



THE UNIVERSITY *of* EDINBURGH

This thesis has been submitted in fulfilment of the requirements for a postgraduate degree (e.g. PhD, MPhil, DClinPsychol) at the University of Edinburgh. Please note the following terms and conditions of use:

This work is protected by copyright and other intellectual property rights, which are retained by the thesis author, unless otherwise stated.

A copy can be downloaded for personal non-commercial research or study, without prior permission or charge.

This thesis cannot be reproduced or quoted extensively from without first obtaining permission in writing from the author.

The content must not be changed in any way or sold commercially in any format or medium without the formal permission of the author.

When referring to this work, full bibliographic details including the author, title, awarding institution and date of the thesis must be given.

**Prenatal methadone exposure and the developing
brain**

Victoria J. Monnelly



Degree of Doctor of Medicine

The University of Edinburgh

2018

ABSTRACT

Opioid use globally is increasing resulting in rising numbers of pregnant women exposing their unborn babies to opioid drugs, with an estimated 30,000 children each year in Europe born to mothers who have used opioids during pregnancy. Methadone is the recommended opioid substitute during pregnancy. Although maternal and perinatal outcomes for pregnant opioid users can be improved by maintenance methadone, there are increasing uncertainties about the safety of methadone on the developing brain and subsequent neurodevelopmental outcomes of children who are exposed prenatally to methadone. Advanced MRI techniques, such as diffusion MRI (dMRI), allow detailed non-invasive investigation of brain microstructure. Imaging biomarkers such as fractional anisotropy (FA), mean diffusivity (MD), axial diffusivity (AD) and radial diffusivity (RD) derived from dMRI, are robust markers of white matter development, with FA associated with later neurodevelopmental outcome. Tract-based spatial statistics (TBSS) enables group-wise comparison of dMRI data and has been optimised for the neonatal brain.

The aim of this thesis was to test the hypotheses that: (i) systematic review of existing published literature shows that prenatal methadone exposure is associated with altered neurodevelopment, visual development and / or brain tissue injury apparent on imaging; (ii) infants with prenatal methadone exposure have altered brain white matter microstructure when compared with term born unexposed control infants, which is manifest by a reduced FA and increased MD and increased RD; (iii) infants with prenatal methadone exposure have reduced total brain volumes and regional brain volumes when compared with term born unexposed infants.

A systematic review of the literature was performed and where amenable data were pooled in random effects meta-analysis. 43 studies were included, 29 reported neurodevelopmental outcomes, 12 reported visual, and 2 reported neuroimaging data. 8 studies were meta-analysed and effect size was expressed as weighted mean difference (WMD) in developmental quotient scores. Childhood neurodevelopment, visual development and behaviour were altered in association with prenatal methadone exposure. Meta-analysis point estimates of cognitive indices (WMD of -4.43, 95% CI -7.24 to -1.63) and motor indices (PDI scores: WMD of -5.42, 95% CI -10.55 to -0.28) in 2 year old children exposed to prenatal methadone were lower than in age matched children with no prenatal drug exposure.

Term born methadone-exposed infants were recruited and underwent an MRI scan shortly after birth. These were compared with 20 non-exposed term controls. An optimised Tract-based Spatial Statistics (TBSS) pipeline was used to perform voxel-wise statistical comparison of FA data. Prenatal methadone exposure was associated with microstructural alteration in major white matter tracts which were present at birth and when head circumference was adjusted for; these changes persisted in the anterior and posterior limbs of the internal capsule and the inferior longitudinal fasciculus ($p < 0.05$). In a subgroup of prenatal methadone exposed infants, lower white matter volumes ($p = 0.049$) and higher cerebrospinal fluid volumes ($p = 0.001$) were demonstrated when compared to healthy term control infants.

This thesis has shown that through systematic review of the literature, there is an association between prenatal methadone exposure and both lower neurodevelopmental scores and visual dysfunction. TBSS showed that prenatal methadone exposure is associated with atypical white matter development compared with unexposed controls. Reduced FA in the white matter skeleton is apparent soon after birth, indicating an association between methadone exposure and brain development in utero; polydrug use among maternal cases limits causal inference. The data do not confirm the safety of methadone for medically assisted treatment of heroin addiction in pregnancy.

LAY SUMMARY

Opioid drugs such as heroin are extremely addictive. Methadone is a medication which is prescribed to help addicts not take illicit opioid drugs and is recommended during pregnancy, as it results in less pregnancy-related complications, such as being born early. However, methadone crosses the placenta, resulting in unborn babies being exposed to methadone whilst they are still developing in the womb. There are concerns that methadone might affect the way brain connections develop and that children exposed to methadone whilst in the womb might not develop normally through childhood as a result. Special MRI scans, known as diffusion MRI, assess the movement of water in the brain, and provide an indication of how well formed the brain white matter tracts (connections) are. White matter tracts that are well developed (mature) and healthy are linked to normal childhood development and less mature tracts are linked to more developmental problems.

This thesis was undertaken to answer the following questions: (i) Is there evidence from studies already published, that exposing unborn babies to methadone causes problems with childhood development? (ii) Are the white matter tracts of the brain different in otherwise healthy babies who were exposed to methadone in the womb, when compared with healthy babies not exposed to any drugs? (iii) Are the whole brains, or specific parts of the brain of babies exposed to methadone in the womb smaller than healthy babies not exposed to any drugs?

A thorough review of the medical literature using specific search words was performed, and where possible, results from studies were combined and statistically

analysed (meta-analysis). 43 studies in total were included, 29 reported development, 12 reported vision and 2 reported brain imaging studies. 8 studies were combined in meta-analysis. Altered childhood development, and problems with vision and behaviour, are more common in children who were exposed to methadone in the womb. Combining the studies (meta-analysis) confirmed this, with developmental scores being on average 4 or 5 points lower if children had been exposed to methadone before birth.

Methadone-exposed babies born around their due date (term) were recruited and underwent an MRI scan shortly after birth. These were compared with 20 term healthy babies not exposed to any drugs. Specialised MRI scans showed that major white matter tracts were less well developed in the babies who were exposed to methadone in the womb, and in a smaller group of babies, the amount of white matter in the brain was less than in healthy babies. Many of the mothers taking methadone also took other prescribed medications such as sedatives or illicit drugs such as heroin, therefore this study was not able to definitively conclude that methadone itself is responsible for the differences described.

The long-term implications of differences in brain connections and worse childhood development in children exposed to methadone in the womb are worthy of further study. In particular, whether alternative strategies or medications to treat drug addiction during pregnancy can improve the outcomes for mothers and babies.

DECLARATION

Except where acknowledgement has been made, the studies undertaken in this thesis were undertaken solely by the author. No part of the work described in this thesis has been previously accepted for or is currently being submitted in candidature for another degree. The publication included in this thesis is the work of the author unless otherwise indicated. Supplementary material relating to Chapter Three can be found in Appendix I and II. Two first author publications can be found in Chapters Three and Four, and the published papers in original formats are found in Appendix III and IV.

Chapter 3:

Within this publication, I conceptualized and designed the study, designed the data collection instruments, collected data, carried out the initial analyses, coordinated and supervised data collection, applied quality assessment, drafted the initial manuscript and critically reviewed the manuscript. Drs Helen Mactier and Ruth Hamilton helped with data collection and drafting of the manuscript for publication. Dr Chappell carried out meta-analysis of a subset of data and critically reviewed the manuscript. I also acknowledge the work of Prof. Boardman, who conceptualized and designed the study, supervised my design of the data collection instruments, applied quality assessment as the second rater, and reviewed and revised the manuscript.

Chapter 4:

Within this Chapter, I acknowledge the work of Dr Sarah Cooper, who assisted in identifying potential participants, Dr Devasuda Anblagan and Dr Manuel Blesa who

assisted with the TBSS analysis and Drs Graham Wilkinson and Alan Quigley who provided a clinical radiology report for all study participants. I acknowledge the work of Dr Ahmed Serag, who performed the volumetric analysis of a subgroup of MRI data. I collaborated with Dr Donata Favoretto and team (Italy) for drug analysis of meconium samples. Prof Boardman and Dr Bastin supervised the study and reviewed and revised the manuscript.

Victoria J Monnelly

DEDICATION

I dedicate this thesis to my amazingly strong and inspirational mother, Molly without whom I (literally) would not be here, or be the person I am; to my wonderful husband, Mike, who keeps me grounded and makes me a better person; to my beautiful children, Emma and George, who make me want to live forever and to my best friend, Georgina, for being 'my person'.

ACKNOWLEDGMENTS

I would firstly like to thank my supervisors, Professor James Boardman and Dr Mark Bastin who have been a constant source of knowledge, support, inspiration and motivation throughout the last four years. I would also like to thank Theirworld (www.theirworld.org) for the generous funding of the study, and Simpson's Special Care Babies charity who funded my MD fees, and finally to the University of Edinburgh for sponsoring the study.

A special thank you goes to Louise Croan and Stephanie Cameron, specialist midwives with an interest in substance misuse during pregnancy, and Dr Sarah Cooper, consultant obstetrician who helped identify potential participants for the study, and to Dr Devasuda Anblagan and Dr Manuel Blesa for their help analyzing the TBSS data. I would like to thank Thorsten Feiweier at Siemens Healthcare for collaborating with dMRI acquisitions (Works-in-Progress Package for Advanced EPI Diffusion Imaging) and Dr Ahmed Serag for his help analyzing the sMRI data and calculating brain volumes.

My colleagues in the Clinical Research Imaging Centre in Edinburgh, in particular the radiographers and Dr Scott Semple, who have been a constant source of encouragement and their efforts to obtain optimal images even when the babies were moving in the MRI scanner were thoroughly appreciated. I would also like to thank Drs Sarah Sparrow, Rozalia Pataky, and Emma Telford for their help recruiting healthy term control babies, Drs Graham Wilkinson and Alan Quigley for providing a clinical report of the MRI scans, and Dr Kamath Tallur for his help and advice

regarding follow up imaging for the patients with incidental MRI findings discovered during this study.

I would like to acknowledge Ms Marshal Dozier, librarian at the University of Edinburgh, for her help and guidance with the original literature search for the systematic review, and Dr Oliver Koch for his help translating two articles written in German.

A special thank you to my extremely tolerant husband Mike and our beautiful children, Emma and George, who make it all seem worthwhile. Their love, support and encouragement has allowed me to get through some difficult times over the last few years. And finally, a huge thank you to all the families and babies who took part in this research. Without their involvement, none of this would have been possible.

PUBLICATIONS, PRESENTATIONS AND PRIZES

First author publications:

Monnelly VJ, Anblagan D, Quigley A, Cabez MB, Cooper ES, Mactier H, Semple SI, Bastin ME, Boardman JP: Prenatal methadone exposure is associated with altered neonatal brain development. *Neuroimage Clin* 2018;18:9-14.

Monnelly VJ, Hamilton R, Chappell FM, Mactier H, Boardman JP: Childhood neurodevelopment after prescription of maintenance methadone for opioid-dependency in pregnancy: a systematic review and meta-analysis.

Dev Med Child Neurol. 2018 Dec 3. doi: 10.1111/14117. [Epub ahead of print]

Oral presentations:

1. Prenatal methadone exposure and neurodevelopmental and neuroimaging outcomes: a systematic review. Neonatal Society Summer Meeting, Brighton, UK, June 2017
2. Atypical brain development in neonates exposed prenatally to methadone. Neonatal Society Summer Meeting, Brighton, UK, June 2017
3. Prenatal methadone exposure and neurodevelopmental and neuroimaging outcomes: a systematic review. Joint European Neonatal Societies Meeting, Venice, November 2017
4. Atypical brain development in neonates exposed prenatally to methadone. Joint European Neonatal Societies Meeting, Venice, November 2017

Prizes:

1. Joint winner of best presentation by a trainee at Neonatal Society Summer Meeting, Brighton, 2017.

TABLE OF CONTENTS

ABSTRACT	ii
LAY SUMMARY	v
DECLARATION	vii
DEDICATION	ix
ACKNOWLEDGMENTS.....	x
PUBLICATIONS, PRESENTATIONS AND PRIZES.....	xii
TABLE OF CONTENTS.....	xiv
LIST OF FIGURES	xix
LIST OF TABLES.....	xxi
ABBREVIATIONS	xxii
CHAPTER 1: INTRODUCTION.....	1
1.1 Opioid use disorder.....	1
1.1.1 Drug use disorders and Opioids.....	1
1.1.2 Opioid use disorder in pregnancy	2
1.1.3 Treatment of OUD in pregnancy	4
1.1.4 Polydrug use and alcohol use in OUD.....	5
1.1.4.1 Meconium for exposure profiling in OUD	6
1.1.5 Challenges of research in pregnant women with OUD.....	7
1.2 Consequences of prenatal opioid exposure.....	8
1.2.1 Fetal neurobehaviour.....	8
1.2.3 Neonatal Abstinence Syndrome.....	9
1.2.4 Sudden infant death syndrome	11
1.2.5 Pre-clinical studies	15
1.2.5 Developmental effects of prenatal opioid exposure in childhood and beyond.....	17
1.2.6 Nature versus Nurture	20
1.3 Cerebral white matter and opioids.....	25
1.3.1 The oligodendrocyte	26
1.3.2 Myelination.....	28
1.3.2.1 Normal pattern of myelination	28
1.3.2.2 Disruption of ‘normal’ myelination.....	29
1.3.3 Neural effects of opioid drugs	30

1.4 Neuroimaging.....	32
1.4.1 Conventional MRI	32
1.4.1.1 Neonatal MRI.....	32
1.4.2 Quantitative MRI.....	35
1.4.2.1 Diffusion MRI	35
1.4.2.1.1 The diffusion tensor	36
1.4.2.1.1 Fractional anisotropy.....	37
1.4.2.1.2 Axial diffusivity.....	38
1.4.2.1.3 Radial diffusivity	38
1.4.2.1.4 Mean diffusivity.....	38
1.4.3 Analysis using dMRI metrics	39
1.4.3.1 Region of Interest	40
1.4.3.2 Tractography	40
1.4.3.3 Tract-based spatial statistics.....	41
1.4.4 TBSS in neonatal imaging.....	42
1.4.4.1 Power calculations for neonatal imaging studies using TBSS.....	45
1.4.5 Brain volume	46
1.4.6 Neuroimaging in opioid exposure.....	47
1.4.6.1 Infants and children	47
1.4.6.2 Adult MRI studies	51
1.5 Summary.....	53
CHAPTER 2: AIMS AND HYPOTHESES	55
2.1 Aims:	55
2.2 Hypotheses:.....	55
CHAPTER 3 : PRENATAL METHADONE AND CHILDHOOD OUTCOMES ...	57
3.1 General introduction.....	57
3.1.1 The Bayley Scale of Infant Development	57
3.2 Background.....	58
3.3 Aims.....	60
3.4 Materials and methods.....	60
3.4.1 Systematic review search information	60
3.4.2 Quality assessment of included studies.....	62
3.4.3 Meta-analysis.....	62
3.5 Results.....	63

3.5.1 Characteristics of included studies	63
3.5.2 Neurodevelopmental outcome.....	65
3.5.2.1 Meta-analysis.....	65
3.5.2.1 Meta-analysis of neurodevelopmental scores at 6 months.....	70
3.5.2.2 Meta-analysis of neurodevelopmental scores at 2 years.....	72
3.5.2.3 Qualitative analysis.....	75
3.5.2.4 Children beyond 2 years of age.....	76
3.5.3 Visual development and function	82
3.5.4 Neuroimaging.....	92
3.6 Discussion	95
3.7 Study strengths	96
3.8 Study limitations.....	96
3.9 Clinical implications of this study.....	98
3.10 Future research	100
CHAPTER 4: PRENATAL METHADONE EXPOSURE AND BRAIN DEVELOPMENT.....	102
4.1 General introduction.....	102
4.2 Background.....	104
4.3 Hypothesis.....	105
4.4 Methods and Materials	106
4.4.1 Participants	106
4.4.2 MRI acquisition	108
4.4.2.1 TBSS.....	109
4.4.3 Statistics	109
4.5 Results.....	110
4.5.1 All participants.....	110
4.5.2 MRI features.....	115
4.5.3. Incidental MRI findings	115
4.5.4 White matter correlates of prenatal methadone exposure	117
4.6 Discussion	121
4.7 Study strengths	122
4.8 Study limitations.....	123
4.9 Clinical implications and future research	124
4.10 Meconium analysis.....	125

