indication of the integrity and functioning of CNS development (Salihagic-Kadic et al., 2005). Future work on this topic should examine all facial movements, incorporate gross fetal behaviours, as well as assessing both fine-grained and general body movements postnatally.

The qualitative study (Chapter 8) was designed to understand smoking mothers' perceptions of risk associated with both cigarettes and e-cigarettes in terms of the impact on the fetus and infant. Two key themes were identified for cigarette smoking: health and justifications. Six themes emerged for e-cigarette use: the unknown, experience, comparison to cigarettes, the product, advice, and a healthier option. From the data, a number of conclusions were drawn. With respect to cigarette smoking, women expressed their knowledge of the associated health-related risks yet provided justifications for their continued use during pregnancy, thereby rationalising

their own behaviour, with the knowledge of the negative potential impact not enough to initiate quitting attempts. Regarding e-cigarette use, the majority of themes discussed included negative evaluations for the use of e-cigarettes during pregnancy, concerns which are warranted given the growing body of evidence regarding the safety of use (Spindel & McEvoy, 2016). These women expressed the view that e-cigarettes are 'worse' than cigarettes because they contain nicotine, but ignore the fact that cigarettes also contain nicotine. There needs to be clearer communication regarding the chemicals and toxins that are present in both of these products alongside the associated risks with use, which is essential in allowing women to make an informed choice regarding their nicotine intake during pregnancy. Conclusions from this study provide support for clinicians to develop interventions focusing on the justifications for continued smoking and to address the concerns regarding e-cigarette use during pregnancy.

Effects of CO and nicotine exposure

The research presented in this thesis can provide an insight into the effects that CO and nicotine exposure during pregnancy can have on behavioural development. By including e-cigarette users, we can begin to understand the unique effects of CO exposure in comparison to nicotine which is present in both cigarettes and e-cigarettes. Of particular importance is the postnatal study (Chapter 6) given that birth outcomes were negatively affected for the cigarette exposed group only, but neurobehaviour was similar for both the cigarette and e-cigarette exposed infants.

Carbon monoxide is a particularly important substance when considering the effects of maternal smoking during pregnancy. As CO is within the air, it was anticipated that women involved in the research would be exposed to CO, even if they did not smoke due to the heavily industrialised area and social context with 17.2% of adults (+18) (Public Health England, 2020) smoking cigarettes in Middlesbrough, UK. A study assessing air pollution over a 24-hour period in regions in Northern Italy assessed the effects of CO and nitrogen dioxide ($NO_{2)}$ on birth outcomes (Giovannini et al., 2020). Interestingly, results indicated that there was a positive correlation between low levels of CO (environmental exposure such as car pollution and urban environment) within 10 days prior to birth and birthweight (Giovannini et al., 2020). These results reflect the findings presented in the research as part of this thesis (Chapter 6) as low levels of CO exposure, via the environment, did not have a negative impact on birth weight. These two pieces of research taken together could that indicate it is only CO exposure that is above that of the environment (urban areas) such as cigarette smoking that leads to negative birth outcomes, such as low birth weight.

When assessing levels of breath CO early in pregnancy, women who had scores greater than 3ppm (same cut off as in the present series of studies), were more likely to have a C-section, low birth weight and below the 25th centile, small for gestational age, adverse pregnancy events and fetal distress (Reynolds et al., 2019). Results of CO leading to a reduction in birthweight and small for gestational age is thought to be associated to CO binding to fetal blood leading to a reduction of oxygenation (Reynolds et al., 2019), which is caused by the increase in carboxyhaemoglobin. CO crosses through the placenta and enters the fetal circulatory system (Bednarczuk,

Milner, & Greenough, 2020). These results support the current findings from the postnatal study (Chapter 6) that indicates that infants born to cigarette smokers had a lower birth weight and smaller head circumference, suggesting this is a result of CO. Non-exposed and e-cigarette exposed infants did not have a lower birth weight or a reduction in head circumference as they were not exposed to CO levels that were comparable to the cigarette exposed infants, indicating that CO is possibly the main responsible cause for having a significant negative impact on birth outcomes.

Recent government updates state that e-cigarette use combined with stop smoking support is an option that should be available to all (McNeill, Brose, Calder, Simonavicius, & Robson, 2021), however, there is limited guidance for use during pregnancy. Although recognised that stopping smoking during pregnancy without the use of NRT is preferable, any method of licensed NRT is preferred in comparison to cigarette smoking as there is a significant reduction in the amount of chemicals (Public Health Agency., 2016). A concern is that the majority of research assessing the effects of nicotine, NRT or e-cigarettes, during pregnancy focus on the acute effects on fetal development and birth outcomes (e.g., McDonnell, Bergin, & Regan, 2019), opposed to the longitudinal and behavioural effects. The problem is that nicotine alternatives are recommended for women during pregnancy; that advice is inadvertently recommended for the unborn child at critical stages of brain development. This is a problem as the fetal brain may be more affected by nicotine in comparison to other toxicants found in cigarettes (e.g., Wickstrom, 2007). The effects that nicotine causes on the fetal system and development are exacerbated due to the long half-life, crossing the blood-brain barrier, with problems such as the extensive expression of nAChRs throughout the CNS (Benowtiz, 2010). Due to the

convenience of providing NRT, individuals may assume it is therefore completely safe. This may lead to the assumption that the risks associated with nicotine are manageable (Ginzel et al., 2007).

The general consensus amongst healthcare professionals is that further research is required on the use of nicotine during pregnancy (Bruin, Gerstein, & Holloway, 2010). Such claims are supported by the findings from the postnatal study (Chapter 6) that e-cigarette exposed infants display similar negative neurobehavioural outcomes to cigarette exposed infants. These finding are in line with predictions from animal models. Much of the animal literature reviewed concludes that prenatal exposure to nicotine, in particular in rodents, leads to poor cognitive performance, hyperactivity and an increase in physiological anxiety (Bruin et al., 2010). These studies highlight the contribution of nicotine to the developmental long-term effects. Further studies indicate that e-cigarette users and NRT users have the same level of nicotine in their body, as measured by urine and saliva, as cigarette smokers (Shahab et al., 2017), thereby explaining why postnatal neurobehavioural results are similar for cigarette and e-cigarette exposed infants (Chapter 6). This would suggest that CO leads to the health-related outcomes (e.g., lower birth weight, preterm delivery and smaller head circumference), but nicotine may be responsible for the cognitive and behavioural outcomes. This has implications for not only the recommendation of e-cigarette use, but also nicotine delivered by patches, gum and inhalators during pregnancy.

A review was conducted by the Committee on Toxicity of Chemicals in Food, Consumer Products and the Environment assessing the safety of e-cigarettes concluding that any health effects as a result of e-cigarette use would be much lower

than that of cigarettes (Committee on Toxicity of Chemicals in Food, 2020). There is also a UK-wide ambition for a smoke free population by 2030 (McNeill et al., 2021), with individuals quitting or using e-cigarettes for example in order to reduce their risk associated with continued cigarette smoking. According to some there is strong evidence that e-cigarettes containing nicotine are effective to help individuals stop smoking (McNeill et al., 2021). However, again, such reports are reflective of the general adult population and neglect the pregnant women and their fetuses who are susceptible to alterations in brain and CNS development via nicotine exposure. Whilst cigarette alternatives may be suitable for the mother during pregnancy, this is not necessarily the case for the fetus, with the recommendation that mothers abstain from both tobacco and nicotine throughout the entirety of their pregnancy (Nordenstam, 2019).

In sum, the series of studies reported in this thesis suggest that there may be differing effects of CO and nicotine for the developing fetus/infant. Therefore, future research needs to focus on the effects of nicotine as this may be the most important factor leading to the negative behavioural outcomes.

The association between pre and postnatal behaviour

One of the central aims of the research was to establish the relationship between prenatal mouth movements and infant neurobehaviour in the hopes to provide an insight into what different profiles of mouth movement may mean for subsequent behaviour and development.

It is well established that gross abnormal fetal movement profiles are likely to indicate abnormal postnatal development (Einspieler & Prechtl, 2005; Reissland et al., 2019; Talic et al., 2012). However, fine grained movements, using the FOMS coding might not readily translate into postnatal movement and behaviour. Furthermore, it is essential to define the parameters of what is considered normal and abnormal development.

Although it is well established that prenatal programming affects postnatal behaviour (Barker, 1995), it appears that individuals with the same level of exposure prenatally, whether that be stress, depression, anxiety or toxin exposure such as nicotine, may be affected differentially. This is likely due to genetics also playing a vital role in fetal programming. As a result of such exposure these infants may be susceptible to adversity later in life. However, a supportive parental environment postnatally may lead to the infant being able to adapt to adversity, thus explaining why there is variation in behaviour (Pluess & Belsky, 2011). Here it is important to mention early postnatal plasticity, in which very early experiences have the ability hinder or promote subsequent development (Belsky & Pluess, 2009). Hence, although the current study was unable to show differences in prenatal mouth movement frequencies in relation to exposure group (Chapter 4), it was evident there were differences postnatally by assessing neurobehaviour (Chapter 6), which may be a result of postnatal plasticity.

The neurobehavioural outcomes are not just reliant on the prenatal period and the mothers stress, depression, anxiety or smoking status, but also what happens in the first month of life. A parent's sensitivity to an infant's needs can influence their

neurobehavioural development, similarly, so can a reduction in parental contact. For example, an infant who does not receive parental stimulation or physical contact is likely to exhibit delays in motor development (Gutman & Feinstein, 2010) which can therefore lead to further dysregulation of an infant's neurobehaviour (Mansi et al., 2007). Adverse experiences can lead to altered brain function during both the fetal and infant period (Gudsnuk & Champagne, 2011).

Due to the vast variability in fetal movements that may be affected by a range of factors, it could be difficult to associate this to postnatal behaviour. Additionally, there is a greater degree of precision for assessing postnatal behaviour which is not as precise in the prenatal period (DiPietro et al., 2002).

Implications for policy

One key finding of the research conducted as part of this thesis that is likely to have an impact on policy is that when assessing infants postnatally using the NBAS those infants prenatally exposed to cigarettes or e-cigarettes display similar negative behavioural outcomes.

Whilst appreciating the risk versus benefit aspect of the argument, it is imperative that studies are beginning to be conducted to assess not only the health implications of using nicotine during pregnancy, but also the well-known behavioural affects that are evidently affected for infants who are prenatally exposed to cigarettes (Froggatt, Covey, & Reissland, 2020a)[13]. Any way of reducing cigarette consumption, including

[13] Chapter 5 in the thesis.

the use of e-cigarettes and other forms NRT will always likely be favourable in comparison to smoking (Bar-Zeev, Lim, Bonevski, Gruppetta, & Gould, 2018), with this argument not being disputed. However, simply because e-cigarettes and other forms of NRT are more favourable in comparison to smoking during pregnancy, the argument concerning the safety for use during pregnancy should not be neglected. The research presented in this thesis is the first known study to assess the impact of prenatal e-cigarette exposure on infant neurobehavioural outcomes at one month of age.

As highlighted by the qualitative research conducted as part of this thesis, mothers often justify their smoking behaviours, and therefore there needs to be a change in the way interventions are designed in order to target the justifications provided by the mothers. This is of particular importance, given that these women were already undergoing a risk perception-based intervention as part of their routine antenatal care.

Critique

The limitations of each study have been discussed in each respective chapter. However, there are a number of general limitations across all the studies that warrant further exploration and are discussed below.

A concern for research assessing smoking during pregnancy is the reliance on maternal self-report. One study attempted to validate maternal self-report levels of smoking via cotinine measures, and out of 737 women who claimed to stop smoking prior to pregnancy, 21.6% were reclassified as active smokers following the cotinine

assessment due to evidence that they continued to smoke (England et al., 2007). Authors of the study claim that misclassification led to bias in their study, with an overestimation of negative birth outcomes. This appears to be the case in other countries, for example a study of 1,239 women from Estonia who self-reported as non-smokers, 20.9% of these women were active smokers as identified by cotinine measures (Pärna et al., 2005).

In attempts to address the issue of maternal self-report in the present research, a CO breath test was conducted in addition to asking the mothers about the number of cigarettes smoked per day. A CO breath test can provide an indication as to whether the women were likely smokers. However, the results provide an immediate reading at the time of measurement and therefore cannot provide an indication of the long-term use or quantity of cigarette smoking. Levels of CO decrease rapidly up to 50% within 4 hours, and thus smoking can sometimes go undetected (NICE, 2010). To highlight this issue, a woman who smokes <10 per day, but had a cigarette 20 minutes before the CO reading, the test might show a higher ppm reading in comparison to someone who smokes 11-20 per day but hadn't smoked that morning prior to the CO breath test. A study assessing the validity of CO measurement assessing both parents during pregnancy, obtained a CO breath test reading (Smokerlyzer) and conducted an interview to assess level of smoking. The results of the study indicated that CO measurement was a good indicator for smoking with specificity of 97-100% (Christensen et al., 2004). However, the cut off for smoking in Christensen et al. (2004) was 8ppm, which is much higher than what was conducted in the series of research studies in this thesis, as the NICE (2010) guidance indicator level of cigarette smoking of 3ppm was used.

In the current research it was not assessed when the mother last had a cigarette prior to the scan, and therefore the CO breath test was relied on to provide an indication of this. However, when analysing fetal behavioural patterns, there was no relation to maternal or fetal CO and fetal mouth movements. Therefore, it could be argued that rather than smoking cigarettes having an immediate impact on fetal behaviour, although not established in the current series of studies, it could be argued that cigarette smoking has a cumulative effect (Vesterinen, Morello-Frosch, Sen, & Woodruff, 2017) rather than an immediate effect. This needs to be examined in future research.

Given the results that behavioural outcomes are similar for cigarette and e-cigarette exposed infants, assessing nicotine intake is vitally important but difficult to measure. Due to CO not being in e-cigarettes, nor other methods of NRT, this method cannot be relied upon to provide an indication of toxin exposure, with scores on the smokerlyser being comparable to non-smokers. In the present research the amount of nicotine in milligrams in the cartridge of their e-cigarette were noted. However, it is impossible to quantify in detail how much of the cartridge is consumed per day compared to cigarettes where this can be ascertained through questioning and CO breath test. Therefore, it is difficult to determine how much nicotine is circulating in fetal blood and amniotic fluid. A large review of the literature between 2007-2017 based on 40 studies by Whittington et al. (2019) assessing the use of e-cigarettes indicated that the amount of nicotine stemming from e-cigarettes consumed during pregnancy is comparable to the levels of nicotine ingested by smoking cigarettes

(Whittington et al., 2018). This is of course problematic, given that there are many known risks associated with nicotine use during pregnancy (e.g., Ng et al., 2019).

E-cigarette users are often smokers who have switched to e-cigarettes in order to quit smoking. However, one concern with the present research is that the first time point at which smoking status was recorded was at recruitment, approximately 20 weeks gestational age. Smoking status was recorded alongside a CO breath test at 32- and 36-weeks gestational age and then again at the one month follow up. There are a number of implications to this schedule of testing. Smoking at different times during pregnancy will lead to different developmental trajectories due to sensitive or critical periods for various parts of development. For example, critical brain developments occur within the 2nd and 3rd trimester, whereas neurobehavior is likely to be implicated if continued smoking into the last part of the 3rd trimester, whereas quitting smoking in the first trimester can protect against growth restriction (Pickett, Wakschlag, Dai, & Leventhal, 2003). A study examining the fluctuations of smoking intensity (not smoking, light, moderate and heavy smoking) throughout pregnancy based on 60 women, indicated that only 7% remained stable in their smoking intensity, whereas there was vast within person variability for the remaining 93% of women (Pickett et al., 2003).