4.10.1 Background.....	125
4.10.2 Methods.....	126
4.10.3 Results.....	126
4.10.4 Interpretation.....	128
4.11 Brain volume analysis.....	129
4.11.1 Background.....	129
4.11.2 Methods.....	130
4.11.3 Results.....	131
4.11.3.1 Whole brain volume.....	131
4.11.3.2 Intracranial segmentation volume analysis.....	136
4.11.4 Interpretation.....	141
4.12 Conclusion.....	142
CHAPTER 5: DISCUSSION.....	143
5.1 Summary of key study findings.....	143
5.2 Challenges of research in OUD.....	145
5.2.1 Polydrug use.....	145
5.2.2 Concomitant alcohol use.....	146
5.2.3 Inherent population challenges.....	148
5.3 MRI to investigate potential effects of prenatal drug exposure.....	151
5.3.1 The advantages.....	151
5.3.2 The challenges.....	152
5.4 Prenatal methadone exposure and developmental venous anomalies.....	157
5.5 VEP and MRI findings from this study.....	158
5.6 Prenatal opioid exposure and congenital abnormalities.....	159
5.7 Clinical implications of this study.....	161
5.7.1 Management of OUD in pregnancy.....	161
5.7.1.1 Methadone dosing regimens.....	162
5.7.1.2 Alternative opioid substitutes: Buprenorphine.....	162
5.7.1.3 Medically assisted opioid detoxification.....	163
5.7.2 Neurodevelopmental follow up for all infants with prenatal methadone exposure.....	168
5.8 Study strengths.....	169
5.9 Study limitations.....	170
5.10 The future.....	173

5.10.1 The impact of prescription opioids during pregnancy	173
5.10.2 Future directions	176
5.11 Conclusions	177
BIBLIOGRAPHY.....	179
APPENDIX I.....	203
APPENDIX II.....	207
APPENDIX III	227
APPENDIX IV	239

LIST OF FIGURES

Figure 1 : Oligodendrocyte maturation and methadone	27
Figure 2 :The movement of water within the brain	35
Figure 3 : The diffusion tensor	37
Figure 4 : dMRI metrics.....	39
Figure 5 : PRISMA flowchart showing process of inclusion and exclusion of studies	63
Figure 6 :Quality rating of all included studies. ND, neurodevelopmental outcome studies	64
Figure 7 : WMD in MDI (panel A) and PDI (panel B) at age 6 months between infants exposed to prenatal methadone and unexposed infants.....	71
Figure 8 : WMD in MDI (panel A) and PDI (panel B) at age 2 years between children exposed to prenatal methadone and unexposed children	74
Figure 9 : Details of participant flow through the study	111
Figure 10 : Euler diagram indicating prenatal drug exposures	113
Figure 11 : Left DVA with associated cavernoma	116
Figure 12 : Mean FA map of the subjects in transverse, coronal and sagittal planes	118
Figure 13 : Mean RD map of the subjects in transverse, coronal and sagittal planes	119
Figure 14 : Mean FA across the white matter skeleton of neonates with prenatal methadone exposure compared with unexposed controls.....	120
Figure 15 : Euler diagram illustrating the drug exposure profiles for the 10 methadone-exposed participants with sMRI data used for whole brain volumes....	132
Figure 16 : Box plot showing median intracranial volumes in methadone-exposed (green) and unexposed controls (yellow).....	134
Figure 17 : Correlation between head circumference and total intracranial volume	135
Figure 18 : Euler diagram illustrating the drug exposure profiles for the subgroup with sMRI data used for brain segmentation	137

Figure 19 : Box plot showing the relative volume of cerebral white matter and CSF in participants with full intracranial segmentation in methadone-exposed (green) and un-exposed control infants (red).....140

LIST OF TABLES

Table 1 : Advantages of using TBSS to analyse dMRI data in neonates	41
Table 2 : Summary of the eight studies included in the meta-analysis.....	66
Table 3 : Summary of the twelve studies reporting neurodevelopmental outcomes in children beyond age 2 years	79
Table 4 : Twelve studies reporting childhood visual outcomes after prenatal methadone exposure	84
Table 5 : Two studies reporting childhood neuroimaging outcomes after prenatal methadone exposure	93
Table 6 :Maternal characteristics of participants with dMRI.....	112
Table 7: Infant characteristics of participants with dMRI	114
Table 8 : Results of meconium samples	127
Table 9 : Maternal characteristics of participants with sMRI data used for whole brain volumes	131
Table 10 : Infant characteristics of participants with sMRI data used for whole brain volumes	133
Table 11 : Maternal characteristics of participants with sMRI data amenable to full segmentation.....	136
Table 12 : Infant characteristics of those with sMRI data amenable to full segmentation.....	138
Table 13 : Table showing relative brain proportions in the 15 infants with full intracranial segmentation	139
Table 14 : Appendix I: All studies reporting childhood neurodevelopmental outcomes after prenatal methadone exposure	203
Table 15 : Appendix II: All studies reporting childhood neurodevelopmental outcomes after prenatal methadone exposure	207

ABBREVIATIONS

AD	Axial Diffusion
ALFA	Accurate Learning with Few Atlases
AMP	Amphetamine
BDZ	Benzodiazepine,
BMI	Body Mass Index
BSID	Bayley Scales of Infant Development (1969)
BSID III	Bayley Scale of Infant Development (third version)
BW	Birth weight
CBAQ	Child Behaviour Attitude Questionnaire
CBCL	Child Behaviour Checklist
CGPQ	Child Game Participation Questionnaire
CI	Confidence intervals
CNS	Central nervous system
CRIC	Clinical Research Imaging Centre
CrUSS	Cranial ultrasound,
CSF	Cerebrospinal fluid
CVI	Cerebral visual impairment,
d	Days
DBM	Deformation based morphometry
dMRI	Diffusion magnetic resonance imaging
DTI	Diffusion tensor imaging
DVA	Developmental venous anomaly
FA	Fractional anisotropy

FASD	Fetal alcohol spectrum disorder
FFAE	Free fatty acyl esters
EtG	Ethyl glucuronide
EPI	Echo planar X
EPO	Erythropoetin
ERP	Event-related potentials
FADS2	Fatty acid desaturase 2 gene
FLAIR	Fluid attenuation inversion recovery
GA	Gestational age
GCI	General Cognitive Index
HC	Head circumference,
HPA	Hypothalamic-Pituitary Axis
IBR	Infant behaviour record
ICHD	Intracerebral hemi-diameter
IF	Incidental findings
ILF	Inferior longitudinal fasciculus
IQ	Intelligence quotient
IUS	Infant urine screening
KABC-A	Kaufman Assessment Battery for Children, achievement component
MAT	Medication assisted treatment
MD	Mean diffusivity
MDI	Mental Developmental Index
mg	Milligrams
M-P	Merril-Palmer Scale

MRI	Magnetic resonance imaging
m	Months
ms	Milliseconds
MSCA	McCarthy Scales of Childhood abilities
MeSH	Medical subject headings
MSCA	McCarthy Scales of Childhood Abilities,
MUS	Maternal urine screening
NDI	non-verbal developmental index,
NAS	Neonatal abstinence syndrome
ns	Not significant
OFC	Occipitofrontal circumference
OD	Opioid use disorder
PD	Polydrug use
PDI	Psychomotor Developmental Index
PLIC	Posterior limb of the internal capsule
PMA	Post menstrual age
PROSPERO	International Prospective Register of Systematic Reviews
RA	Robert's Apperception
RAKIT	Revision of the Amsterdam Children's Intelligence Test
RATC	Robert's Apperception Test for Children
RC	Reynell Developmental Language Scales (Comprehensive)
RD	Radial diffusivity
RE	Reynell Developmental Language Scales (Expressive)
SBIS	Stanford-Binet Intellectual scale

SES	Socio-economic status,
SIDS	Sudden Infant Death Syndrome
SIMD	Scottish Index of Multiple Deprivation
sMRI	Structural magnetic resonance imaging
SNR	Signal to noise ratio
SON-IQ	Snijders-Oomen Nonverbal Intelligence Test
SSRI	Selective Serotonin Re-uptake Inhibitor
SUDI	Sudden unexplained death in infancy
SL	Saccade latency
SNP	Spatial negative priming
SLF	Superior longitudinal fasciculus,
TBSS	Tract-based spatial statistics.
T	Tesla
TCA	tricyclic antidepressant
TEA	Term-equivalent age
UK	United Kingdom
US	United States
VA	Visual acuity
VEP	Visual evoked potential
VL	Vineland Social Maturity Scale
w	Weeks
WISC-R	Wechsler Intelligence Scale for Children – Revised
WWPA	Werry-Weiss Peters Activity Scale
yr	Years

μV	microvolts
2D	2-dimensional
3D	3-dimensional

CHAPTER 1: INTRODUCTION

1.1 Opioid use disorder

1.1.1 Drug use disorders and Opioids

Five per cent of the global adult population used drugs at least once in 2015, equating to approximately a quarter of a billion people worldwide, and almost 30 million adults suffer harmful effects from their drug use, either resulting in drug dependence, or necessitating treatment, or both (World Drug Report, 2017). Opioid use accounts for the heaviest burden of disease attributable to drug use disorders, and there are an estimated 35 million past-year users of opioids worldwide.

The term ‘opioid’ includes drugs such as heroin and morphine, but also encompasses a range of synthetic medications such as fentanyl, oxycodone, codeine, methadone and buprenorphine. Opioid drugs act via the opioid receptor and are involved in pain modulation, but they also produce euphoria, a state of intense happiness or excitement, which makes them prone to misuse and dependence. Opioids remain the most harmful drug type in terms of health effects because their use is associated with fatal overdose, addiction, blood borne virus acquisition and medical and psychiatric co-morbidities.

Although heroin is the commonest opioid on the European market, mostly originating from Afghanistan, Iran or Pakistan, other commonly seized opioids include buprenorphine, methadone and tramadol, all of which have are subject to misuse and illicit sales (European Drug Report, 2017). This is consistent with the observation that heroin use has been increasing since 2007 against the backdrop of a prescription

opioids epidemic and whilst 1.3 million opioid users in Europe are deemed ‘high risk’, synthetic opioids such as methadone and oxycodone are increasingly seen in high risk opioid use(European Drug Report, 2017) not just heroin.

1.1.2 Opioid use disorder in pregnancy

Although opioid use is more common in men, once women have initiated substance misuse, they progress more rapidly to drug use disorders. There are estimated to be between 250,000 and 350,000 children with a problem drug-using parent in the UK. In Scotland, there are between 41,000 and 59,000 children with a problem drug using parent, which represents 4 – 6% of all children under 16 years in Scotland and is double the rate in England and Wales(Hidden Harm, 2003). Whilst parental problem drug use can cause serious harm to children of all ages, the effects of maternal drug use during pregnancy poses a unique threat by exposing the unborn child to exogenous drugs at a critical point in their formation and early development.

Rates of heroin use have more than doubled between 2002–4 and 2013–5, from 0.8 to 2.0 per 1000 women(World Drug Report, 2017) and this has resulted in a rise in the number of pregnant women with opioid use disorder (OUD). A study from the United States (US) reports an annual average of 21 000 pregnant women using opioids during their pregnancy(Smith and Lipari, 2013), but exact prevalence is difficult to ascertain due to under-reporting during pregnancy(McGlone et al., 2012). It is estimated that across Europe, over 30,000 children each year are born to mothers who have used opioid drugs during pregnancy(Gyarmathy et al., 2009). US figures pertaining to neonatal abstinence syndrome (NAS), a drug withdrawal syndrome most commonly

used as a proxy measure of prenatal drug exposure, suggest a 5-fold increase in the number of babies prenatally exposed to opioid medications with 5.8 per 1000 births affected by NAS in 2012(Patrick et al., 2015a). This cost the state approximately \$1.5 billion in 2012, as the majority of cases were billed through Medicaid. Furthermore, NAS incidence is likely to be an under-estimate of true prenatal opioid exposure rates, as not all babies with a prenatal drug exposure will experience NAS.

In Scotland, the Information Services Division (ISD), a branch of NHS National Services Scotland, state that 1.3% of all maternities (a pregnancy which results in a live birth or a still birth) reported drug misuse (13.2 per 1000 maternities)in 2014/2015(ISD Scotland, 2014). Of these, 42% were opioids, estimated at 299 pregnant women admitting to prenatal opioid exposure. Similar to the reports from the US, considerable geographic variation in the rates of drug misuse in Scotland have also been observed, with the highest rates correlating to the most deprived areas of Scotland – as high as 22 per 1000 in the most deprived areas.

The risks of opioid use during pregnancy relate to both the health and wellbeing of the mother and the potential adverse effects on their unborn child. Drug and alcohol addiction are important risk factors for maternal death through suicide, accidental overdose and medical complications(Cantwell et al., 2011).

1.1.3 Treatment of OUD in pregnancy

Pregnant women who use heroin, or who have problem opioid use, are recommended medically assisted treatment (MAT) with a long acting opioid substitute, such as methadone(ACOG, 2012). Methadone is a synthetic mu-receptor opioid agonist with a long half-life, making it suitable for once daily dosing. It was introduced as an opioid substitute in the 1960s for treatment of heroin addiction. Methadone maintenance treatment was extended to pregnant heroin addicts in an attempt to reduce the high perinatal morbidity and mortality for infants born to addicted mothers. As part of a comprehensive antenatal care plan, methadone is associated with improved use of antenatal services, reduced use of heroin during pregnancy, and reduced risk of preterm delivery when compared with no treatment (Burns et al., 2007, Mattick et al., 2009, Zelson et al., 1973). Fetal benefits of methadone use include improved growth(Hulse et al., 1997, Kandall et al., 1976) and less risk of intrauterine death(Kandall et al., 1977).

However, the introduction of methadone into clinical practice during pregnancy was not accompanied by any randomised trials assessing infant outcome. Thus, the long-term effects of prenatal methadone on the infant through childhood remain unknown. Methadone freely crosses the placenta, exposing the fetus to methadone at a critical time during brain development. Given the role of endogenous opioids in normal brain development, fetal exposure to exogenous opioids raises concerns about the potential detrimental effects this may have on brain development. Such effects will be explored in more detail throughout this Chapter.

1.1.4 Polydrug use and alcohol use in OUD

Polydrug use is acknowledged as one of the major challenges when undertaking research involving drug misusing populations (Konijnenberg, 2015) because it prevents assessment of causation. In addition, pregnant women often underreport substance misuse (Kelly et al., 2001, McGlone et al., 2012). Studies attempting to quantify polydrug rates in methadone maintained pregnant women report rates of between 50% (Delano et al., 2013) and 80% (McGlone et al., 2012, McGlone et al., 2013b) and importantly, maintenance methadone therapy does not appear to decrease polydrug use (Delano et al., 2013).

A retrospective case review of 444 infants whose mothers were prescribed methadone found that 80% used illicit drugs (Dryden et al., 2009), with benzodiazepines and heroin being the most commonly used, followed by cannabis. Another study reported similar rates, with 74% of infants exposed to opiates other than methadone, followed by 66% benzodiazepines and 62% cannabis exposure (McGlone et al., 2013a). In this cohort of 100 methadone exposed infants, only 9 infants were exposed to methadone alone. In another study from the same region in Scotland, the rate of excess alcohol exposure was 47%, calculated by detectable alcohol metabolites in meconium samples of 21/47 infants born to mothers prescribed methadone (McGlone et al., 2012). Therefore, assessing potential consequences of a single drug, such as methadone, becomes challenging in the context of polydrug use, alcohol use and OUD.

The possibility that prenatal alcohol and opioid exposure in combination may have a synergistically deleterious effect should be considered. Aside from one small study which reported more severe NAS in an infant with FAS (Kreitinger et al., 2013) this remains speculative, but further research in this area is warranted.

1.1.4.1 Meconium for exposure profiling in OUD

Meconium analysis for prenatal substance exposure profiling is a recognised and sensitive method of ascertaining prenatal drug and alcohol exposure. It is superior to maternal interview, and more sensitive than urine analysis (McGlone et al., 2012). Meconium formation begins at approximately 12 weeks' gestation and continues throughout pregnancy. It is a stable matrix and has the benefit of allowing longer drug and alcohol detection windows representing the second and third trimester exposures (Lozano et al., 2007), although meconium likely is most representative of the last trimester exposures (Gray et al., 2010).