Although research suggests that if women stop smoking in early pregnancy, the negative effects, particularly in terms of miscarriage, small for gestational age, growth restriction and low birth weight lead to outcomes similar to non-smokers (McCowan et al., 2009; Vardavas et al., 2010). However, there is a suggestion that smoking at any time point during pregnancy can lead to negative effects, such as

reduced fetal growth, with the odds of this occurring increasing the longer the mother smokes during her pregnancy (Blatt, Moore, Chen, Van Hook, & DeFranco, 2015). However, ideally the mother should stop smoking in the preconception period. It is considered that the preconception period is typically thought as six months prior to conception and until the 10th gestational week and it is well known that factors such as cigarette smoking can lead to epigenetic changes which can result in negative pregnancy and child outcomes (Amoako, Nafee, & Ola, 2017). Whilst the preconception period lasts a number of months, there are two key critical time points (during gametogenesis and pre-implantation) which may lead to epigenetic reprogramming (Amoako et al., 2017). Therefore, smoking prior to and throughout pregnancy, not just at three time points, needs to be assessed.

In addition, and general to the discussion of behavioural research methodology, is that snapshots of behaviour are assessed, 20 minutes at both 32- and 36-weeks gestational age. Similarly, the infant was only assessed once lasting approximately 40-60 minutes. Whilst it is considered that 10-minute observations are sufficient enough to capture behavioural data to make conclusions, as evidenced by Reissland et al. (2015) pilot study, 15-minute observations are considered to be reliable (Heyman et al., 2001). However, there are a number of factors that can influence fetal and infant behaviour such as maternal caffeine intake (Mulder, Tegaldo, Bruschettini, & Visser, 2010), maternal fasting (Abd-El-Aal, Shahin, & Hamed, 2009) and infant sleep (Sadeh, 2007) for example, all of which can have an effect throughout pregnancy and early infancy and the effects of such will depend on when the fetus/infant was assessed in relation to the influencing factor. Sleep states for example are of particular importance when considering fetal behavioural analysis. There are four distinct states. State 1 in which periods of inactivity are spontaneously

interrupted by gross body movements. State 2 whereby movements are mainly stretches and limb movements. State 3 is a period of quiet wakefulness, and state 4 is where the fetus is active with continual movements (Reissland & Kisilevsky, 2016). Within the observation times of the present series of studies, it is likely that the fetuses may have transition through such states, however, it was ensured that the fetuses were at least in state 2, as measured by assessing limb movements.

There is a suggestion that prenatal care, as it is currently practised in the UK, is in fact too late to reverse some negative outcomes. This is likely linked to the fact that by the time the woman realises she is pregnant and has her first prenatal appointment, vital fetal development has already taken place. The focus should be on preconception care (Atrash, Johnson, Adams, Cordero, & Howse, 2006), as smoking during this time can lead to an increase in preterm births (Haas et al., 2005). Additionally, smoking in the periconception period (1 month prior to pregnancy until end 1st trimester) can lead to a three-fold increase in heart defects (Karatza et al., 2011). Therefore, it appears that it may be too late to change health behaviours once pregnant. Although a number of risk factors are associated with preterm birth, that are often addressed during pregnancy, they cannot account for the cumulative effects of smoking prior to pregnancy. Therefore, health status prior to pregnancy should be addressed. Such research highlights a problem with the research presented in the thesis as smoking status was simply ascertained at point of recruitment (after the 20-week anomaly scan), and then reported and verified by CO breath test at 32, 36 and one month after birth. Smoking status was not assessed throughout the course of pregnancy.

Longitudinal research is beneficial in examining the causality of a research topic and understanding an effect over time and therefore is an important method used in developmental psychology for example (Marcellus, 2004). However, some of the benefits are often reduced due to attrition rates (Barry, 2005). This is highly problematic in research, as this can have a significant impact upon the findings in that those who discontinue with the research may have certain characteristics leading to a bias sample. Attrition rates have the potential to introduce bias into the data set and lead to incorrect interpretations of the data, especially if the attrition is non-random (Eisner, Murray, Eisner, & Ribeaud, 2019). By dropping out of the research, it could be that the remaining cases are no longer representative of the original sample. Throughout the prenatal period of the research, there was attrition linked to quality of the scans, which was random, and at 36 weeks there was drop out in addition to poor quality scans. Two women did not participate due to already giving birth, with 13 women who could not attend the scan (see Chapter 2 for further details). Assessing levels of stress, depression, anxiety, attachment, maternal age and smoking status (5 non-smokers, 7 smokers and 1 e-cigarette user) in these women in comparison to those who attended the appointment, with no differences found. This suggests that drop out at this stage was random. By the postnatal follow up, out of 123 recruited, 40 women either dropped out or were not eligible to participate. Nine were excluded due to medical complications following birth. Six could not be contacted and 25 declined to participate. For these 31 women there was no difference between maternal age, stress, depression, anxiety or attachment levels, or gestation at birth and head circumference. However, there was a significantly smaller mean birthweight for those who did not participate. Out of these women 8 were non-smokers, 5 were e-cigarette

users and 18 were smokers. In some studies, attrition rates have been as high as 70%, with it being suggested that attrition rates over 20% is considered concerning and has the potential to introduce bias (Marcellus, 2004). Attrition rate in the present postnatal study was 25% and therefore only just over the threshold to what may be considered concerning, and therefore likely to have little bias. Because attrition does occur, it is important to highlight the original sample and then the final sample within a research paper.

In addition to attrition, some of the ultrasound scans could not be analysed due to poor quality or lack of mouth area visibility. Due to the limited time and capacity the NHS could afford to dedicate to the scans (20-minutes per participant), there were often times when the fetus was in a poor position for visibility of the fetal face, therefore there was occasions when the scan was not sufficient to capture the data required for the research (see Chapter 2 for further details).

One of the central aims of the research was to begin to establish what prenatal mouth movements might mean for subsequent behaviour. It is challenging to begin to address this question for a number of reasons. Firstly, the environment is vastly different between pre and postnatal life. Secondly, there are a number of other environmental factors that may play a role, such as smoking, parenting interaction, attachment style and postnatal nutrition. To highlight such issues, a study assessed scores on the Bayley Scales of Infant Development at 2 years of age and found this was predicted by parental behaviours such as positive affect, sensitivity and parent-child synchrony, indicating that the interaction with a parent can shape a child's cognitive, motor, social and emotional development (Treyvaud et al., 2009). There is

some research to suggest that skin to skin contact immediately after birth leads to better motor modulation and state organisation as it benefits the newborn due to the transition between in utero and outside environment. Therefore, it could be that such factors leads to different neurobehavior and should be taken into account (Ferber & Makhoul, 2004). It seems apparent that there is a relationship between mother-infant interaction and neurobehavior of an infant (Costa & Figueiredo, 2012).

In comparison to other fetal assessment methods, the FOMS is not a standardised assessment and at present there is no identification of what is considered normal or abnormal levels of facial movements, with the parameters of such not being defined. This is in contrast to assessment measures such as the KANET, which is a standardised assessment tool that is used in clinical settings that can identify normal and abnormal behaviour in the fetus (see Chapter 3 for further details) (Antsaklis, Kurjak, & Izebegovic, 2013). When using an assessment measure such as the FOMS, it is important to first determine what is considered normal behaviour and the parameters for this.

Future research

In order to address the critiques of the research associated with the issue of CO and self-reporting, a biological measure could be introduced, such as a cotinine measurement in the mother and subsequently the newborn infant, by obtaining urine or saliva samples throughout the course of pregnancy and in the immediate postpartum period. This would provide an indication of levels of nicotine ingested during pregnancy for both cigarette smokers and e-cigarette users. Measures of

cotinine could then be used to assess the association to both prenatal and postnatal behaviours. Cotinine is the most predominant metabolite of nicotine, that can be assessed through a variety of methods, in particular saliva, urine and blood, with great sensitivity and specificity (Kim, 2016). Urine samples are considered a better method of measuring cotinine due to being approximately 6 times higher in concentration in comparison to blood or salvia. Cotinine is reflective of the amount of nicotine exposure. A biomarker would be a more objective measure of establishing smoke exposure. Cotinine measures from cord blood at birth and urine up to at least 4 years of age have been shown to accurately classify children who are exposed to cigarette smoke (Puig et al., 2008). However, one problem with using cotinine samples is the high cost associated with processing and sending of the samples (Raja, Garg, Yadav, Jha, & Handa, 2016).

Observing the general population, men and women between the ages of 25 to 64 years old, there was high validity between self-reported smoking and serum cotinine measures. For those who are regular smokers, cotinine levels were 10ng/ml or higher for 97.2% of men and 94.9% of women, which is in contrast to those who used to smoke but hadn't in the previous months (6.3% and 5.2%) and those claiming to be non-smokers (2.5% and 2.7%) (Vartiainen, Seppälä, Lillsunde, & Puska, 2002). In a study of 998 pregnant women, there were fluctuations of smoking throughout pregnancy, but generally a high correlation between self-report and cotinine measures, although these correlations were weaker when assessing on an individual basis (Pickett, Rathouz, Kasza, Wakschlag, & Wright, 2005). Examining cotinine will allow researchers to identify the impact of this chemical in a dose-dependent manner in order to effectively guide policy on e-cigarette use during pregnancy to help

mothers make an informed choice during their pregnancy. Cotinine samples have already been proven useful with research indicating a dose-response relationship between prenatal nicotine exposure and ADHD (Sourander, Sucksdorff, Chudal, Surcel, Hinkka-Yli-Salomäki, Gyllenberg …& Brown, 2019). However, in the present studies the current thesis, smoking status was only asked upon recruitment at 20 weeks, then examined in further detail and CO at 32 and 36 weeks and then again at the one month follow up, and considering there are variations in smoking status, future research should attempt to assess smoking status throughout the entirety of pregnancy.

However, there are a number of limitations to this approach, such as funding for cotinine measurement and potential difficulty in obtaining a measure at birth. This is why this method was not considered for the research presented in the thesis. However, given the results that e-cigarette exposed infants display similar neurobehaviour to cigarette exposed infants, this research could be used as a starting point to fund future research with a more controlled way of establishing nicotine exposure.

Assessment of other forms of NRT was beyond the scope of this thesis, but none the less the findings of this present study would indicate the importance of research into the behavioural effects of those exposed to other forms of NRT, such as patches, gum and inhalators.

Although beyond the scope of this thesis, future research should attempt to assess the complete fetal facial movement profiles of the fetuses to further explore complexity

of facial movements, alongside assessing body movements and touch behaviours. Examining facial gestalts might be able to provide a better indication of complexity.

Additionally, continuity of pre to postnatal mouth movements should be assessed, this was not an aim of this research, but stripping back the research aims may provide a better approach to understanding how pre to postnatal behaviour are related.

Rates of smoking during pregnancy are declining (2.4% reduction from 2018/2019 to 2019/2020) (Public Health England, 2020), however, one concern is that the decreases are occurring at slower rates for those mothers with lower socio-economic status, which is contributing to the health inequalities. This poses an issue that smoking status is tied to health inequalities and therefore it can be difficult to assess the true effect of smoking outside of these vast inequalities (Stock & Bauld, 2020). Therefore, future research should take this into consideration.

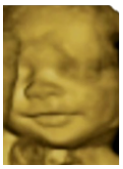
Conclusions

The five studies presented in this thesis highlight a mixed method approach to understanding the impact of prenatal cigarette and e-cigarette exposure on fetal mouth movements, infant neurobehaviour, attempts to begin to explore the relationship between pre to postnatal behaviour and a maternal understanding of risks associated with both cigarette and e-cigarette use during pregnancy. Although results indicate no significant differences in fetal behaviour in contrast to previous research and found no relationship between prenatal mouth movements and postnatal behaviour as assessed by the NBAS, the null results are still important. As e-cigarette exposed

infants display similar neurobehaviour as cigarette exposed infants and the interview study provided insight into reasoning for continued smoking, this has important implications for policy and guidance for the recommendations of e-cigarette use and smoking cessation interventions during pregnancy.

Appendices

Appendix 1



The PEN Study

Prenatal Effects of Nicotine

South Tees Hospitals NHS

NHS Foundation Trust

The James Cook University Hospital
Marton Road
Middlesbrough
TS4 3BW

www.southtees.nhs.uk
Tel: 01642 850850

The PEN Study: Prenatal effects of nicotine

Information Leaflet for Parents

We would like to invite you to take part in a study looking at facial movement before birth. Your participation will help us to learn about how the unborn child's facial expressions develop in the womb.

What is the purpose of this study?
The purpose of this study is to establish whether babies of mothers who smoke show different facial movements in the womb compared with mothers who do not smoke. We want to look at babies when they are 32 and 36 weeks into the pregnancy. We do this by videotaping scans of facial movements of the unborn baby.

Why did we ask you?
We will invite the first 50 mothers who are pregnant and who do not smoke and the first 50 mothers who do smoke. We only ask mothers who have had their 20-week anomaly scan showing a healthy baby.

What is involved for the participant in this study?
If you choose to take part, we will scan your unborn baby using the ultrasound scanner in the Unit of the James Cook Hospital. We will scan the baby twice at 32 and 36 weeks into your pregnancy. Although we will not reimburse your travel expenses, we shall give you a copy of your scans on DVD. You will be asked if you would like to be contacted after the birth of your child to take part in future research.

How long does my participation in the study last and what is the procedure?
You will be involved in this study for a minimum of four months in the latter part of your pregnancy. The scanning will last about 15-20 minutes. We will look at the baby's face and point out to you the baby's movements. You can see the baby on the screen as you could when you got your 20-week anomaly scan. Sometimes, when the baby is in the wrong position (e.g. hiding the face behind an arm) you cannot see much. At other times, you can see mouth movements or even the baby sucking his or her thumb. You will be lying on your

back as you did in your 12 and 20-week scans. An experienced person will do the scan in order to see the face of your baby. After the scans, we will ask you to fill out four questionnaires relating to whether you are stressed and how positive/negative your mood is now in order to see whether this might have an effect on how the baby moves. These will take about 5-10 minutes each. We will also ask you to blow in a tube in order to assess your Carbon Monoxide level. This will take just a few minutes.

What are reasons why you might not be able to participate in the study?
We want to establish the normal range of movements that the unborn baby shows in mothers who smoke and those who do not. In the unlikely event that you develop any complications during the pregnancy, you will not be able to continue in the study.

What happens if you give up smoking?
If you are assigned to the smoking group and you give up smoking we can still use your scans and you will continue being part of the study. We will then be able to identify whether giving up smoking later in pregnancy affects fetal facial movements.

What happens if we find an anomaly?
The scan is not intended to look for problems with your baby. If any problems were observed during the non-medical scans, you would be referred to the scan clinic and a doctor in the clinic would make appropriate arrangements for follow-up as per normal hospital guidelines.

Confidentiality:
All data will be anonymous and no names will appear in any published results. Data will be kept in a locked cabinet in the Department of Psychology, University of Durham, for 5 years after publication of all results.

What happens if you no longer want to participate?
It is your decision to take part in this study. Participation in the study will not affect your care in any way. You are free to withdraw from the study at any time, without penalty or loss of benefits. If you decide not to take part in this study, you still receive the highest level of care and attention by staff.

Results of this study
All participants get a summary of the results on request. We aim to publish the study. We may show pictures of the scan in publications. The picture will not include any information that would allow identification of you or your baby. We will also collect information about your delivery for the purpose of the study.

Participation in this study:
If you would like to participate in this study, please contact Mrs. Kendra Exley, The James Cook University Hospital. Ultrasound scanning unit, e.mail: Kendra.Exley@stees.nhs.uk, tel: 01642 854884 who will make appointment. Or contact the researcher, Suzanne Lisa Froggatt, email: suzanne.l.froggatt@durham.ac.uk.

Questions or concerns
Please read the attached consent form. If you have any questions or concerns please contact S. Froggatt, N. Reissland, K. Kumarendran or K. Exley.

Before signing the consent form, you will have the opportunity to ask any questions and address any concerns.

We thank you for your time and if you would like any more information please feel free to ask.

This study is covered by normal NHS indemnity.