Fatty acid ethyl esters (FAEE) and Ethyl-glucuronide (EtG) are products of non-oxidative ethanol metabolism and have been established as biomarkers of fetal ethanol exposure (Vaiano et al., 2016). FAEE and EtG do not cross the placenta, therefore their presence in meconium is due to fetal synthesis, largely from transplacental movement of ethanol ingested by the mother, and hence they can quantify prenatal alcohol exposure. Small amounts of FAEEs are derived endogenously and so validated cut-off values to discriminate between endogenous FAEEs and those derived from maternal alcohol intake are used. As meconium is a stable matrix and has high

sensitivity, it lends itself to be the gold standard for prenatal drug and alcohol exposure profiling.

1.1.5 Challenges of research in pregnant women with OUD

Women with OUD are a vulnerable population. There is a high rate of abuse, in particular early sexual abuse, amongst women with OUD, reported to range between 30 and 50% among women with OUD (Hans, 1999), which may result in a distrust of people in authority, or those with perceived ‘power’.

In addition, women with OUD are more likely to have co-existing mental health problems, particularly anxiety and/or depression, experience domestic violence and poor physical health, have low education, and be socially isolated (Konijnenberg, 2015) than women without OUD. It is widely acknowledged that children with prenatal drug exposure are at increased risk of experiencing instability in their home lives (Konijnenberg, 2015) and studies have tried to disentangle the ‘nature vs nurture’ aspect, which is explored later in this Chapter. These factors are extremely important to acknowledge because they have implications when recruiting such women to enrol their infants in research trials and may provide, in part, an explanation as to why the studying this population of women and their children so challenging and also why the literature is so sparse in this area.

The thesis will explore the inherent challenges posed when undertaking research in pregnant women with OUD in more detail in Chapter Five, based on the experience gained during the MRI study.

1.2 Consequences of prenatal opioid exposure

1.2.1 Fetal neurobehaviour

Opioids ingested by pregnant women cross the placenta and enter the fetal circulation. Thus, effects of maternal drugs, and possible withdrawal from these drugs, can be observed in the fetus. Fetal neurobehaviour describes patterns in fetal movements and fetal heart rate patterns, including baseline heart rate, beat to beat variability and accelerations or decelerations. Fetal neurobehaviour provides integral information about fetal wellbeing and health(Nijhuis, 2003), and also provides a window into the developing nervous system. Any disruption to fetal neurobehaviour warrants particular consideration, as it may represent a threat to normal fetal development.

Maternal methadone ingestion results in altered neurobehaviour in the fetus. Reduced quality and quantity of movement, assessed by ultrasound visualised behaviours, is described after methadone ingestion (Wouldes et al., 2004). Other studies have also reported slower, less variable fetal heart rate, with fewer accelerations and less motor activity after maternal methadone(Jansson et al., 2005). Less fetal neurobehavioural suppression occurs with divided daily dosing of methadone compared with single daily dose of methadone (Jansson et al., 2009, Wittmann and Segal, 1991) suggesting that dosing frequency and peak levels in maternal blood are important factors to consider. However, single daily dosing of methadone continues to form the mainstay of treatment.

Different neurobehavioural effects are seen with different opioids. Buprenorphine, a partial mu and kappa opioid agonist also used for OUD in pregnancy, appears to induce

less fetal effects compared with methadone(Jansson et al., 2011, Salisbury et al., 2012). This may in part reflect the partial mu agonist and kappa antagonist action that buprenorphine has compared to the pure mu actions of methadone(Belcheva et al., 1998). Buprenorphine is also associated with reduced rates of drug withdrawal after birth(Jones et al., 2010), and the same reasons may underlie this observation.

Finally, polydrug use in addition to methadone use appears to potentiate effects of methadone on baseline heart rate and heart rate variability(Jansson et al., 2012).

1.2.3 Neonatal Abstinence Syndrome

A well described consequence of prenatal opioid exposure is NAS, a newborn drug withdrawal syndrome. NAS is a manifestation of abrupt drug cessation in the newborn, characterised by sympathetic and autonomic nervous system overdrive(Kocherlakota, 2014). Affected infants often show signs of drug withdrawal within the first few days of life, but this is variable and is dependent on the drug the mother has been taking, timing of her last dose in relation to the birth of the baby and also on genetic factors that influence infant metabolism of drugs(Wachman et al., 2014, Wachman et al., 2017). Characteristic features of NAS include a high-pitched cry, jitters and tremors, diarrhoea, irritability, incoordinate sucking leading to poor feeding and excessive weight loss. At its most severe, NAS can cause seizures.

NAS is believed to be a self-limiting condition, which can be treated with pharmacological measures to ameliorate the withdrawal symptoms. First line pharmacological agents commonly used to treat NAS include opioid drugs such as oral

morphine, methadone, buprenorphine. The use of phenobarbitone to treat NAS has reduced significantly, although many centres still use it as a second line agent if the infant continues to show signs of withdrawal despite an opiate medication. Although NAS has been extensively studied over many decades, critical knowledge gaps remain in terms of optimal management of infants exposed prenatally to opioid drugs. Of particular importance, and yet frequently overlooked, is the key question of whether treating NAS, and by doing so further exposing the developing brain to exogenous opioids, is better and/or safer than adopting a more conservative approach to managing the withdrawal syndrome.

There is a lack of long term data on the effects of prenatal methadone exposure on children, perhaps because it was introduced into clinical practice five decades ago during an era when trial methodology was less well-developed and less emphasis was given to fetal effects of maternal medical treatments. Whilst NAS could be considered an inevitable consequence of prenatal drug exposure, it has acted as somewhat of a distraction in the research field, as it is not an outcome, rather a symptom of an underlying exposure. Most trials assessing prenatal drug exposure report only short-term outcomes such as length of hospital stay, rates of NAS, or are non-inferiority trials to assess pharmacological agents or different dosing regimens of various drug therapies (Jones et al., 2010, Mattick et al., 2014). There is a need for studies to assess the long term outcomes of infants treated for NAS (Kocherlakota, 2014) to help bridge the critical knowledge gaps in this area.

1.2.4 Sudden infant death syndrome

Sudden infant death syndrome (SIDS), more recently re-named sudden unexplained death in infancy (SUDI) refers to the sudden death of an infant younger than one year, which remains unexplained after formal examination. It is the leading cause of death in the post-neonatal period(Blair et al., 2006). An association with maternal substance misuse and SIDS was first described in 1972 by Pierson and colleagues, with a case series of three infants who had sudden unexplained deaths in infancy(Pierson et al., 1972). They noted that although maternal health and lifestyle factors may have contributed in part to the deaths, the association between SIDS and maternal heroin use had not been described, and this raised the suspicion that methadone may be in some way implicated.

Several other studies went on to report higher rates of SIDS in prenatal drug exposure: Rajegowda and colleagues in a retrospective cohort study reported that of 383 infants who died of SIDS, 8 were prenatally exposed to drugs, resulting in a SIDS rate of 5.5 per 1000(Rajegowda et al., 1978). They postulated that the underlying mechanism may be the effect of narcotics on the respiratory centre. Chavez in 1979 reported 17 deaths out of 688 infants born to addicted mothers (2.5%), compared to 2 deaths out of 388 matched unexposed infants(0.5%) with a mean age of death in the drug exposed group of 9.2 months (Chavez et al., 1979). Of the 17 infants who died in the addicted mother group, 14 mothers were enrolled in the methadone programme during their pregnancy. At post-mortem, there was no evidence of physical abuse or trauma in any of the infants. The authors conclude that SIDS is a major medical complication of prenatal drug misuse.

Further data supporting an association between prenatal drug exposure and SIDS came in 1990 from Ward and colleagues (Davidson Ward et al., 1990) who reported a SIDS rate of 8.87 per 1000 cases in the prenatal drug group (19/2143) compared with a rate of 1.22 per 1000 amongst control infants (396 / 325,372). They commented on the difficulty of separating the effects of the prenatal substance use from the other confounders associated with SIDS, such as preterm birth, intrauterine growth restriction, maternal smoking and deprivation.

A large retrospective cohort study from New York City attempted to address the issues of confounders common in prenatal drug users. They studied more than 1.2 million infants and identified 1760 cases of SIDS over a ten-year period (Kandall et al., 1993). There were 33 cases of SIDS out of 3416 births with prenatal methadone exposure. When adjusting for high risk variables, the adjusted SIDS rate was 5.83 per 1000 births in prenatal drug exposed compared with 1.39 per 1000 births for unexposed infants.

In 2010, another large retrospective cohort study reported infant mortality in prenatal methadone exposure in Australia, and the single main cause of death was SIDS (Burns et al., 2010). They reported unadjusted infant mortality rates of 24.3 per 1000 compared to infant mortality rates of 4 per 1000 in unexposed infants. They also reported a higher rate of smoking in the methadone-maintained mothers, which may in part have contributed to the higher rates of SIDS.

UK data also confirmed an unexpectedly high proportion of infants with prenatal drug exposure, most commonly methadone, dying of SIDS(Cohen et al., 2015) when post-mortem findings were investigated. There were 138 neonatal deaths over an 8 year period, and 32 were cases of SIDS. Of the 32, 12 (37.5%) were exposed prenatally to drugs, and 10 (31%) were exposed to prenatally to methadone. All cases had other risk factors for SIDS, including heavy smoking, sharing a sleeping surface with mother and low birth weight. This data did not capture deaths in the post-neonatal period, which may have underestimated the incidence, as the mean age of death in previous reports was 9 months(Chavez et al., 1979).

Interestingly, when ‘drug-exposed’ cohorts were broken down into drug class, 272 out of the 2143 were exposed to opiates, and there were 5 SIDS deaths in the opiate-exposed group, giving a SIDS rate of 18.38 per 1000(Davidson Ward et al., 1990). This finding was replicated by Kandall, who also reported the higher risk associated with opiates, even higher than cocaine, with a risk ratio for SIDS after prenatal methadone exposure of 3.6(Kandall et al., 1993).

The pathophysiological process underlying the observation of increased risk of SIDS and prenatal opiate exposure has been partially explored. Olsen and colleagues in 1980 describe a depressed ventilatory response to hypercarbia in infants prenatally exposed to methadone(Olsen and Lees, 1980). More recently Ali and colleagues describe an impaired response to a hypoxic challenge in a prospective study of infants prenatally exposed to drugs and of infants of smoking mothers compared to control unexposed infants(Ali et al., 2016, Ali et al., 2017). There is also a suggestion that myelination

is delayed in infants with SIDS(Kinney et al., 1991) when compared to infants who have died of other causes.

1.2.5 Pre-clinical studies

The endogenous opioid system is a central mediator of pain modulation, reward and stress responsivity (Byrnes and Vassoler, 2017). It consists of three families of opioid peptides: beta-endorphin, enkephalins and dynorphins and three receptor types: mu, kappa and delta. These are distributed throughout the central and peripheral nervous system, allowing for regulation of a wide range of behavioural and physiological effects. The neural effects of opioids will be explored later in this chapter in section 1.3.3. Many pre-clinical studies have attempted to describe the effects of opioid drugs on growth, behaviour, memory and on neural cells. Most studies use the rodent model because it is the most similar in terms of human brain development to replicate, although mice, chick and lambs have also been used. Different opioid drugs have been studied; most commonly morphine, but methadone and buprenorphine, and more recently oxycodone have all been studied.

Opiate exposure, either prenatally or in early postnatal life, has several potential consequences including adverse development (Enters et al., 1990, Steingart et al., 2000), impaired spatial learning (Davis et al., 2010), impaired memory (Wang and Han, 2009, Che et al., 2005) and abnormal social function (Niesink et al., 1999) and increased vulnerability to developing neuropsychiatric conditions in later life (Tan et al., 2015). Prenatal opioid exposure alters reward-related behaviours, which has been postulated to enhance susceptibility to later addiction (Wong et al., 2014).

Opioids appear to have a noticeable influence on the activity of hippocampal neurons: synaptic plasticity in the hippocampus is modulated by endogenous opioids (Simmons

and Chavkin, 1996); prenatal morphine exposure in rats results in lowered levels of post-synaptic density protein-95, an important component of the cytoskeleton of neurons (Yang et al., 2006) and affect hippocampal neurogenesis causing loss of neural progenitor cells in the subgranular zone of dentate gyrus, either through modulating proliferation or interfering with differentiation and maturation(Zhang et al., 2016). This is important, because the hippocampus has an important role in spatial learning, and several animal studies have noted disruption to spatial learning in the context of prenatal opioid exposure(Davis et al., 2010, Eisch et al., 2000, Wang and Han, 2009). Validated tests, such as the Morris water maze testing (MWM), designed to test spatial memory with particular sensitivity to hippocampal lesions have shown that mice exposed prenatally to heroin had neuro-behavioural deficit in memory and learning and authors attributed this to alterations in the apoptotic pathway in the developing hippocampus(Wang and Han, 2009). Another study confirmed these findings when mice exposed prenatally to heroin performed significantly worse on a test indicative of spatial memory and discrimination, related specifically to the septo-hippocampal cholinergic projection(Steingart et al., 2000). They also showed that these changes were reversible if fetal cholinergic cells were injected into the hippocampus after prenatal exposure.

Animal studies have shown alterations in pain perception in later life, with prenatal morphine exposed rats displaying enhanced nociceptive response in the adult life(Rozisky et al., 2011). Endocrine effects relating to prenatal opioid exposure have also been reported in rat models. These include increased levels of serotonin after prenatal morphine exposure(Laborie et al., 2005), increased circulating levels of

adrenaline but reduced adrenal levels of adrenaline and noradrenaline (Dutriez-Casteloot et al., 1999). These findings can be partially explained by the role of endogenous opioids in the stress response, mediated by the Hypothalamic-Pituitary-Adrenal (HPA) axis.

Pre-clinical studies provide a useful window, as they can control for confounders such as polydrug use that human studies cannot. However, the limitations of pre-clinical systems for making inference about possible human effects include difference in the timing of neurodevelopment, with rodents having a much shorter gestation than humans, meaning that exposing a dam to opioid throughout pregnancy only equates to first and second trimester exposure in humans; differences in the timing of drugs; the route of administration; stress caused by injections; the dosage of drug; and species-specific differences in drug metabolism.

1.2.5 Developmental effects of prenatal opioid exposure in childhood and beyond

Literature reporting neurodevelopmental outcome after prenatal opioid exposure is conflicting (Konijnenberg and Melinder, 2011). Many studies report 'normal outcomes' where scores fall within the normal range, when in fact the opioid-exposed group performed consistently worse than control groups across a number of developmental domains (Doberczak et al., 1988, Wilson et al., 1979, Hans and Jeremy, 2001). A systematic review into the developmental effects of prenatal opioid exposure reported a tendency towards poorer neurodevelopmental outcome (Baldacchino et al.,

2015), and more recent studies were more likely to report worse neurodevelopmental outcomes(Hunt et al., 2008, McGlone and Mactier, 2015).

Long term childhood data relating to outcomes after prenatal opioid exposure is sparse. One longitudinal study followed 72 opioid-exposed children up to the age of 8 years and compared them with 58 children with no prenatal exposures and found that developmental trajectory worsens rather than improves in the opioid exposed group, and that this difference is more marked in girls(Nygaard et al., 2016). Another longitudinal study from the same Norwegian group examined a cohort of 45 prenatally exposed 17-22 year old young adults and compared them to 48 young adults without prenatal opioid exposure. This study found that although cognitive scores were within the normal range, the cognitive problems were more evident in the drug exposed group compared with controls, and this did not disappear with time(Nygaard et al., 2017).

A further preliminary study using functional MRI to assess working memory examined 11 children with prenatal opioid exposure and compared them to 12 children without prenatal opioid exposure. They describe memory impairments in those with prenatal opioid exposure(Sirnes et al., 2018) adding weight to the argument that developmental deficits do occur in association with prenatal opioid exposure and that these are evident in later childhood. Childhood neurodevelopmental deficits commonly become more apparent with increasing age, which reflects the ontogeny cognitive and motor skills over time.

Increased addictive behaviour is also reported in children of addicts, highlighting a particular vulnerability and possible intergenerational transmission of drug abuse. (Glantz and Chambers, 2006). The direct mechanism is unknown, but it is concerning nonetheless. It could represent either a genetic predisposition, modulation of the areas of the brain involved in addiction pathways which are activated in utero (DiNieri et al., 2011) or environmental factors resulting in poor coping mechanisms and learned behaviour.