Contact details:

Suzanne L Froggatt (PhD researcher) University of Durham, suzanne.l.froggatt@durham.ac.uk, tel (a dedicated number for this study):

Dr. Kumar Kumarendran, (consultant) The James Cook University Hospital. Email: Kumar.Kumarendran, tel 01642 850850 ext 52777

Mrs. Kendra Exley, (radiographer) The James Cook University Hospital.Ultrasound scanning unit, e.mail: Kendra.Exley@stees.nhs.uk, tel: 01642 854884

Dr Nadja Reissland, (psychologist) University of Durham n.n.reissland@durham.ac.uk, tel 0191-3343287

Appendix 2

Smoking Assessment

1. Age (years): _____

2. What is your highest level of education? (*Please circle*)
   None GCSE College/A-level Degree Masters degree PhD

3. How many units (e.g half a glass of wine or a $^{1/3}$ of a pint of beer) of alcohol per week do you drink?
   None 1-3 4-6 7-9 10-14 15 or more

4. Who in your household smokes cigarettes?_____

5. Do you smoke? (*Please circle*) Yes/No (if yes, please continue)

6. How old were you when you started smoking cigarettes? _____age

7. If you used to smoke but have given up, when did you quit?
   ……..

   Did you use NRT to help you quit? *(Please circle)* Yes/No

8. Do you use nicotine replacement therapy? (e.g. e-cigarettes, nicotine patches, nicotine gum)
   Yes / No *(If yes, please circle the type of NRT used)*

   e-cigarettes Nicotine patches nicotine gum nicotine spray other……

9. How many milligrams of nicotine are in the NRT product you use*? (e.g. how many mg of nicotine is in the e-cigarette cartridge you are using)*
   _____

10. **If you currently smoke**: Have you been referred to smoking cessation?_____

11. Have you contacted the smoking cessation during your pregnancy?_____

12. How many years have you smoked cigarettes **regularly**?_____years

13. How many cigarettes a day do you smoke? **Circle One**

0	**1**	**2**	**3**
10 or less	11 to 20	21-30	31 or more

14. Would you like to give up smoking if you could do so easily? *(please circle)*
    Yes / No

15. On the following scale from 1 to 10, what number best reflects how ready you are now to quit smoking? *(Please circle)*

Definitely not ready to quit				**ready**					**Definitely to quit**
1	2	3	4	5	6	7	8	9	10

Appendix 3

Prenatal Attachment Questionnaire

Please highlight only one answer

1.Over the past two weeks I have thought about or been preoccupied with the baby inside me:

Almost all the time
Very frequently
Frequently
Occasionally
Not at all

2.Over the past two weeks when I have spoken about, or thought about the baby inside me I got emotionally feelings which were:

Very weak or non-existent
Fairly weak
In between strong and weak
Fairly strong
Very strong

3.Over the past two weeks my feelings about the baby inside me have been:

Very positive
Mainly positive
Mixed positive and negative
Mainly negative
Very negative

4.Over the past two weeks I have had the desire to read about or get information about the developing baby. This desire is:

Very weak or non-existent
Fairly weak
Neither strong nor weak
Moderately strong
Very strong

5.Over the past two weeks I have been trying to picture in my mind what the developing baby actually looks like in my womb:

Almost all the time
Very frequently
Frequently
Occasionally
Not at all

6.Over the past two weeks I think of the developing baby mostly as:

As a real little person with special characteristics
A baby like any other baby
A human being
A living thing

A thing not yet really alive

7.Over the past two weeks I have felt that the baby inside me is dependant on me for its well-being:

Totally
A great deal
Moderately
Slightly
Not at all

8.Over the past two weeks I have found myself talking to my baby when I am alone

Not at all
Occasionally
Frequently
Very frequently
Almost all the time I am alone

9.Over the past two weeks when I think about (or talk to) my baby inside me, my thoughts:

Are always tender and loving
Are mostly tender and loving
Are a mixture of both tenderness and irritation
Contain a fair bit or irritation
Contain a lot of irritation

10.The picture in my mind of what the baby at this stage actually looks like inside the womb is:

Very clear
Fairly clear
Fairly vague
Very vague
I have no idea at all

11. Over the past two weeks when I think about the baby inside me I get feelings which are:

Very sad
Moderately sad
A mixture of happiness and sadness
Moderately happy
Very happy

12.Some pregnant women sometimes get so irritated by the baby inside them that they feel like they want to hurt it or punish it:

I couldn't imagine I would ever feel like this
I could imagine I might sometimes feel like this, but I never actually have
I have felt like this once or twice myself
I have occasionally felt like this myself
I have often felt like this myself

13.Over the past two weeks I have felt:

Very emotionally distant from my baby
Moderately emotionally distant from my baby
Not particularly emotionally close to my baby
Moderately close emotionally to my baby
Very close emotionally to my baby

14.Over the past two weeks I have taken care with what I eat to make sure the baby gets a good diet:
Not at all
Once or twice when I ate
Occasionally when I ate
Quite often when I ate
Every time I are

15.When I first see my baby after the birth I expect I will feel:
Intense affection
Mostly affection
Dislike about one or two aspects of the baby
Dislike about quite a few aspects of the baby
Mostly dislike

16. When my baby is born I would like to hold the baby:
Immediately
After it has been wrapped in a blanket
After it has been washed
After a few hours for things to settle down
The next day

17.Over the past two weeks I have had dreams about the pregnancy or baby:
Not at all
Occasionally
Frequently
Very frequently
Almost every night

18.Over the past two weeks I have found myself feeling, or rubbing with my hand, the outside of my stomach where the baby is:
A lot of times each day
At least once per day
Occasionally
Once only
Not at all

19.If the pregnancy was lost at this time (due to miscarriage or other accidental event) without any pain or injury to myself, I expect I would feel:
Very pleased
Moderately pleased
Neutral (i.e. neither sad nor pleased; or mixed feelings)
Moderately sad
Very sad

Appendix 4

Postnatal Attachment Questionnaire

Anonymous code: Baby's age:

Smoking status: Cigarette smoker
E-cigarette smoker
Nicotine patches/gum
Combination
Non-smoker *Please highlight only one answer*

1.When I am caring for the baby, I get feelings of annoyance or irritation:
Very frequently
Frequently
Occasionally
Very rarely
Never

2.When I am caring for the baby I get feelings that the child is deliberately being difficult or trying to upset me:
Very frequently
Frequently
Occasionally
Very rarely
Never

3.Over the last two weeks I would describe my feelings for the baby as:
Dislike
No strong feelings toward the baby
Slight affection
Moderate affection
Intense affection

4. Regarding my overall level of interaction with the baby I:
Feel very guilty that I am not more involved
Feel moderately guilty that I am not more involved
Feel slightly guilty that I am not more involved
I don't have any guilty feelings regarding this

5.When I interact with the baby I feel:
Very incompetent and lacking in confidence
Moderately incompetent and lacking in confidence
Moderately competent and confident
Very competent and confident

6.When I am with the baby I feel intense and anxious:
Very frequently
Frequently
Occasionally
Almost never

7.When I am with the baby and other people are present, I feel proud of the baby:
Very frequently
Frequently
Occasionally
Almost never

8.I try to involve myself as much as I possibly can PLAYING with the baby:
This is true
This is untrue

9.When I have to leave the baby:
I usually feel rather sad (or its difficult to leave)
I often feel rather sad (or its difficult to leave)
I have mixed feelings of both sadness and relief
I often feel rather relieved (and it's easy to leave)
I usually feel rather relieved (and its easy to leave)

10.When I am with the baby:
I always get a lot of enjoyment/satisfaction
I frequently get a lot of enjoyment and satisfaction
I occasionally get a lot of enjoyment/satisfaction
I very rarely get a lot of enjoyment/satisfaction

11.When I am not with the baby, I find myself thinking about the baby:
Almost all the time
Very frequently
Frequently
Occasionally
Not at all

12.When I am with the baby:
I usually try to prolong the time with him/her
I usually try to shorten the time I spend with her/her

13.When I have been away from the baby for awhile and I am about to be with him/her again, I usually feel:
Intense pleasure at the idea
Moderate pleasure at the idea
Mild pleasure at the idea
No feelings at all about the idea
Negative feelings about the idea

14.I now think of the baby as:
Very much my own baby
A bit like my own baby
Not yet really my own baby

15.Regarding the things that we have had to give up because of the baby:
I find that I resent it quite a lot

I find that I resent it a moderate amount
I find that I resent it a bit
I don't resent it at all

16.Over the past three month, I have felt that I do not have enough time for myself or to pursue my own interests:
Almost all the time
Very frequently
Occasionally
Not at all

17.Taking care of this baby is a heavy burden of responsibility. I believe this is:
Very much so
Somewhat do
Slightly so
Not at all

18.I trust my own judgement in deciding what the baby needs:
Almost never
Occasionally
Most of the time
Almost all the time

19.Usually when I am with the baby:
I am very impatient
I am a bit impatient
I am moderately patient
I am extremely patient

Appendix 5

Perceived Stress Scale

The questions in this scale ask you about your feelings and thoughts during the last month. In each case, you will be asked to indicate by highlighting how often you felt or thought a certain way.

0 = Never 1= Almost never 2= Sometimes 3= Fairly often 4= Very often

1.In the last month, how often have you been upset because of something that happened unexpectedly?	0 1 2 3 4
2.In the last month, how often have you felt that you were unable to control the important things in your life?	0 1 2 3 4
3.In the last month, how often have you felt nervous and "stressed'?	0 1 2 3 4
4.In the last month, how often have you felt confident about your ability to handle your personal problems?	0 1 2 3 4
5.In the last month, how often have you felt that things were going your way?	0 1 2 3 4
6.In the last month, how often have you found that you could not cope with all the things that you had to do?	0 1 2 3 4
7.In the last month, how often have you been able to control irritations in your life?	0 1 2 3 4
8.In the last months, how often have you felt that you were on top of things?	0 1 2 3 4
9.In the last month, how often have you been angered because of things that were outside of your control?	0 1 2 3 4
10.In the last month, how often have you felt difficulties were piling up so high that you could not overcome them?	0 1 2 3 4

Appendix 6

Hospital Anxiety and Depression Scale

Tick the box besides the reply that is closet to how you have been feeling in the past week. Don't take too long over your replies: your immediate response is best.

I feel tense or 'wound up'':		**I feel as if I am slowed down:**	
Most of the time		Nearly all the time	
A lot of the time		Very often	
From time to time, occasionally		Sometimes	
Not at all		Not at all	
I still enjoy the things I used to enjoy:		**I get sort of frightened feeling like 'butterflies' in the stomach:**	
Definitely as much		Not at all	
Not quite so much		Occasionally	
Only a little		Quite often	
Hardly at all		Very often	
I get a sort of frightened feeling as if something awful is about to happen:		**I have lost interest in my appearance:**	
Very definitely and quite badly		Definitely	
Yes, but not too badly		I don't take as much care as I should	
A little. But it doesn't worry me		I may not take quite as much care	
Not at all		I take just as much care as ever	
I can laugh and see the funny side of things:		**I feel restless as I have to be on the move:**	
As much as I always could		Very much indeed	
Not quite so much now		Quite a lot	
Definitely not so much now		Not very much	
Not at all		Not at all	
Worrying thoughts go through my mind:		**I look forward with enjoyment to things:**	
A great deal of the time		As much as I ever did	
A lot of the time		Rather less than I used to	
From time to time, but not too often		Definitely less than I used to	
Only occasionally		Hardly at all	
I feel cheerful:		**I get sudden feelings of panic:**	
Not at all		Very often indeed	
Not often		Quite often	
Sometimes		Not very often	
Most of the time		Not at all	

I can sit at ease and feel relaxed:		**I can enjoy a good book or radio or TV program:**	
Definitely		Often	
Usually		Sometimes	
Not often		Not often	
Not at all		Very seldom	

Appendix 7

Consent Form for parents

Initial

I have read the information for parents leaflet ☐

I have had the opportunity to ask questions and discuss this study ☐

I have received satisfactory answers to my questions ☐

I have received enough information about the study ☐

Who have you spoken to about the study? _____

I understand that I am free to leave the study: ☐

- at any time
- without having to give a reason for leaving ☐
- and without affecting my medical care? ☐

I give my consent for video images of the scan to be made of my unborn child. ☐

I also give my consent for material to be shown for research and teaching purposes, used in publications, journals and textbooks. ☐

I agree that the scans are only used for research purposes and cannot be used to identify any specific conditions. ☐

I agree that you can obtain delivery details of my baby from my medical records. ☐

I agree that if I score highly on the questionnaires the clinic would make appropriate arrangements for follow-up as per normal hospital guidelines. ☐

I understand that data will be anonymous and no names will appear in any published results ☐

I can review the material by arrangement with the University of Durham, Psychology department (Suzanne L Froggatt suzanne.l.froggatt@durham.ac.uk). ☐

I give my permission to be contacted by S.Frogggatt after the birth of my baby to take part in future research. ☐

I agree to participate in this study as explained to me by the person named above:
Signed: _____Date: (dd/mm/yy)_____

Name: _____
(BLOCK CAPITALS please)

Email address:_____

Phone number (Moblie):
_____(Home):_____

Home address:
_____

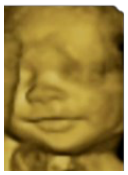
_____

Partner name:

Witnessed by:_____Date: (dd/mm/yy) ____
_____

Appendix 8

Postnatal information leaflet



The PEN Study

Prenatal Effects of Nicotine

Following the birth of your baby please contact us to make an appointment

Telephone Suzanne (a dedicated number to this research: 07843707236

Email: suzanne.l.froggatt@durham.ac.uk

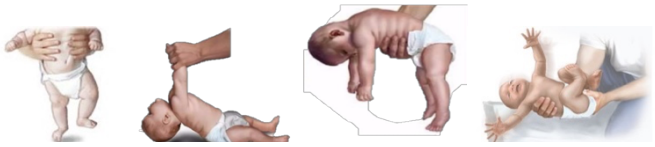
Invitation for the follow-up study post-birth

We would like to invite you to continue with our research and assess your baby at 4 weeks old.

The Neonatal Behavioural Assessment Scale is used across the world to establish strengths and areas where support may be needed in the early infancy stage.

Following the birth of your baby please contact us to make an Appointment

Telephone Suzanne (a dedicated number to this research: 07843707236 Email: suzanne.l.froggatt@durham.ac.uk



What is the purpose of this study?

There are two aims to this study. Firstly, similar to research you took part in during pregnancy, we want to see if there are differences in babies who are exposed to nicotine and those who are not exposed at one month old. Secondly, we want to see how prenatal movements relate to postnatal development.

What is involved in the follow-up research?

Questionnaires—A range of questionnaires you will have previously filled out during pregnancy e.g. stress, depression and anxiety questionnaire. These will take about 5-10 minutes each. We will also ask you to blow in a tube in order to assess your Carbon Monoxide level.

Interview— An audio recorded interview asking about your experience viewing the 4D scan and how you think nicotine impacts on fetal and infant development.

Baby Assessment—Neonatal Behavioural Assessment Scale (NBAS) which is similar to an assessment that your health visitor may do. Throughout the assessment your baby will be assessed on their response to light and sound during sleep and once awake their social abilities and reflexes including the stepping and walking reflex.

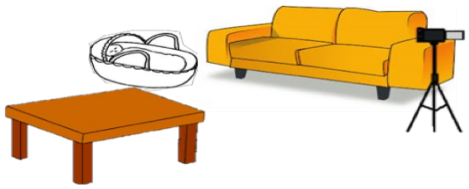
How does the assessment start?

- Your baby will be undressed throughout the assessment (to their vest) so it is possible to see the reflexes and see their response to this.
- The best time to carry out this assessment is in-between feeds and ideally will start when your baby is asleep in a quiet semi-darkened room.

Below are some pictures of the NBAS assessment to show you the types of things we do. This is very similar to what a health visitor assesses.

The assessment identifies strengths and areas where extra support may be re- quired for your baby. This is not a medical assessment and will be used for re- search purposes only. Should the assessment show your baby is having a particu- lar difficulty, it is our duty of care to inform a healthcare professional.