A study of adolescents has shown that increased risk-taking behaviour, executive dysfunction and early sexualised behaviour are all associated with prenatal drug exposure, although this study was from those predominantly exposed to cocaine in utero (Miguel-Hidalgo, 2009, Conradt et al., 2014). Children of alcoholics have been more extensively studied, and are at increased risk of behavioural, psychological and cognitive deficits (Johnson and Leff, 1999), independent of fetal alcohol spectrum disorder (FASD).

Although there is limited data about the outcome of adults who were prenatally exposed to drugs, one study reports increased rates of substance misuse, crime and unemployment in a cohort of adults with known prenatal opiate exposure (Skinner et al., 2009). Whilst the current evidence is by no means robust, studies are using increasingly reliable methodology, and building up a picture suggestive of harm. Taken together, the findings of pre-clinical studies and human studies raise significant concerns about the long-term effects of prenatal opioid exposure on the developing

brain and subsequent behaviour, learning and memory. Further longitudinal studies are needed.

1.2.6 Nature versus Nurture

One of the widely acknowledged challenges when performing research with substance misuse is the myriad of environmental, social and health confounders that can accompany drug misuse to some degree. These include social deprivation, unemployment, crime, poor health and nutrition, co-existence of mental health disorders and inadequate access to health services including prenatal care. Studies have attempted to disentangle the complicated interactions between biological (nature) and environmental factors (nurture) influencing child development and these are summarised below.

Mental health disorders are commonly associated with substance misuse (Jane-Llopis and Matytsina, 2006). Depression in women with OUD can result in less accessing of prenatal care, longer postnatal stay and increased likelihood of NAS (Hensley et al., 2018). Depression during pregnancy predicts postnatal depression, which is known to impact on childhood neurodevelopment, in particular behaviour and IQ (Nulman et al., 2012). In addition, drugs commonly used to treat depression, such as selective serotonin reuptake inhibitors (SSRIs) are recognised as a cause of neonatal abstinence syndrome (Klinger and Merlob, 2008), with neonatal symptoms in between 10 – 30% of all pregnant women treated with SSRIs (Jefferies, 2011). This may make the baby more challenging to look after in the early weeks and months and have an additive effect with the withdrawal related to methadone. Prevalence of substance use disorders

and comorbid mental health problems are highest for women abusing opiates(Conway et al., 2006), again identifying women as a particularly vulnerable group.

Children at high risk of developmental problems have been shown to benefit from stable home environments (Ornoy, 2003) and it has been hypothesised that a stable home environment may mitigate some of the adverse effects associated with prenatal opioid exposure. However, drug dependent parents are also more likely to physically and/or sexually abuse their children(Smith et al., 2007, Walsh et al., 2003). Children who suffer abuse have delayed cognitive development which is likely to result from under stimulating environment, poor learning environment, or less commonly as a direct result of brain trauma (Carrey et al., 1995).

Ornoy and colleagues believed that the home environment was much more important than the specific role of prenatal heroin exposure in determining long term developmental outcome. They report that children prenatally exposed to heroin raised in adopted families had normal development compared with those also exposed prenatally but raised at home (Ornoy et al., 1996). They also compared development with a control group who had matched social deprivation and found that these children had worse developmental scores than those prenatally exposed to heroin, implying that environmental and social factors are more important than prenatal drug exposure *per se* for cognitive development. In a later study, they reported a high rate of attention deficit hyperactivity disorder (ADHD) amongst all children with prenatal heroin exposure, the ADHD rate being twice as high in children raised at home compared with those raised in foster care (Ornoy et al., 2001). This finding of increased ADHD

and impulsivity in 42 children in foster care with prenatal drug exposure compared to 50 non-exposed children was corroborated by a Norwegian study (Slinning, 2004).

The concept that being raised at home by biological parents leading to worse outcomes was reinforced by an Italian study who assessed spatial, mnemonic, visuo-perceptive, linguistic and learning abilities in twenty-nine children with prenatal heroin exposure. They compared the abilities of 19 children who were raised by biological parents at home with 10 children raised in foster care and reported a significantly worse performance in the group raised at home, especially with visuo-spatial and mnemonic abilities (Fundaro et al., 2002). They hypothesised that the prenatal drug may itself have a direct effect on functions such as visuo-spatial processing, but that this could be counteracted by a stable home environment, which allows the child to develop compensatory strategies.

Reports that outcomes were worse in drug exposed children if fostered (Soepatmi, 1994) were supported by another study which assessed children prenatally exposed to drugs during adolescence, which showed that adoption did not appear to mitigate the negative effects of prenatal drug exposure (Ornoy et al., 2010). Interestingly, in this study the 'control' children were matched for social and environmental deprivation but were not drug exposed, and they performed similarly to the drug exposed children, suggesting that environment might be more important than the drug exposure (Ornoy et al., 2010). However, this may reflect the fact that children going into foster care are likely to be from more chaotic and unstable backgrounds and therefore their inherent risk of a poorer outcome may be already increased.

More recently, Nygaard and colleagues investigated the effect on cognitive function of 45 adolescents with prenatal drug exposure, in the context of early placement with stable foster or adoptive parents and compared this to the same assessment in 48 control adolescents who had no prenatal opioid exposure. They report a positive effect of undergoing fewer changes in caregiver but were unable to assess the role of maternal upbringing versus foster care as their sample only included one child who was reared by the biological mother(Nygaard et al., 2017).

Many of these studies have compared outcomes of children with prenatal drug exposure raised either by their biological parents or by foster/ adopted parents, in an attempt to isolate the prenatal drug exposure by comparing only the postnatal environmental. One problem with this approach, is that foster care or adoption success can be affected by many confounding variables, such as the age at which the child enters the care system-with younger ages generally being associated with more favourable outcomes- the number of placements that a child is exposed to, and how the child becomes involved in the care system in the first instance.

Most studies assume that foster care and or adoption is providing a more stable home environment than remaining with their biological parents, and this may not be the case. One study acknowledging this compared the outcomes of a cohort of children in foster care, where only some had been drug exposed prenatally(Brooks and Barth, 1998), although the authors unfortunately provided minimal details regarding the prenatal drug exposure. However, this study also investigated the effect of being placed either

with strangers, or with family (kinship care) in a questionnaire-based study, to try to discern whether an outcome was related to drug exposure, kinship status or both. Although educational performance was similar between groups, drug exposed children were more likely to have special educational needs, irrespective of whether in kinship care or non-relative foster care. Emotional development was generally worse in drug exposed children, and more so for those in non-relative foster care. Behavioural problems were three times more likely in the drug exposed group, but more so when placed with family. Authors acknowledge that placing some drug-exposed children with family may involve placing them in environments that do not adequately meet their emotional needs and conclude that further research is needed.

It is extremely difficult to separate and control for the social and environmental confounders frequently observed in association with drug misuse. Removing children from their unstable home environment and placing them in foster care may improve their outcomes if the situation they were removed from was particularly detrimental, or if they were placed within a nurturing loving family, or both. However, removing a child from their mother and potentially their siblings, especially at a slightly older age, could be extremely traumatic, and may not be beneficial for the child in the long term.

A recent study has illustrated the complex interaction between adverse life events of the mother and subsequent effects on her children, by demonstrating intergenerational effects of maternal childhood maltreatment which manifest in reduced intracranial volumes on MRI imaging in offspring (Moog et al., 2018). These reductions were primarily due to reduced cortical gray matter and occurred independently of potential

confounders such as maternal socioeconomic status, recent violence, obstetric risk factors, and age at MRI. This study suggests that effects which occur in a child's intrauterine life may have important downstream consequences for later life and illustrate a common and gross over-simplification that exists when attempting to attribute cause and effect or elucidate associations.

It is likely that biological risk factors of prenatal drug exposure determine vulnerability to later neurodevelopmental effects, but that postnatal environmental factors modulate the severity of the effects(Moe, 2002). As there is no consensus in the published literature, reflecting the numerous factors contributing to each complex case, it would seem that an individualised approach is required to ensure the basic needs of the child are met in a suitable environment, whilst remaining aware of the unknown effect of intrauterine programming and epi-genetic phenomena.

1.3 Cerebral white matter and opioids

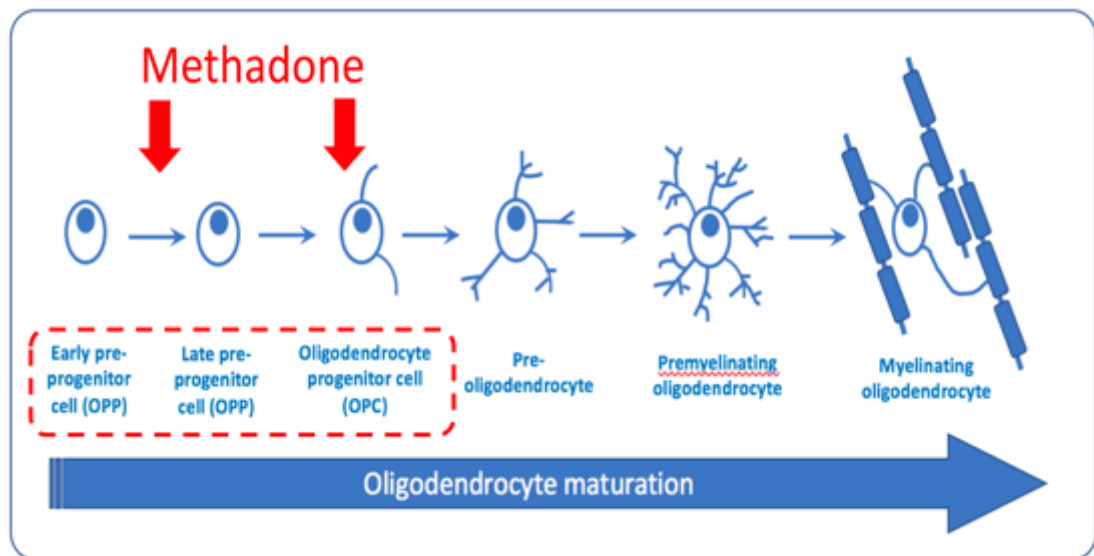
White matter is comprised of myelinated axons, comprising more than half of the volume of the cerebral hemispheres(Feldman et al., 2010). Axons are encased in a lipid-rich sheath called myelin, which appears white, hence the term white matter. Axons are the long thin segments of nerves that conduct electrical impulses from neuron to neuron and are responsible for propagating an electrical signal either to another are of the central nervous system, or to the peripheral nervous system, and hence are integral to the connectivity of the brain.

1.3.1 The oligodendrocyte

The oligodendrocyte is a neural cell responsible for production of myelin. Myelin is a lipid-rich structure which provides axonal insulation and increases the speed of conduction down nerves. Oligodendrocytes have to undergo a complex and precisely timed cell lineage involving proliferation, migration and differentiation before becoming mature oligodendrocytes capable of producing myelin. Premyelinating oligodendrocytes are particularly vulnerable to damage or disruption during early brain development, see Figure 1.

Oligodendrocytes express mu and kappa opioid receptors, implicating endogenous opioids in their regulation. Mu-opioid receptors are expressed by both immature and mature oligodendrocytes, whereas kappa-opioid receptors are only expressed in differentiated oligodendrocytes (Knapp and Hauser, 1996). This could have important clinical implications, as methadone is a mu-agonist whereas buprenorphine is a partial mu and partial kappa agonist.

Figure 1 : Oligodendrocyte maturation and methadone



Historically it was believed that oligodendrocytes had only one function - to produce myelin - and that myelin in turn had only one function: to insulate axons, resulting in improved speed of axonal transmission, essentially improving the information transfer efficiency of the brain. However, there is increasing evidence that oligodendrocytes have a much more important and varied role than initially thought.

Oligodendrocytes provide trophic support to axons and promote their activity (Mitew et al., 2014). Myelin also appears to have important regulatory roles. Pre-clinical models have implicated myelin in the maturation and viability of axons. Yin et al showed that mice deficient in myelin associated glycoprotein (Yin et al., 1998), had reduced axonal calibers and decreased neurofilament spacing. Yamazaki and colleagues describe myelin having a dynamic role regulating impulse transmissions through axons in a rat model; the depolarisation of oligodendrocytes increased the conduction velocity of an action potential along the axon, indicating that white matter

can have functional as well as structural plasticity(Yamazaki et al., 2014). They go on to propose that myelination may be regulated according to functional activity in axons. Fields et al describe neural synchrony among the multiple axons under the domain of an individual oligodendrocyte promoted by myelin(Fields, 2008). In addition, there is a sub category of oligodendrocyte precursor cells (OPCs) that can generate action potentials, receive synaptic input and are preferentially damaged by ischaemia(Ragnhildur et al., 2008).

1.3.2 Myelination

1.3.2.1 Normal pattern of myelination

Developmentally, myelination is considered the last stage of white matter development. Myelination is an exquisitely complex process, which starts *in utero* from the mid-trimester, and continues for several decades(Dubois et al., 2014). It follows a predictable pattern, termed regional asynchrony. This refers to the earlier myelination of areas more centrally and inferiorly, and earlier maturation of sensory pipelines rather than motor. Asynchrony of the maturational process reflects the hierarchy of connections between cortical areas. The early maturation of sensory areas responsible for low level processing, enables stabilisation of the information used for integrative areas, used for high level processing, which develop later on.

Myelinated axons form white matter fibre tracts, or bundles. White matter tracts connect the cerebral cortex to other areas of the brain and carry both sensory and motor fibres. The majority of myelination occurs within the first 2 years of life, with some areas of the brain such as parts of the prefrontal cortex remaining unmyelinated until

late adolescence. This is related to the function of the frontal lobe, which is involved in higher cognitive functions, such as executive function, emotions and behaviour and therefore on the basis of regional asynchrony, it is logical that these areas myelinate much later in life.

1.3.2.2 Disruption of ‘normal’ myelination

The complex sequence of oligodendrocyte differentiation and maturation and subsequent axonal myelination makes the oligodendrocyte an extremely vulnerable cell. Whilst it is clear that endogenous opioids play a role in myelination, the exact mechanism remains unclear. Studies investigating oligodendrocytes and precursor cells show that they are affected by exogenous opiates perhaps because both mu-opioid receptors and kappa-opioid receptors are expressed by oligodendrocytes (Knapp et al., 1998, Knapp and Hauser, 1996). Methadone affects early lineage of the oligodendrocyte by stimulating the proliferation of progenitor cells and accelerating the maturation of immature pre-oligodendrocytes (Vestal-Laborde et al., 2014), which may in turn disrupt the complex sequence of myelination and brain maturation. Prenatal buprenorphine exposure has also been shown to adversely affect the number and calibre of myelinated axons in the developing brain (Sanchez et al., 2008).

Historically, myelination was only ascertained post mortem, by histological staining for myelin. However, nowadays, magnetic resonance imaging is able to demonstrate myelin and allows detailed investigation of brain microstructure.

1.3.3 Neural effects of opioid drugs

Opioid drugs affect the developing central nervous system. As opioid receptors are expressed by the neural progenitor cells that are common precursors of all central nervous system (CNS) neurons and microglia, prenatal opioid exposure might directly influence very early lineage and fate decisions via paracrine or autocrine feedback loops(Hauser and Knapp, 2018). Studies suggest that prenatal opioid exposure may modify developing dopaminergic, cholinergic and serotonergic systems, alter myelination and exert damaging neurotoxic effects and these studies are discussed below.

Morphine induces a general neurotoxic effect on chick embryonic brain cultures(Sakellaridis et al., 1986) and reduces neuronal cell packing density and total number of neurons, implying that morphine might affect cortical cell proliferation and maturation(Seatriz and Hammer, 1993). Prenatal morphine exposure suppresses neuronal proliferation(Sadraie et al., 2008), inhibits purkinje cell survival and dendritic differentiation in the mouse cerebellum(Hauser et al., 1994) and reduces total dendritic length in the rat brain(Ricalde and Hammer, 1990), an effect which can be reversed by opiate receptor blockade. It also disrupts neuronal migration and adversely affects neuronal survival, with increasing evidence of this in areas on the brain containing higher numbers of mu-opiate receptors(Harlan and Song, 1994).

Methadone can affect levels of neurotransmitters variably depending on brain region, gender and whether exposed prenatally or postnatally(Robinson et al., 1997), with reduced levels of norepinephrine and increased levels of serotonin in the rat

hippocampus. Methadone also reduces striatal acetyl choline (ACh) levels(Robinson et al., 1996, Guo et al., 1990) which could impact on later motor development. Direct neurotoxicity has also been demonstrated(Droblenkov et al., 2010) including neuronal apoptosis in human fetal brain neurons induced by morphine(Hu et al., 2002).

A recent review of the effects of opioid drugs on the endogenous opioid system summarised the neural effects of opioids largely derived from pre-clinical studies, and commented that whilst many animal studies show the response to acute administration of opioids, chronic opioid exposure leads to compensatory mechanisms(Hauser and Knapp, 2018), which is likely mediated by up or down-regulation of opioid receptors(Belcheva et al., 1998, De Vries et al., 1991) and it is these consequences that could be responsible for differences seen in prenatal opioid exposure.

Finally, a review summarising the pre-clinical literature around prenatal opioid exposure highlights the current knowledge gaps, specifically with regards to the effects of methadone and buprenorphine, which are commonly used for medically assisted programmes and the lack of data on effect of polydrug use(Byrnes and Vassoler, 2017).

1.4 Neuroimaging

1.4.1 Conventional MRI

The first human MRI scan was performed in 1977. Initially described as nuclear magnetic resonance imaging, over time the ‘nuclear’ has been dropped due to negative public connotations and MRI has now become a powerful diagnostic and prognostic tool. MRI requires a magnetic field that is strong and uniform, with increasing strength being represented by increasing Tesla (T) value. Most clinical MRI scanners are 1.5T, although 3T scanners are increasingly being used.

MRI is safe, has no ionising radiation exposure and can acquire detailed images in multiple planes, with better soft tissue contrast than other imaging modalities such as computed tomography (CT). Furthermore, advanced techniques such as diffusion and MR spectroscopy allow for tissue characterisation, whilst functional MRI allows visualisation of active parts of the brain during activities. These characteristics explain why MRI has become the imaging modality of choice for many medical specialities, particularly neonates. Some disadvantages include the inability to perform an MRI on a patient with metal artefacts and devices in their body and the longer scanning time and increased cost incurred by MRI when compared with other imaging modalities.

1.4.1.1 Neonatal MRI

Neonatal MRI was introduced into clinical practice in the 1980s, shortly after first being used in adults, although it was not widely available until the 1990’s. Of

particular importance for the developing brain, MRI is the only imaging modality that can distinguish the presence or absence of myelin within the brain.

Neonatal MRI is now an integral part of modern neonatology, with a clear framework for the indications for neonatal MRI and standards for reporting (British Association of Perinatal Medicine, 2016). There are many factors that affect neonatal MR image acquisition, quality and interpretation, including scan time, signal to noise ratio (SNR), increased water content of the neonatal brain, movement artefact and the dynamic nature of myelination in the first years of life.

SNR refers to the difference between the signal intensity of the area of interest and the background noise and determines how grainy the image is. SNR is proportional to the volume of the voxel and is closely related to acquisition time and field strength. Spatial resolution is defined by the size of the imaging voxel, a voxel representing a small arbitrary unit of volume in a magnetic resonance scan, and it determines how sharp the image looks. Higher resolution images result in crisper images, therefore images obtained at 3T have higher SNR and higher resolution when compared to images obtained at 1.5T. Alternatively, the images are of similar quality in terms of spatial resolution, but are obtained over a shorter acquisition time (Rutherford et al., 2004).

Because the neonatal brain is much smaller than the adult brain but of similar complexity (e.g. cortical configuration at birth resembles that of the adult human), higher-resolution scans are needed to depict anatomic features in detail, and therefore a high SNR is a prerequisite for the increased resolution needed for MR imaging of

newborn infants(Hillenbrand and Reykowski, 2011). Bespoke neonatal phased array head coils are available which dramatically improve SNR, resulting in better quality images and / or shorter acquisition times.

The immature brain has a much higher water content than the adult brain(Huppi and Dubois, 2006). This increased water content results in a marked increase in T1 and T2 relaxation times, occurring most noticeably from term until age 1, in association with myelination.

Imaging of the newborn brain typically takes between 30 and 60 minutes, depending on the specific MRI protocol. MRI makes characteristic noises which are sequence dependent and although ear protection is routinely used (Minimuffs, Natus), the noise and vibration risk waking the infants. Previously, neonates undergoing MRI scan would have been routinely sedated using a rapidly absorbed oral sedation such as chloral hydrate (Ibrahim et al., 2015, Finnemore et al., 2014, Delgado et al., 2015). There has been a move away from routine sedation of healthy babies involved in research, and most infants less than 3 months, are scanned during natural sleep, after a feed. This technique is commonly referred to as a ‘feed and swaddle’ and is used with varying levels of success, although successful acquisition of longer MRI sequences can be challenging in neonates without sedation (Ibrahim et al., 2015, Vasu et al., 2014).

Because brain maturation occurs in an organized and predictable fashion, neonatal-specific brain atlases exist (Blesa et al., 2016) to provide a context to allow

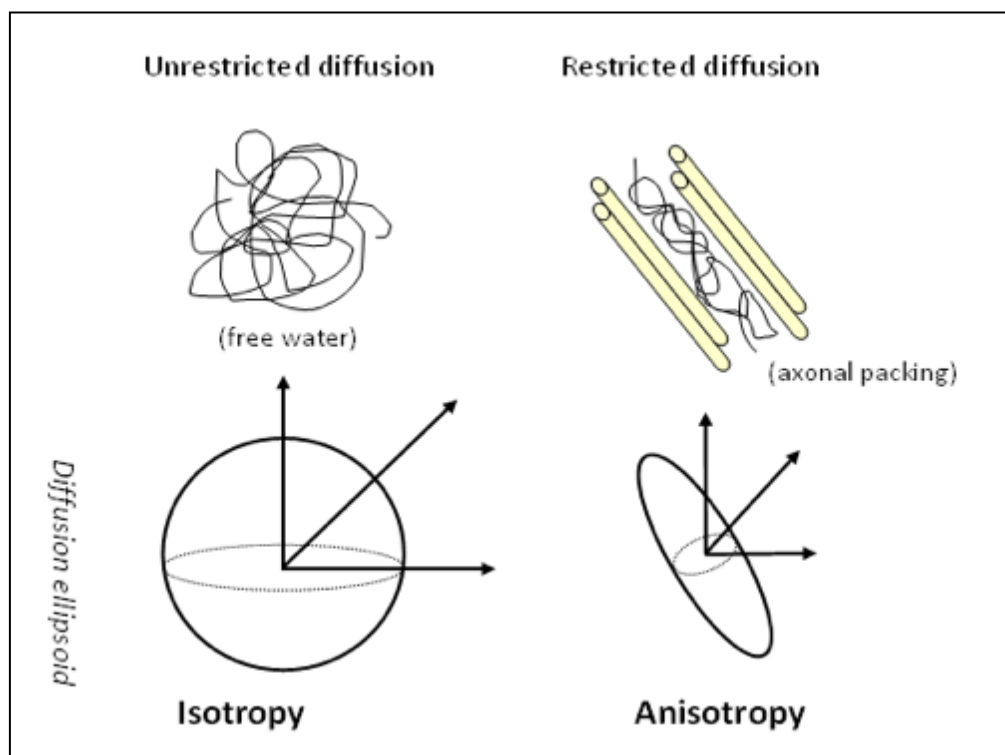
interpretation. Furthermore, with the increasing diagnostic and prognostic capabilities of MRI scans and the complexities specifically relating to neonatal MRI interpretation described above, neonatal neuroradiology is an evolving radiological sub-specialty.

1.4.2 Quantitative MRI

1.4.2.1 Diffusion MRI

Diffusion MRI (dMRI), also referred to as diffusion tensor imaging (DTI), is an advanced MRI technique that measures the movement of water in tissues. At body temperature, water molecules within the brain are in a constant state of random Brownian motion, see Figure 2. dMRI uses this directional dependence of water to allow inference about underlying brain microstructure to be made.

Figure 2 :The movement of water within the brain



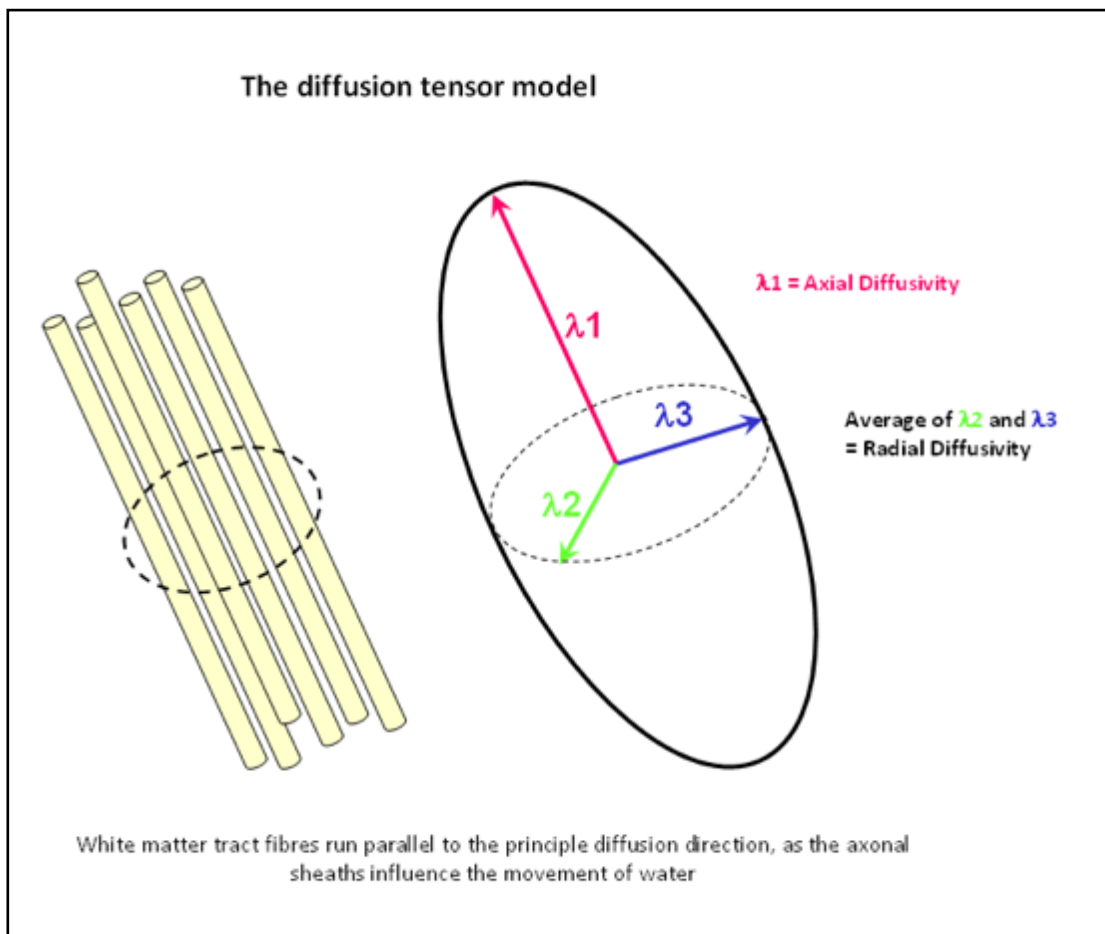
1.4.2.1.1 The diffusion tensor

A tensor is a 3D version of a vector, and so it provides both magnitude and directional information in 3 orthogonal directions. The magnitude data is provided by the eigenvalues of the tensor, which are used to generate FA/MD, while the directional information is given by the three eigenvectors.

The diffusion tensor model is best visualised as an ellipsoid, see figure 3. The tensor assumes there is one fibre population in a voxel and that water diffuses mostly down the fibre direction. Thus, the eigenvector which corresponds to the largest eigenvalue, referred to as AD is taken to be the principal fibre direction. The two other eigenvectors are orthogonal to this with the magnitude of water diffusion in these directions given by the two remaining eigenvalues, which are averaged to give the RD.

Isotropic diffusion is represented by unrestricted random water molecule movement in cerebrospinal fluid (CSF). In cerebral grey matter internal cellular structures slow the rate of water diffusion, but it is still isotropic in nature. In cerebral white matter, the axonal structure impedes free diffusion and directs water molecules preferentially to diffuse in the direction parallel to the axon, known as anisotropic diffusion. FA is frequently used in neonatal dMRI and indicates the degree of directional coherence of water molecule diffusion. Other measures include mean (MD), axial (AD) and radial (RD) diffusivity which indicate the overall magnitude of water molecule diffusion, and that occurring parallel and perpendicular to the main fibre direction.

Figure 3 : The diffusion tensor model



The main parameters from the diffusion tensor model are the three eigenvalues, λ_1 , λ_2 , λ_3 representing diffusion along the three axes (eigenvectors) of an ellipsoid in each voxel. The first eigenvalue represents axial diffusivity (AD) along the first eigenvector which is assumed to be orientated along the fibre direction. The rate of water diffusion perpendicular to λ_1 is an average of the second and third eigenvalues and is referred to as radial diffusivity (RD).

1.4.2.1.1 Fractional anisotropy

FA takes scalar values between 0 and 1, where 0 represents isotropic and 1 completely anisotropic water diffusion. Higher FA values indicate more mature and well myelinated white matter tracts, whereas lower FA values often indicate structurally compromised white matter. Normal neonatal FA values range between 0.13 to 0.61,

the higher values representing FA in areas such as the PLIC and corpus callosum(Kunz et al., 2014). In regions of complex fibre structure, FA can also take low values as it is unable to resolve crossing fibres, for example. FA is the most commonly used biomarker of diffusion anisotropy, and has found use in describing white matter structure in the developing brain(Hasan et al., 2004).

1.4.2.1.2 Axial diffusivity

The eigenvector which corresponds to the largest eigenvalue is the AD and represents the principal fibre direction.

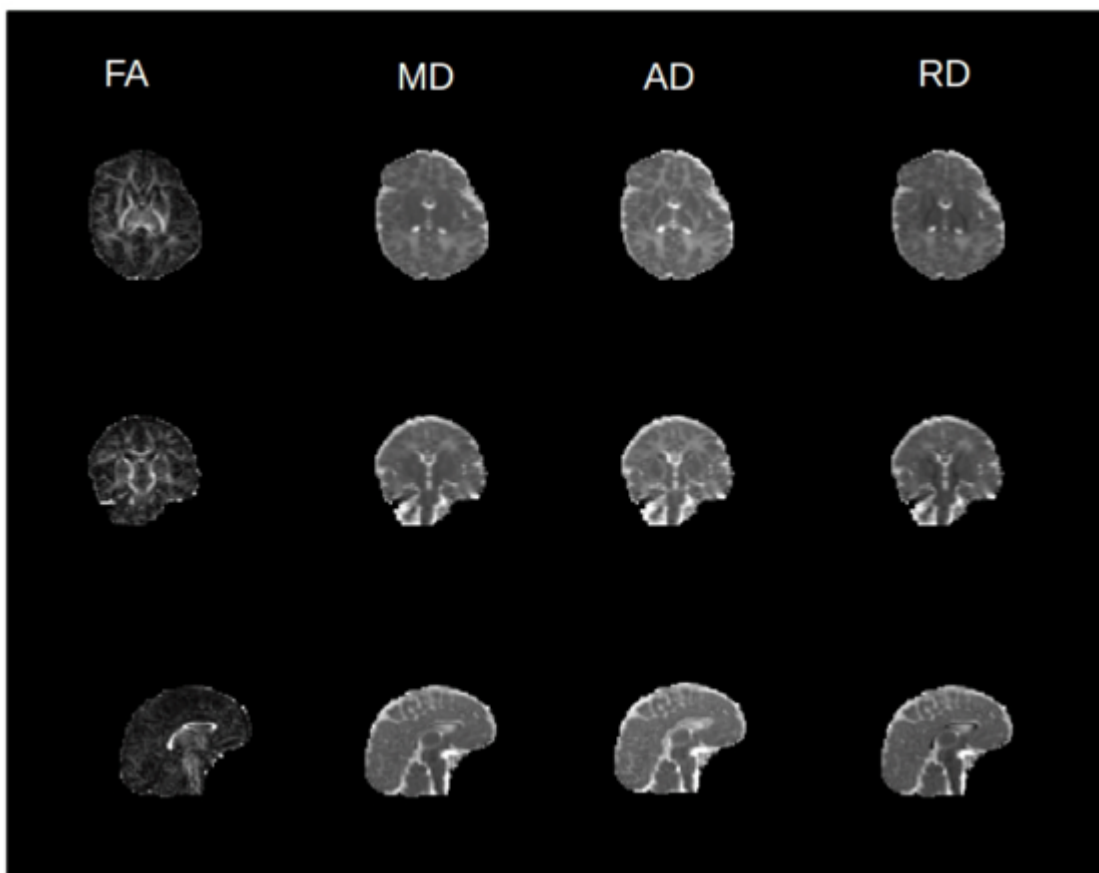
1.4.2.1.3 Radial diffusivity

RD is calculated as an average of the 2 eigenvectors orthogonal to the principal diffusion direction, see Figure 3. It provides useful information when used in conjunction with FA. RD typically is low in densely packed, well myelinated tracts and conversely it is high in less well packed or poorly myelinated tracts.