Ideally, your baby will need to be asleep in a quiet room where the assessment will be carried out in, in a mosses basket or similar. Here is an example of a room layout. Your sleeping baby and a surface (sofa, table or floor space) in view of the camera.



Why you may not be able to participate

If your baby is receiving treatment or in the NICU at 4 weeks old you will not be able to participate.

Please read the privacy notice on the next couple of pages that informs you about how your data will be stored, processed and share.

You have the right to withdraw at any point and all data is confidential.

PART 1 – GENERIC PRIVACY NOTICE

Durham University's responsibilities under data protection legislation include the duty to en- sure that we provide individuals with information about how we process personal data. We do this in a number of ways, one of which is the publication of privacy notices. Our privacy notices comprise two parts – a generic part and a part tailored to the specific processing activity being undertaken.

Data Controller

The Data Controller is Durham University. If you would like more information about how the University uses your personal data, please see the University's Information Governance webpages or contact:

Information Governance Unit Telephone: (0191 33) 46246 or 46103 E-mail: info.access@durham.ac.uk

Data Protection Officer

The Data Protection Officer is responsible for advising the University on compliance with Data Protection legislation and monitoring its performance against it. If you have any concerns regarding the way in which the University is processing your personal data, please contact the Data Protection Officer:

Jennifer Sewel
University Secretary
Telephone: (0191 33) 46144
E-mail: jennifer.sewel@durham.ac.uk Retention

The University keeps personal data for as long as it is needed for the purpose for which it was originally collected. Most of these time periods are set out in the University Records Retention Schedule.

Your rights in relation to your personal data

Privacy notices and/or consent

You have the right to be provided with information about how and why we process your per- sonal data. Where you have the choice to determine how your personal data will be used, we will ask you for consent. Where you do not have a choice (for example, where we have a legal obligation to process the personal data), we will provide you with a privacy notice. A privacy notice is a verbal or written statement that explains how we use personal data.

Whenever you give your consent for the processing of your personal data, you receive the right to withdraw that consent at any time. Where withdrawal of consent will have an impact on the services we are able to provide, this will be explained to you, so that you can determine wheth- er it is the right decision for you.

Right to rectification

If you believe that personal data we hold about you is inaccurate, please contact us and we will investigate. You can also request that we complete any incomplete data.

Once we have determined what we are going to do, we will contact you to let you know.

Right to erasure

You can ask us to erase your personal data in any of the following circumstances:

We no longer need the personal data for the purpose it was originally collected

You withdraw your consent and there is no other legal basis for the processing

You object to the processing and there are no overriding legitimate grounds for the pro- cessing

The personal data have been unlawfully processed

The personal data have to be erased for compliance with a legal obligation

The personal data have been collected in relation to the offer of information society ser- vices (information society services are online services such as banking or social media sites).

Once we have determined whether we will erase the personal data, we will contact you to let you know.

Right to restriction of processing

You can ask us to restrict the processing of your personal data in the following circumstances:

You believe that the data is inaccurate and you want us to restrict processing until we de- termine whether it is indeed inaccurate

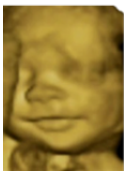
The processing is unlawful and you want us to restrict processing rather than erase it

We no longer need the data for the purpose we originally collected it but you need it in order to establish, exercise or defend a legal claim and

You have objected to the processing and you want us to restrict processing until we deter- mine whether our legitimate interests in processing the data override your objection.

Once we have determined how we propose to restrict processing of the data, we will contact you to discuss and, where possible, agree this with you.

Appendix 9



The PEN Study

Prenatal Effects of Nicotine

Debrief sheet

Thank-you for taking part in the research.

The aim of the research is to assess whether there are longitudinal differences in fetal and early infant behaviour between those who were exposed to nicotine and those who were not exposed to nicotine. Additionally, we want to assess whether fetal facial movements and self-touches are predictive of early infant behaviour.

We hope to publish our results in academic journals and present the findings at an international conference. Whilst I am unable to provide you with individual results due to anonymity of data, I would be happy to provide you with a research summary for the entire project upon request. Due to the longitudinal approach to the research, a summary and link to the papers will not be available until approximately March 2021.

All the data we collected from you will be stored in locked premises at Durham University and only members of the research team will have access to this for further analysis. You have the right to withdraw from the study and your data will be destroyed. If you wish to withdraw your data please contact me before January 2020 otherwise the results may have been published with your data included.

Should you require further information or have any questions please contact Suzanne L Froggatt.

Email: suzanne.l.froggatt@durham.ac.uk
Tel (a dedicated research number): 07843707236

Appendix 10

Feedback from participants

The research was important and of interest to me. We have been told for years about risks of smoking during pregnancy leading to low birth weight and premature births, but haven't heard about reduction in facial expressions whilst in the womb or impacts after birth. I have never been tempted to smoke, but as a scientist and mum to be, I found it incredibly interesting.
The 4d scan was surprisingly beautiful to see and to see my babies face was a very special thing. Instead of an outline it was a definite little human in there and made an even stronger bond and as a result wanted to look after my baby more.
I also found that family were incredibly interested in the images and videos of our baby. Was important to them too.

2) As a researcher, I cannot recommend Suzanne highly enough. She is incredibly professional and puts you at ease. Suzanne is easy to talk to and comfortable to be around. She is also confident and an expert in what she is doing, so was very happy for her to carry out checks on my 4 week old baby.

Hi Suzanne was lovely to take part in the project i think it was important as it i was able to see what my little girl looked like and put me at peace of mind that everything was okay with her facial features was lovely to see her movements while i was carrying her i didnt really think about exercising during my pregnancy. I also felt comftable around you as you explained everything that you were doing throughout and you were very respectful during everything. Thank you for the oppourtunity x

Hi Suzanne,
I felt the research was important and very interesting and seeing the baby in 4D definitely helped to strengthen the bond with baby and therefore impacted upon choices I made in relation to food, allowing myself time to rest etc. I feel that for me this was particularly important with having a toddler to care for also- as there did not seem as much time to relax and bond with baby, as there was in my first pregnancy.
As a researcher you were really lovely, relaxed and approachable and always happy to help, which made participation easier. It was particularly nice that you were so enthusiastic about your research and willing to discuss all aspects and explain why you were doing what you were doing.
Thank you for allowing us to participate in your study.

I found the research very interesting. It would be interesting to see if there was a difference from smoking to non smoking mothers in terms of the baby. The 4D scan defiantly helped me to visualise my baby, giving me an idea visually of how my baby would look when born. I always thought about healthy choices prior to being a part of the research. I have always felt it was important to eat healthy giving my baby the nutrition she needs.
In terms of Suzanne she was so lovely. Respectful, kind and understanding. I felt very comfortable and would certainly do this again. The whole experience was a positive one, knowing I was contributing to a study I found interesting and also, seeing my little girls face was fabulous. When Suzanne came out to my home after having my daughter for the follow up, again she was so polite and friendly, very gentle with my daughter and asked permission for everything she did.