1.4.2.1.4 Mean diffusivity

MD is an inverse measure of membrane density and characterises the net degree of displacement of water molecules. MD is measured in micrometres² per second. MD is sensitive to cellularity, oedema and necrosis and it decreases with age in childhood, so MD values are higher for neonatal brains than for paediatric and young adult brains. This may in part represent increasing complexity of white matter structures with increasing myelination.

Figure 4 : dMRI metrics



1.4.3 Analysis using dMRI metrics

There are several ways of analysing dMRI data, broadly categorised into regional or whole brain analyses. Regional analyses are where water diffusion measures are obtained in one or more pre-defined area of the brain. There are two main approaches; region of interest analysis (ROI) and tractography. Whole brain analyses can also include tractography, see Figure 5, along with other techniques such as tract-based spatial statistics(TBSS).

1.4.3.1 Region of Interest

ROI obtains water diffusion measures from a specific brain area, which can be defined manually or automatically. It has the advantage of assessing specific regions of the brain, but it requires a hypothesis about which regions might be affected. It also introduces bias if manual delineation of the ROI is used, as these are inherently subjective. If multiple ROIs are used, this increases the number of statistical tests and it is important to correct for multiple comparisons.

1.4.3.2 Tractography

Tractography is a method of identifying and visualising a white matter tract by connecting estimates of fibre orientation derived from the principal eigenvector of the diffusion tensor. Once the tract has been identified, the measures of diffusion previously described can be averaged over the entire tract or calculated for individual segments of the same tract.

Figure 5 : Whole brain tractography in the neonatal brain

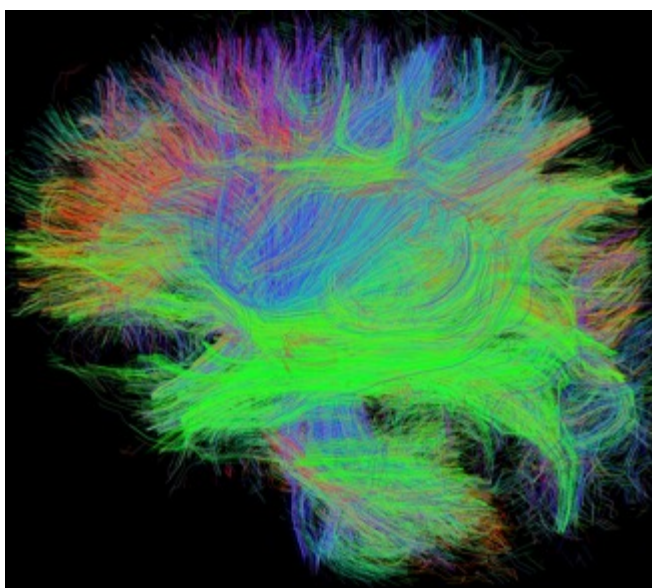


Image reproduced with thanks to
Dr Mark Bastin.

1.4.3.3 Tract-based spatial statistics

TBSS is an unbiased observer-independent mechanism of analysing dMRI data. First described in 2006 by Smith et al and developed in Oxford, UK, TBSS aims to improve the sensitivity, objectivity and interpretability of multi-subject dMRI data (Smith et al., 2006). It does this by projecting FA values onto a subject-mean FA tract skeleton (centre of the white matter tracts) prior to statistical voxel-wise analysis. The FA skeleton data is then analysed in the same way as VBM. See Table 1 for benefits of using TBSS to analyse neonatal dMRI data.

Table 1 : Advantages of using TBSS to analyse dMRI data in neonates

Unbiased and automated	Optimised processing pipeline
Whole brain analysis technique negates need for pre-defined areas of interest	Reduced group sizes needed to show a difference
Validated in neonates	Maps to later neurodevelopmental outcome

TBSS removes the potential bias of having to delineate areas by hand, which is required for some regional analyses, and is still sensitive and robust in the estimates of FA being taken from the relevant voxels. A slight limitation is that it might miss variations in the peripheries of white matter tracts which are excluded from the analyses.

1.4.4 TBSS in neonatal imaging

Neonatal dMRI data sets are frequently of lower resolution compared with adult data and have relatively poor signal to noise. In addition, the neonatal brain undergoes significant changes in the period after birth, with large increases in brain volume and complexity during the neonatal period and these can present further technical challenges when analysing dMRI data (Ball et al., 2010).

With respect to TBSS in particular, the main difference between neonatal and adult TBSS is that they use different target images to register all the FA volumes to. In adult TBSS, all the FA volumes are registered to a standard template. A key prerequisite for TBSS is the need for accurate spatial alignment of individual data, and if an adult template is used to analyse neonatal data it results in poorly aligned data sets that prevents meaningful statistical analysis and may result in individual data sets being removed from studies (Bassi et al., 2008). To overcome this, in neonatal TBSS analysis, the most representative subject of the cohort is used as the target. In this option every FA volume is registered to every other one to identify the "most representative" subject (one with the minimum mean displacement score from all other subjects in the group), and this is used as the target image.

A further minor difference between adult and neonatal TBSS is that the mean FA skeleton image has a threshold of 0.2 in adults and 0.15 in neonates. This is to remove voxels with lower anisotropy at the edges of the skeleton which may be partial volume voxels in grey matter.

Neonatal imaging studies using TBSS have assessed; structural alterations in white matter microstructure in preterm infants; factors which may be associated with or predispose to these alterations; whether dMRI can predict neurodevelopmental outcome; the use of TBSS as a biomarker for treatment effects of neuroprotective agents.

TBSS has significantly improved understanding of preterm brain injury. Studies have investigated the effect of factors such as lower gestation(Anjari et al., 2007), bronchopulmonary dysplasia(Ball et al., 2010), punctate white matter lesions(Bassi et al., 2011) and chorioamnionitis(Anblagan et al., 2016) on white matter microstructure and have found that all of these factors negatively impact white matter development.

The first study to identify the link between genetic effects and preterm brain injury used TBSS in combination with DNA analysis and described significant alterations in white matter FA with genetic variants in 2 single nucleotide polymorphisms within candidate genes(Boardman et al., 2014). One was found in the Armadillo Repeat gene deleted in Velo-Cardio-Facial syndrome (ARVCF) gene which has been linked to neuronal migration and schizophrenia, and one in the fatty acid desaturase 2 (FADS2) gene, which has been linked to intelligence.

TBSS can also map to later developmental outcome both in preterm infants and in term asphyxiated infants. Van Kooij and colleagues showed increased FA in the corpus callosum in preterm infants undergoing MRI at term-equivalent age (TEA) was

associated with improved cognitive performance and increased FA in the posterior limb of the internal capsule (PLIC); FA values in the fornix and thalamus were associated with improved gross motor performance at 2 years (van Kooij et al., 2012). These findings were corroborated by Duerden and colleagues who reported increased cognitive scores at 18 months were associated with higher FA at TEA (Duerden et al., 2015). They also reported that early MRI, around 32 weeks postmenstrual age can predict motor outcomes but not cognitive outcomes.

Even with only 10 infants per group, Porter and colleagues were able to demonstrate that widespread microstructural abnormalities in white matter tracts in full term infants after asphyxia were reduced after treatment with therapeutic hypothermia (Porter et al., 2010). Comparing the cooled group with healthy controls, the only abnormality was that FA was significantly reduced in the internal capsule.

Tusor and colleagues reported significantly lower FA values in asphyxiated infants treated with therapeutic hypothermia who went on to have an unfavourable outcome, defined as death or significant neurodisability (Tusor et al., 2012). They report a significant linear correlation between developmental scores and FA; a correlation between locomotor function and FA in the corpus callosum and corticospinal tracts; a correlation between developmental scores and FA values in the fornix, cingulum and uncinate fasciculus, the structures associated with cognition and memory.

Finally, TBSS has been used to assess neuroprotective agents. One study investigated the effect of erythropoietin (EPO) in preterm infants in a randomised double-blind

placebo controlled prospective multicentre trial(O'Gorman et al., 2014). This trial reported that preterm infants treated with EPO had significantly higher FA than those in the placebo group. The second trial was a proof-of-concept, open-label, randomised controlled trial using FA in the PLIC, assessed by TBSS, as one of its primary outcome measures to investigate whether adjuvant therapy with inhaled xenon has synergistic benefits when combined with hypothermia for the treatment of perinatal asphyxial encephalopathy(Azzopardi et al.). There was no difference in FA between groups and this trial concluded that it had not proven a beneficial effect of using xenon in combination with therapeutic hypothermia. Interestingly, although this was reported as a negative trial, authors also report a higher FA in the PLIC in the group who had a normal/mildly abnormal neurological examination when compared to the group with very abnormal neurology (mean FA difference 0.03, $p = 0.02$).

1.4.4.1 Power calculations for neonatal imaging studies using TBSS

Traditional power analysis techniques are not directly applicable to TBSS analyses but sensitivity analyses have been performed by modelling a treatment effect as a global increase in FA(Ball et al., 2013). This was compared to a real effect of increasing age at scan since myelination increases as gestation at scan increases and FA is inherently linked to myelination. They demonstrated that a mean increase in FA of 5% is sufficient to detect a widespread 'treatment' effect when comparing equal group sizes of 20 or more. This has provided a guide for future studies with regards to sample size and power to detect changes in FA.

1.4.5 Brain volume

Head circumference (HC), also known as occipitofrontal circumference (OFC), is a surrogate for intracranial brain volume, as calculated by MRI scans, in both infants(Cheong et al., 2008) and adults(Hshieh et al., 2016). Brain volume also correlates with neurodevelopmental outcome in preterm infants(Setanen et al., 2016, Loh et al., 2017, Keunen et al., 2016). Changes evident in infancy persist throughout childhood, with another study of 7 year olds who were born very preterm showing smaller brain volumes were associated with long term poorer functional outcomes(Monson et al., 2016).

Neuroimaging analysis pipelines rely on atlases generated from healthy individuals to provide an anatomical context in which it is possible to interpret structural or diffusion MRI data(Blesa et al., 2016). Until recently, the atlases used were derived from adult data, introducing bias. In addition, volumes were derived from manual delineation of structures by hand, which was time consuming and susceptible to inter and intra-rater variability. Neonatal atlases now exist, an example of one being the Edinburgh Neonatal Atlas (ENA33), which was derived using data from 33 healthy term born infants(Blesa et al., 2016), and automated segmentation processes have been developed (Serag et al., 2016, Serag et al., 2017).

Challenges for segmentation of the neonatal brain include the large changes that occur in brain shape and appearance associated with normal postnatal development, the reduced SNR and movement artefact(Makropoulos et al., 2014). Quantitative volumes

from brain MRI are useful for investigating brain development and understand early life determinants(Serag et al., 2017).

1.4.6 Neuroimaging in opioid exposure

1.4.6.1 Infants and children

There are a limited number of published MRI studies in infants and children with prenatal opioid exposure(Kristine et al., 2009), and one of the crucial difficulties is that studies after the immediate neonatal period have not been able to exclude the effects of postnatal factors, such as socio-economic status, pharmacological treatment of NAS, environmental factors, on brain structure. Therefore, it remains unknown whether the brains of children with prenatal methadone exposure are different to children without such an exposure at birth.

Studies assessing brain volumes in prenatal opioid exposure report reduced volumes in the basal ganglia(Walhovd et al., 2007, Yuan et al., 2014, Sirnes et al., 2017), the thalamus(Sirnes et al., 2017), the cerebellum(Sirnes et al., 2017) and increased volume of the lateral ventricles(Yuan et al., 2014), which is commonly seen in preterm infants as a consequence of white matter loss, although cerebral white matter loss was not reported in any of the studies. Cerebellar growth has been shown to be negatively impacted by postnatal morphine in preterm infants(Zwicker et al., 2016), and this also correlated with poorer neurodevelopmental outcome.

The observation of reduced volumes in the basal ganglia, thalamus and cerebellum is interesting, as mu opiate receptors are distributed mainly in the frontal cortex, the hippocampus, cerebellum and basal ganglia, illustrated by human autopsy studies showing distribution of methadone within the brain (Pertschuk and Sher, 1975, Wehner et al., 2000). Methadone also exerts specific effects in the basal ganglia of rat brains (Martin et al., 2007). All drugs of abuse promote dopamine release, and the basal ganglia is known for its dopamine-rich structure, so its vulnerability as a region that prenatal exposure may adversely affect is plausible. However the underlying mechanism remains unknown.

Several of the published MRI studies are from a Norwegian group undertaking a longitudinal project on the development of children born to mothers who abused drugs during pregnancy, primarily but not exclusively opiates. In 2007 this group reported reduced regional brain volumes in 14 children with prenatal ‘substance-exposure’ (mean age at MRI 11 years), of which 10 were exposed to opioids, and compared them to 14 control children (mean age at MRI 9 years) (Walhovd et al., 2007). They reported reduced volumes in the pallidum and putamen specifically in the opioid-exposed group, all of whom were placed in foster care from a young age, so minimising the effects of postnatal environmental factors.

Subsequently, a pilot study of 16 infants with prenatal opioid exposure reported reduced whole brain volume and specifically reduced basal ganglia volumes and increased lateral ventricle volume (Yuan et al., 2014). This study lacked a comparison group, circumventing this issue by using population means. It also used manual

segmentation, which is prone to bias. More recently, a study in 16 children with prenatal opioid exposure aged 10 – 14 years at imaging reported reduced basal ganglia volumes when compared to sixteen unexposed children (Sirnes et al., 2017). These changes occurred independent of reduced birthweight and the basal ganglia volumes were normalised to intracranial volume. The opioid exposures were variable; seven were exposed to opioids as part of a treatment programme, nine were exposed to illicit heroin use. Polydrug use was high, with benzodiazepines being frequently used (69%) as well as cannabis and amphetamines. All children in the exposed group were in foster care or adopted. Interestingly 11 out of 16 opioid exposed children had a diagnosis of ADHD compared with only 1 out of the control group, and there are previous reports of reduced basal ganglia volumes associated with ADHD (Frodl and Skokauskas, 2012, Greven et al., 2015). Again, direction of causation is not known. A neonatal MRI study which quantified brain volumes before onset of symptoms of ADHD would provide useful information, as neonatal imaging offsets environmental or later postnatal events.

Data from a study investigating the effects of postnatal morphine exposure in the neonatal period in very preterm infants also provided useful insight into the effects of opiates on the developing brain, albeit prematurity is a different clinical context to prenatal exposure, however both encapsulate times of critical brain development (Zwicker et al., 2016). Zwicker and colleagues performed a sophisticated prospective cohort study of very preterm infants (24 weeks to 32 weeks gestational age at birth) who underwent serial MRI scans, had their total morphine exposure calculated and underwent developmental assessment at 18 months corrected gestation.

They reported that morphine exposure is independently associated with impaired cerebellar growth in the neonatal period and poorer neurodevelopmental outcomes in early childhood. These changes were evident even when correcting for multiple clinical confounders, such as the need for surgery, hypotension and other factors known to impact brain growth.

There are two TBSS studies in prenatal drug exposure: the first was published in 2010, and used TBSS in childhood to assess the white matter microstructure, and involved the same cohort of children as detailed in the 2007 volume study by Walhovd and colleagues (Walhovd et al., 2007). The 'substance exposed' group included 14 children, imaged at a mean age of 11 years, and of whom 10 were exposed to heroin, compared to 14 unexposed children imaged at a mean age of 9 years (Walhovd et al., 2010). Authors reported reduced FA in the substance-exposed group in the superior longitudinal fasciculus (SLF) and inferior longitudinal fasciculus (ILF), but this study was limited by small sample size, postnatal confounders and an increase in ADHD symptoms in the exposed group. In 2012, the same Norwegian group published the first neonatal TBSS study of 13 methadone-exposed infants and 7 control infants (Walhovd et al., 2012). They used the previous study as a basis for their pre-defined areas of interest (ILF and SLF) and used mean diffusivity (MD) rather than FA. They reported that MD was increased in the superior longitudinal fasciculus. This study was limited by small sample size, polydrug use and the use of MD rather than FA, which makes comparison with other studies more difficult. Nonetheless this study reported preliminary findings supporting the hypothesis of white matter damage potentially mediated by prenatal opioid exposure.

Although there is emerging evidence that prenatal opioid exposure leads to regional brain volume deficits, the childhood studies are confounded by postnatal diagnoses such as ADHD which can have accompanying brain volume changes associated with it (Frodl and Skokauskas, 2012, Greven et al., 2015). Postnatal morphine exposure negatively impacts cerebellar growth, and this is linked to poorer neurodevelopmental outcome in childhood. The two TBSS studies are both small numbers and report preliminary findings. All the studies acknowledge the need for further MRI studies to further investigate brain volume and white matter microstructure in prenatal substance abuse.

1.4.6.2 Adult MRI studies

There are several MRI studies in the adult literature assessing the impact of opioid drug abuse, and all which use dMRI report extensive disruption to the organisation of white matter tracts (Qiu et al., 2015, Li et al., 2016b, Qiu et al., 2013, Ma et al., 2015, Wang et al., 2011), likely as a manifestation of loss of myelin and axonal integrity indicated by increased RD and reduced FA (Bora et al., 2012). Several studies report an association between duration of drug misuse and severity of white matter disruption (Ma et al., 2015, Bora et al., 2012, Qiu et al., 2013, Qiu et al., 2015, Shen et al., 2012, Liu et al., 2008), although one study found no correlation between FA and duration of heroin use (Li et al., 2013). A study in abstinent heroin addicts reported higher FA in participants with longer periods of abstinence compared to those with shorter periods (Shen et al., 2012), and this suggests an element of reversibility or improvement over time. Lower FA in the PLIC has also been shown to correlate with higher risk of relapse in heroin dependent patients (Li et al., 2016b). Furthermore, a

recent systematic review investigating the white matter changes associated with substance misuse showed studies consistently reported that white matter is impacted early, and that a dose-dependent relationship exists (Baker et al., 2013), although this was mainly related to cannabis or alcohol use.

The studies that have used TBSS to assess the white matter microstructure have all investigated different opioids, including heroin, codeine and methadone. One study included 33 adults who were chronic users of codeine containing cough syrup and compared their MRI scans with 30 matched controls (Qiu et al., 2015). Authors reported reduced FA in the corona radiata, an area previously linked to risk taking and impulsive behaviour (Berns et al., 2009).

Another study investigated the effects of methadone in a longitudinal self-controlled design, whereby 33 adults prescribed methadone were imaged at the start of the study and 1 year later, and the differences in their diffusion metrics (FA, RD) were calculated (Li et al., 2016a). The authors reported significant reductions in FA and increased RD in the second MRI scan in several overlapping areas; the corticospinal tract, the main motor tract of the central nervous system, the corona radiata and the right superior longitudinal fasciculus. They also report a correlation between higher methadone dose and more severe white matter impairment. This study specifically reports the damaging effects of methadone on the white matter integrity, although this study was also limited by polydrug use, with many relapsing with heroin use in the interim between the two scans.

Adult MRI studies have reported other changes in association with opioid use, including reduced frontal grey matter volume in fifteen heroin addicts compared to fifteen healthy control adults(Liu et al., 2009). In addition, the clinical syndrome of heroin-associated encephalopathy has characteristic MRI findings that correlate with histological findings, including low signal on T1 weighted images, and high signal on T2 weighted images, the abnormal signal being largely attributed to demyelination and vacuole formation, with extensive involvement of both cerebral hemispheres, brainstem and cerebellum(Zhang et al., 2007).

1.5 Summary

This chapter has outlined the escalating problem of OUD during pregnancy and described the myriad of challenges and confounders when studying this population and their children and when interpreting the existing literature. The theoretical harm associated with prenatal opioid exposure has been summarised in both pre-clinical and human studies, building a picture suggesting that prenatal opioid exposure could be harmful. The role of the oligodendrocyte in brain white matter development and its potential role in the underlying mechanism of opioid-related harm has been explored. The ability to investigate underlying brain microstructure in the neonatal population and correlate these findings with later developmental outcome with advanced MRI techniques has been described.

The paucity of data relating to the long-term effects of prenatal opioid exposure, and more specifically of prenatal methadone exposure has been highlighted. The widely held belief that methadone is safe during pregnancy needs to be further investigated

and possibly challenged, and this thesis aims to either fill or expose knowledge gaps through systematic review and meta-analysis of the existing literature relating specifically to childhood outcomes after prenatal methadone exposure. Following this, the thesis will describe the use of advanced MR imaging to investigate brain microstructure shortly after birth in a group of infants with prenatal methadone exposure exploring the timing of the microstructural changes, which is crucial: if alterations in brain structure are already evident around the time of birth, before environmental factors such as home environment or postnatal opioid exposure can confound the results, this means the later outcomes of children with prenatal methadone exposure cannot solely be attributable to postnatal events and should focus research attention to prenatal management strategies of OUD.

CHAPTER 2: AIMS AND HYPOTHESES

2.1 Aims:

1. To perform a systematic review of the published literature investigating the effects of prenatal methadone exposure on childhood development
2. Where study design allows, to meta-analyse quantitative developmental scores and produce forest plots
3. To design, set up and recruit pregnant women prescribed methadone into an MRI based research study investigating brain development after prenatal methadone exposure
 - a. To obtain dMRI data from infants with prenatal methadone exposure shortly after birth
 - b. To analyse the dMRI data using TBSS and to compare prenatal methadone exposed infants with unexposed control infants
 - c. To perform volumetric segmentation of the brain and to compare prenatal methadone exposed infants brain volumes with unexposed control infants brain volumes.

2.2 Hypotheses:

- I. Adverse childhood neurodevelopmental outcomes are associated with prenatal methadone exposure
- II. White matter development is altered in infants with prenatal methadone exposure; manifest by dMRI data showing
 - a. Reduced FA across the white matter skeleton

- b. Increased RD across the white matter skeleton
- c. Increased MD across the white matter skeleton

when compared with healthy neonates not exposed to opioids during pregnancy.

- III. Brain volumes from structural MRI data will be smaller in prenatal methadone exposure compared with healthy neonates not exposed to opioids

CHAPTER 3 : PRENATAL METHADONE AND CHILDHOOD

OUTCOMES

3.1 General introduction

The increasing incidence of pregnant women using opioid drugs during pregnancy has been outlined in Chapter 1, as have the potential detrimental effects on animals and humans. The literature commonly refers to ‘substance misuse’ or ‘opioid exposure’ as a homogenous group, often without specific information about the exposure profiles of children who are exposed to potentially harmful drugs *in utero*. This, along with the issue of polydrug use, makes assessing causation extremely difficult.

In this chapter of the thesis, the childhood developmental outcomes associated specifically with maternal methadone therapy during pregnancy will be investigated, through systematic review of the literature and meta-analysis where appropriate to test the hypotheses that (1) adverse childhood neurodevelopmental outcomes are associated with prenatal methadone exposure and (2) adverse visual outcomes are associated with prenatal methadone exposure. It has been appropriately formatted for this thesis.

3.1.1 The Bayley Scale of Infant Development

The Bayley Scale of Infant Development (BSID) original edition is an individually administered developmental assessment which could be performed on infants aged from 1 month to 36 months (Bayley, 1969). It was first described in 1969 by a

psychologist, Nancy Bayley and involves a series of developmental play tasks from which a developmental quotient is derived, and tests two developmental domains; mental and motor development. Raw scores awarded to the child during the assessment for successfully completing tasks are converted to composite scores, referred to as mental developmental index (MDI) which is the childhood equivalent to intelligence quotient (IQ), and a psychomotor developmental index (PDI) assessing motor function. These indices are compared with norms taken from typically developing children of their age in months. As is the case with IQ, both MDI and PDI have a mean score of 100, with one standard deviation below the mean being a score of 85.

The original Bayley Scales of infant development (BSID) was described and used from 1969 until 1993 but has now been superseded. The second edition (Bayley-II) came into use in 1993 (BSID-II) and described three developmental domains; cognitive, motor and behavioural (Bayley, 1993). The third edition (Bayley-III) has been in use since 2006, and describes five developmental domains; cognitive, language, motor, social-emotional, and adaptive behaviour scales (Bayley, 2006).

3.2 Background

Globally, heroin use has been increasing since 2007 against the backdrop of a prescription opioids epidemic (World Drug Report, 2017). Pregnant women who use heroin are recommended medically assisted treatment (MAT) with an opioid substitute, such as methadone, as part of a comprehensive antenatal care plan because it is associated with improved use of antenatal services, reduced use of heroin during pregnancy, and reduced risk of preterm delivery when compared with no

treatment(Burns et al., 2007, Mattick et al., 2009, Zelson et al., 1973). Fetal benefits of methadone use include improved growth(Hulse et al., 1997, Kandall et al., 1976) and less risk of intrauterine death(Kandall et al., 1977).

Methadone is a synthetic long acting μ -opioid agonist that crosses the placenta freely, which exposes the developing fetal brain to exogenous opioid at a critical period. Pre-clinical studies suggest that exogenous opioids exert pleiotropic harmful effects on the central nervous system(Vestal-Laborde et al., 2014, Thompson et al., 2009, Hutchinson et al., 2011) and studies reporting childhood outcomes suggest that prenatal opioid exposure might impair neurodevelopment(Hunt et al., 2008, Baldacchino et al., 2015), but definitive conclusions are hampered by polydrug exposure and difficulties with adequately controlling for socioeconomic status, quality of care and environmental risk factors(Brogly et al., 2014).

Methadone substitution therapy for pregnant women with opioid use disorder was introduced into clinical practice in the 1960s without evaluation of childhood outcomes in randomized trials. Improved understanding of the long-term outcome of children exposed to prenatal methadone is essential for women and their physicians as alternative management strategies may be more suitable, including buprenorphine substitution therapy or medically supervised opioid withdrawal with psychosocial treatment(Jones et al., 2010, Brogly et al., 2014, Minozzi et al., 2013).

3.3 Aims

The goals of this study were: to perform a systematic review of published literature on childhood neurodevelopment, neuroimaging, and visual outcomes following prenatal methadone exposure; and to meta-analyse results of studies that used a common assessment instrument.

3.4 Materials and methods

3.4.1 Systematic review search information

The study protocol was registered with the international prospective register of systematic reviews (PROSPERO), registration number CRD42017063987 (<https://www.crd.york.ac.uk/prospero/>). Methodology is reported according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) statement (Liberati et al., 2009).

We included randomized trials, cohort studies, cross-sectional studies and case series that reported neurodevelopmental outcome, visual development / function, and / or neuroimaging of children whose mothers had taken methadone during pregnancy. There was no language restriction. Exclusion criteria were: studies of children whose mothers were prescribed alternative opioid substitutes during pregnancy and studies reporting neonatal neurodevelopment.

Two reviewers independently searched MEDLINE, EMBASE and PsycINFO for studies published between 1975 and 2017. MeSH terms used were “methadone” and “prenatal” or “prenatal exposure” or “prenatal drug exposure” or “prenatal exposure delayed effects” or “in utero”. Bibliographies of primary studies and review articles meeting the inclusion criteria were hand searched to identify further eligible studies.

Three reviewers independently screened titles and abstracts to identify potentially eligible studies. Where necessary, full text was retrieved and reviewed to confirm eligibility. Duplication was avoided if it was clear that the same cohort was reported in two publications; where more than one publication for a study was retrieved, the report that contained the maximum data points was reviewed in full to ensure inclusion of all relevant data.

Four reviewers independently extracted data from included studies using a standardized template. Extracted information included study setting, design, population and participant demographics, details of methadone exposure if available, control conditions, recruitment and completion rates, age at outcome measurement, assessment instrument and outcome of assessment. No authors were contacted to request missing information. Each study had data extracted by two reviewers independently, and templates were combined to ensure complete data collection. Disagreements were resolved through discussion.

3.4.2 Quality assessment of included studies

A quality assessment instrument was modified from the Grading of Recommendations Assessment Development and Evaluation (GRADE) Guidelines (Guyatt et al., 2011a, Guyatt et al., 2011b, Balshem et al., 2011). It incorporated objective criteria about study design, sample size and characteristics, use of validated outcome measures, risk of bias (blinding, confounding, attrition) and data analysis. Each study was assessed independently by two reviewers and scored as good (A, 6.5–8), intermediate (B, 3.5–6) or poor quality (C, 1–3). Full details of the quality assessment are available in Appendix I.

3.4.3 Meta-analysis

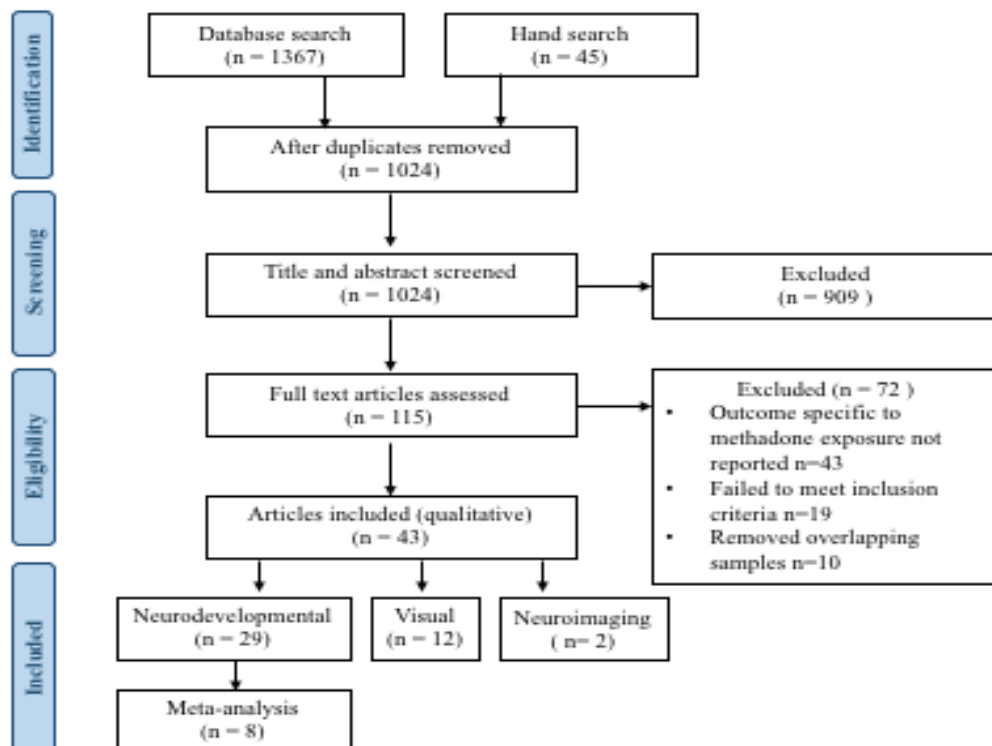
Where studies used the same assessment tool for any outcome domain, quantitative data were pooled in statistical meta-analysis using R 3.2.2 (<https://cran.r-project.org/>). Effect sizes were expressed as weighted mean differences (WMD) and their 95% confidence intervals. For longitudinal studies, data for assessments at 6 months and at 2 years were analysed. Heterogeneity was assessed using the standard I-squared and tau statistics and graphically using forest plots. Where statistical pooling was not possible, data were collated in tables for outcomes across the three domains (neurodevelopmental, visual development and neuroimaging) and statements were generated to represent the body of literature reviewed.