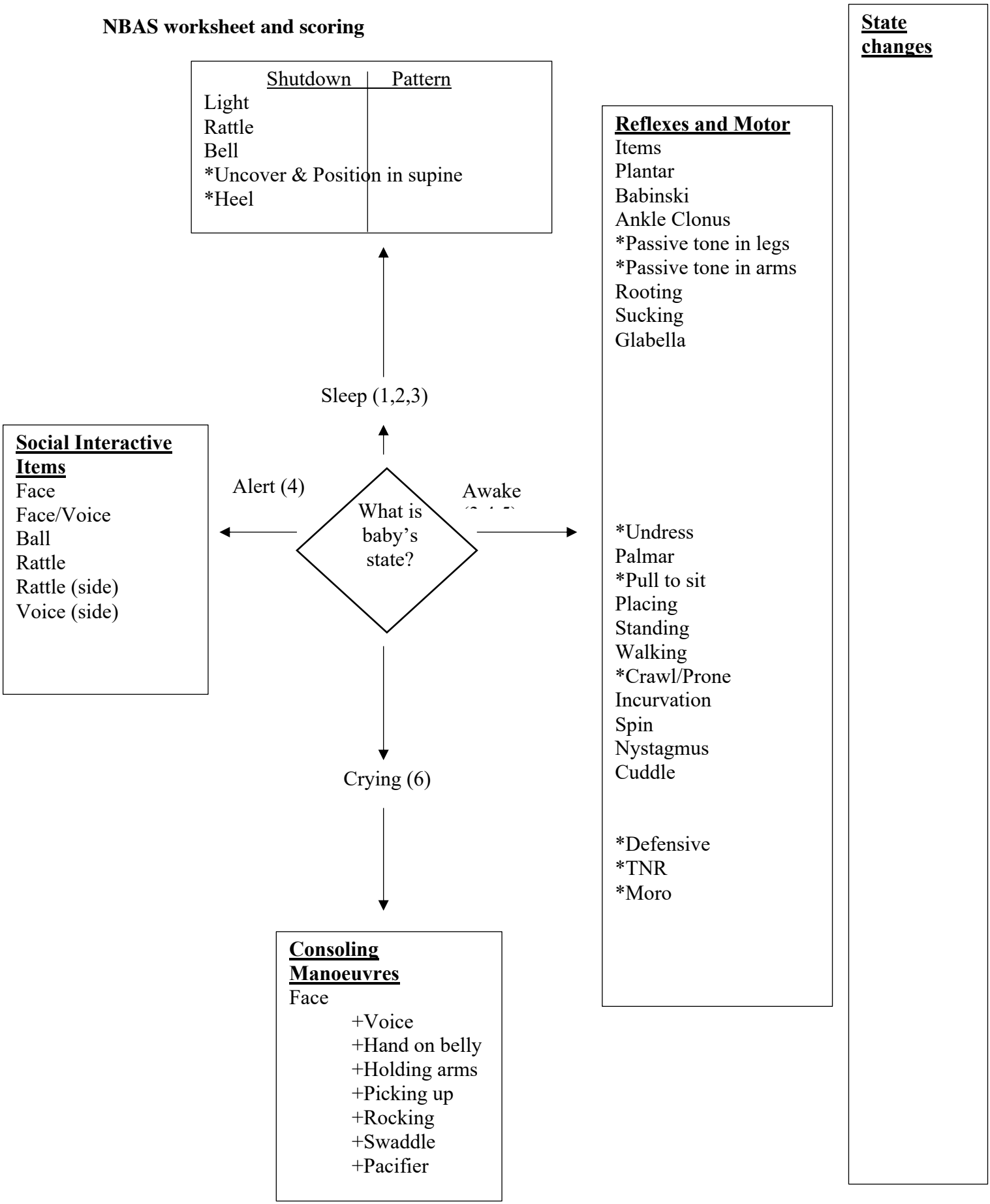
Had my second 4d scan today as part of the study and it was just as special as the first. My partner and I had the opportunity to see our baby before we meet him , see what he gets up to in there and also given photos and videos clips of the whole scans so we are able to look back at our lovely experience. Staff were ever so welcoming and kind which made our experience a pleasant one. Would recommend to anyone to take part and I am so happy I was able to. Thank you again 😌

If this study had not of been on I would never of got a 4 D scan I'm so happy I did now. It was so clear and was brilliant seeing his movements it made me more excited to meet him and the pictures looked the double of him when he was born.
Suzanne was very welcoming and made me feel at ease, she was great with my little boy when I had my home visit ... I would recommend anyone to take the study it made my pregnancy more enjoyable.
Thank you 😊

I would recommend the pen study to every one that gets a chance to be involved. I had both of the 4D scans with them at James cook hospital, they where so welcoming and kind, it was amazing seeing my princess on the screen, thankyous very much for the opportunity to be involved in this amazing research.

Appendix 11

NBAS worksheet and scoring



Alertness

Duration	*Delay*	*Support*
Brief	always	moderate
Moderate	sometimes	minimal
Sustained	never	none

Activity Level (4,5)

Spontaneous	*Elicited*
None	none
Slight	slight
Moderate	moderate
Much	much

Motor Maturity (4,5)

Movements	*Degrees of arcs*
Smooth	45° to 90°
Jerky	45° or less

Tone

Hypotonic
Average
Hypertonic

Consolability (6)

1.

2.

1st Cry (6)

Smiles (All states)

Self Quiet (6,5-4 or lower)

Action	*Number of times*
Brief attempt (<5 seconds)	
Success (5 seconds)	
Sustained success (15 seconds)	

Hand to mouth (all states)

Action	*Number of times*
Swipe	
Hold	
Insert (3+seconds)	
(15 seconds)	

Tremors (all states)

States	*Number of tremors*
1,2,3	
4	
5,6	

Startles (awake)

Number of startles

Baby Name……..Date of Assessment…….Examiner……

Sex…...Dob…….Gestational age……. Birthweight……..Height…….HC……Mode of delivery…….. Length of Labour…….Apgar Scores……..Parity……Type of feeding…….

Infant Behaviour

Habituation	9	8	7	6	5	4	3	2	1	Comments
Response dec. to light										
Reposne dec. to rattle										
Reposnse dec. to bell										
Res. Dec. to foot probe										

Social-Interactive	9	8	7	6	5	4	3	2	1	Comments
Animate visual										
Animate visual & auditory										
Inanimate visual										
Inanimate visual & auditory										
Inanimate auditory										
Animate auditory										
Alertness										

Motor Systems	9	8	7	6	5	4	3	2	1	Comments
General tone										
Motor maturity										
Pull to sit										
Defensive										
Activity level										

State Organisation	9	8	7	6	5	4	3	2	1	Comments
Peak excitement										
Rapidity of build up										
Irritability										
Lability of states										

State Regulation	9	8	7	6	5	4	3	2	1	Comments
Cuddliness										
Consolability										
Self-quieting										
Hand to mouth										

Autonomic System	9	8	7	6	5	4	3	2	1	Comments
Tremulousness										
Startles										
Lability of skin colour										

Supplementary Items	9	8	7	6	5	4	3	2	1	Comments
Quality of alertness										
Cost of attention										
Examiner facilitation										
General irritability										
Robustness & endurance										
State regulation										
E's emotional response										

Reflexes	0	1	2	3	Asym	Comments
Plantar						
Babinski						
Ankle Clonus						
Rooting						
Sucking						
Glabella						
Passive resist – legs						
Passive resist – arms						
Palmer (hand grasp)						
Placing						
Standing						
Walking						
Crawling						
Incurvation						
Tonic dev. -head & eyes						
Nystagmus						
TNR						
Moro						

Summary : Infant	
Strengths	Concerns

Summary : Infant	
Strengths	Concerns

Recommendations for caregiving:

_____

_____

_____

_____

Appendix 12

Semi Structured Interview – for parents who used nicotine during pregnancy

(Questions to be changed depending on whether cigarettes or NRT were used and the answers provided)

During pregnancy did you use cigarettes, nicotine replacement therapy or a combination?

What were you reasons for continuing to smoke/use and NRT during your pregnancy?

Do you believe there is any harm associated with smoking during pregnancy?
- Is there a risk to you?
- Is there a risk to the fetus?
- Is there a risk once the baby is born?

Do you believe there is any harm associated with using e-cigarettes during pregnancy?
- Is there a risk to you?
- Is there a risk to the fetus?
- Is there a risk once the baby is born?

If smoked - Was there anything which might have helped you stop smoking during pregnancy?

How if at all, did looking at your 4D scan change your behaviour? Can you give some examples.

In relation to smoking/NRT what the benefits of viewing your baby via a 4D scan during pregnancy?

Do you think fetal movements differ between non-smokers, smokers and NRT users?

- If so, how?

Do you think nicotine impacts how much your infant cries, is fussy or in general his or her temperament? If yes how and if no why not?

In your opinion does nicotine affect how social (e.g. how much the baby smiles, looks at you and others, plays with others) your baby is? If yes how and if no why not?
In your opinion does nicotine affect how your baby moves his or hers arms, legs and body? If so can you describe the type of movements.

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