3.5 Results

3.5.1 Characteristics of included studies

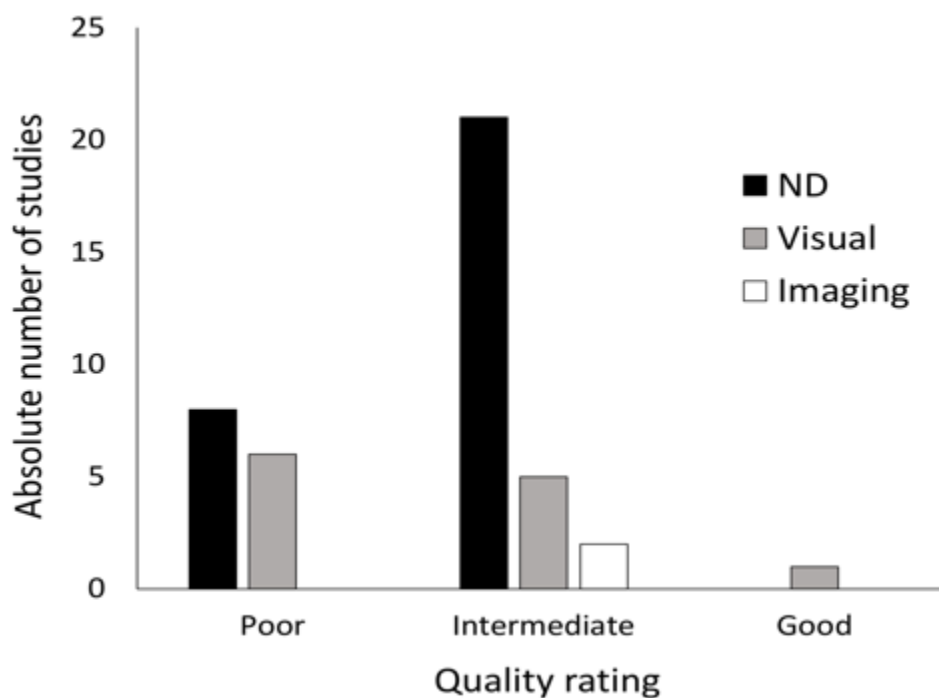
Forty- three eligible studies were identified with a total of 1476 methadone-exposed children compared to 864 unexposed children (Figure 5). 29 studies reported neurodevelopmental outcome (1247 methadone-exposed vs 740 unexposed subjects), 12 reported visual outcome (275 methadone-exposed vs 128 unexposed), and two reported neuroimaging findings (35 methadone-exposed vs 22 unexposed). No randomized trials were found.

Figure 5 : PRISMA flowchart showing process of inclusion and exclusion of studies



Fourteen studies (32.6%) were classified as poor quality, 28 (65.1%) as intermediate and one (2.3%) as good quality (McGlone et al., 2014), see Figure 6. Of those classified as poor quality, the main reasons were lack of blinding, small sample size, high attrition rates, and lack of comparison group validity (Beschner et al., 1977, Ramer and Lodge, 1975, Suffet and Brotman, Davis and Templer, 1988, Doberczak et al., 1988, Bunikowski et al., Paul et al., 2014, Nelson et al., 1987, Gaillard and Borruat, 2002, Hamilton et al., 2010, Gupta et al., 2012, Tinelli et al., 2013, Yoo et al., 2017, Kaltenbach and Finnegan)

Figure 6 :Quality rating of all included studies. ND, neurodevelopmental outcome studies



Twenty of 43 studies provided information about methadone exposure. 24 of 43 studies (597 methadone-exposed children) provided information about maternal

polydrug use during pregnancy: where polydrug use was assessed, most studies reported a majority of subjects to be polydrug exposed. For this reason, when we use the term “methadone exposed” hereafter, it should be taken that a probable majority of those described will have been polydrug exposed.

33 of 43 studies reported infants receiving pharmacological treatment of NAS; in 19 of these the treatment regimen was described, with the most frequently used drugs being morphine, phenobarbital, benzodiazepines, or a combination. Sixteen studies (615 methadone-exposed children) stated that preterm infants were included, while only seven explicitly excluded infants born before 36 weeks’ gestation. In 20 studies, it could not be determined whether preterm infants were included.

3.5.2 Neurodevelopmental outcome

3.5.2.1 Meta-analysis

Of the 29 studies reporting neurodevelopmental outcome, 15 used the original Bayley Scales of Infant Development (Bayley, 1969). Five of these fifteen were uncontrolled and not amenable to meta-analysis (Ramer and Lodge, 1975, Suffet and Brotman, Doberczak et al., 1988, Beschner et al., 1977, Kaltenbach and Finnegan) one study did not report any measure of variance (Kaltenbach and Finnegan, 1987) and one assessed children only at 9 months (Wilson et al., 1981) so these were excluded from meta-analysis. A total of eight studies reported BSID data were therefore eligible for meta-analysis (Table 2).

Table 2 : Summary of the eight studies included in the meta-analysis

Author, Year	Quality rating ^a	Meth	Un-exposed	Age ^b	Drug Info ^c	Assessment tool	Main Findings ^d <i>meth vs un-exposed</i>	Comments ^e
Strauss 1976 *	B	25 25 25	26 26 26	3 m 6 m * 1 yr	No dosing information No drug screening or PD information	BSID (MDI, PDI) at all ages	6 m *: MDI 115.7 (16.8) vs 114.3 (20.9), PDI 109.4 (12.2) vs 111.7 (14.5)	Original cohort 60 methadone-exposed infants vs 53 unexposed infants (no information on matching); Data reported only for infants who underwent Bayley at all 3 time points. Attrition rate at 6 months was 58.4% vs 51%. One case of SIDS in the methadone-exposed group. GA at birth not stated. Assessor blinding not stated. No information about NAS or treatment.
Kaltenbach 1979 ‡	B	26 17	27 24	1 yr 2 yr ‡	Mean dose for 1 yr cohort: 30; Mean dose for 2 yr cohort: 18 No drug screening or PD information	BSID (MDI) at all ages	2 yr ‡: MDI 90.88 (8.26) vs 94.62 (11.93) ns	Original cohort 43 methadone-exposed infants vs 51 unexposed (matched for maternal SES, ethnicity and medical conditions). Attrition rate at 2 years 60.5% vs 53%. Assessors blinded to group. 62% 1 year olds and 67% 2 year olds had been treated for NAS in the neonatal period. No pharmacological agent stated.

Author, Year	Quality rating ^a	Meth	Un-exposed	Age ^b	Drug Info ^c	Assessment tool	Main Findings ^d <i>meth vs un-exposed</i>	Comments ^e
Chasnoff 1984 *‡	B	31 13 11 6	34 29 27 14	3 m 6 m* 1 yr 2 yr‡	Mean dose 14.6 ± 10.2 (5 – 40) Maternal interview and urine screening; 4/31 used drugs in addition to heroin during pregnancy	BSID (MDI, PDI) at all ages	6 m*: MDI 105.9 (12.4) vs 111.0 (12.3); PDI 103.9 (9.0) vs 107.6 (15.1) 2 yr‡: MDI 97.5 (16.1) vs 96.2 (15.9); PDI 99.3 (16.9) vs 98.2 (8.9) <i>No p values stated</i>	Original cohort 39 methadone exposed vs 34 unexposed (matched for maternal age, education, gravidity and smoking). Attrition rate at 6 months was 66.7% vs 14.7%; at 2 years attrition rate was 84.6% vs 58.8%. All infants were term. Assessor blinding not stated. No information about NAS or treatment.
Rosen 1985 *‡	B	41 41 38 34 39	23 22 23 22 21	6 m* ^a 1 yr 18 m 2 yr ‡ ^a 3 yr	42 (mean of original cohort) Maternal urine; 56% of original cohort polydrug use (BDZ, opiates, cocaine, barbiturates, TCA) 15% reported mod-severe alcohol use	BSID (MDI, PDI) at 6m, 12m, 18m and 2yr; M-P at 3yr	6 m*: MDI 95 (2.5) vs 100.7 (4.2), ns, PDI 101 (2.8) vs 105.1 (2.9), ns 2 yr‡: MDI 90.4 (2.6) vs 96.9 (3.1) ns PDI 99.1 (2.7) vs 108 (2.7), p=0.05 <i>All scores are mean (SE)^f</i>	Original cohort 61 methadone-exposed infants vs 32 unexposed infants (matched for maternal ethnicity, SES, infant gender, BW and GA). Attrition rate at 6 months was 32.8% vs 28.1%; at 2 years was 44.3% vs 31.3%. Both groups included preterm infants. Assessor blinding not stated, 75% methadone-exposed had NAS; number treated pharmacologically not stated.
Kaltenbach 1989 *‡	B	27 27 27 27 27	17 17 17 17 17	6 m * 1 yr 2 yr ‡ 3.5 – 4.5 yr	Mean dose 38.42; No drug screening or PD information	BSID (MDI) at 6m, 1 yr and 2 yr; MSCA (GCI) at 3.5-4.5 yr	6 m*: MDI 107.9 (12.23) vs 105.6 (7.31), no p value 2 yr ‡: MDI 100.9 (18.04) vs 103.9 (11.49) no p value;	No information about original cohort, therefore attrition rates unknown. Unexposed group matched for maternal ethnicity and SES. Mean GA infants not stated. Blinding of assessors not stated. 92% treated for NAS; pharmacological agent not stated

Author, Year	Quality rating ^a	Meth	Un-exposed	Age ^b	Drug Info ^c	Assessment tool	Main Findings ^d <i>meth vs un-exposed</i>	Comments ^e
Wilson 1989 ‡	B	33 29 42 26 12	54 42 48 41 12	9 m 18 m 2 yr ‡ 3 – 5 yr 6 – 11 yr	No dosing information Maternal urine screening; 93% used psychoactive drugs	BSID (MDI) at 9m, 18m and 2yr. ^c	2 yr‡: MDI 88.8 (15.5) vs 90.2 (14.6) ns	Original cohort 39 methadone-exposed vs 57 unexposed infants (matched for maternal age, ethnicity, SES and marital status). Attrition rate at 2 years was 18% vs 16%. Mean GA not stated for either group. Assessor blinding not stated. 87% original cohort treated for NAS, pharmacological agent not stated.
Van Baar 1990 *‡	B	21 21 18 21 19	37 34 34 34 34	6 m* 1 yr 18 m 2 yr ‡ 2.5 yr	No dosing or screening information; Original cohort; 6 IV drug users; 94% used multiple drugs; 60% use cocaine	BSID (MDI, PDI, NDI) at all ages WWPA ^f at 18 m (n=14), 2 yr (n=16) and 2.5 yr (n=15)	6 m * MDI 103 (12) vs 107 (13), PDI 116 (18) vs 114 (21), NDI 105 (13) vs 109 (14) 2 yr ‡: MDI 86 (15) vs 98 (16) p<0.05 PDI 102 (16) vs 100 (18) ns, NDI 93 (16) vs 102 (22)	Original cohort 35 methadone-exposed vs 37 unexposed infants (not matched). Attrition rate at 6 months was 19.2% vs 0% and at 2 years was 19.3% vs 8.1%. Assessor blinding not stated. 28/35 (80%) were treated for NAS, pharmacological agent not stated.
Hans 2001 ‡	B	33 33 33 33 33	45 45 45 45 45	4 m 8 m 1 yr 18 m 2 yr ‡	Mean <20 (Range 3 - 40), Maternal interview and MUS; 13/33 cocaine, 18/33 cannabis, 11/33 alcohol use	BSID (MDI, PDI) at all ages	2 yr ‡: MDI 92 (12.7) vs 96 (12.3); PDI 100 (14.2) vs 108 (14.9).	Original cohort 47 methadone-exposed vs 45 unexposed infants (matched for maternal age, SES and IQ). Attrition rate 29.8% vs 0%. Assessors blinded to group. No information about NAS or treatment.

Notes relating to table 2

*indicates studies included in meta-analysis at 6 months, ‡ indicates studies included in meta-analysis at 2 years.

^a Quality rating: A = good, B = intermediate, C = poor, based on modified GRADE criteria; ^b Age expressed in days (d), months (m) or years (yr).^c Drug information includes mean daily methadone dose (in milligrams), maternal urine screening (MUS) and/or infant urine screening (IUS) for drug exposure and information on maternal polydrug (PD) use, where these are reported. Unless otherwise stated, all information in this column refers to methadone-exposed group only; ^d. Scores are presented as mean values (standard deviation) unless otherwise stated; ^e Comments include information on attrition, matching, gestation, blinding, proportion of infants treated for NAS and pharmacological treatment for NAS, where provided in the original study. ^f Standard errors were converted to standard deviation for the meta-analysis.

BDZ = benzodiazepine, BSID = Bayley Scales of Infant Development (1969), BW = birth weight, GA = gestational age, GCI = General Cognitive Index, IQ = intelligence quotient, MDI = Mental Developmental Index, mg = Milligrams, MSCA = McCarthy Scales of Childhood Abilities, NAS = neonatal abstinence syndrome, NDI = non-verbal developmental index, PD = polydrug (defined as methadone plus any other drug use during pregnancy, excluding tobacco), PDI = Psychomotor developmental Index, SES = socio-economic status, SIDS = Sudden Infant Death Syndrome, TCA = tricyclic antidepressant, WWPA = Werry-Weiss Peters Activity Scale.

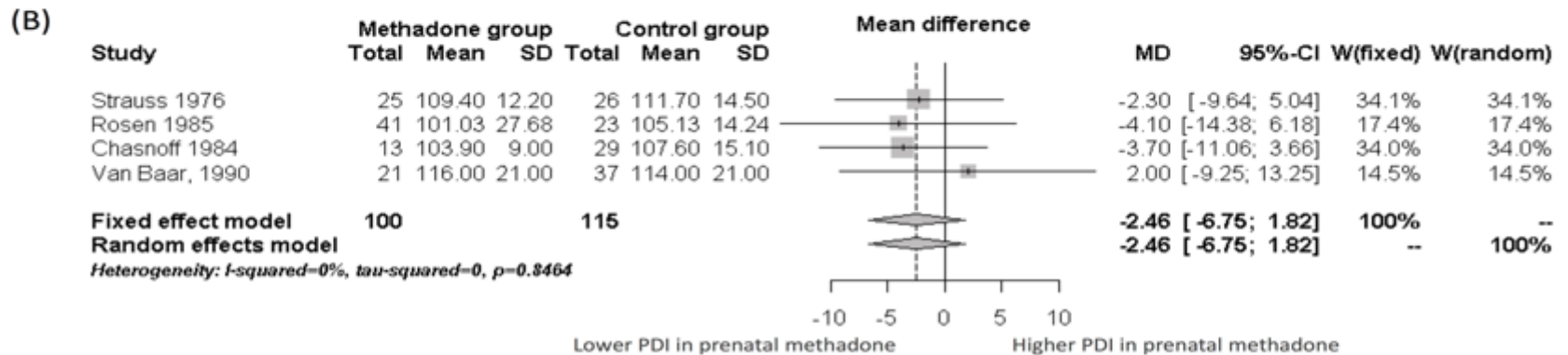
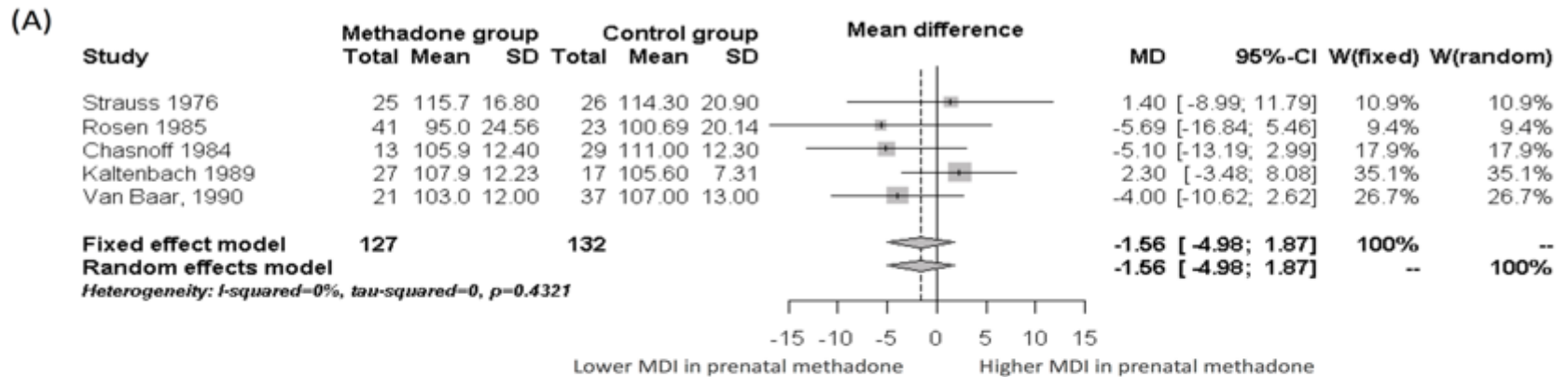
3.5.2.1 Meta-analysis of neurodevelopmental scores at 6 months

At 6 months of age, five studies reported Mental Development Index (MDI)(Strauss et al., 1976, Chasnoff et al., Rosen and Johnson, 1985, Kaltenbach and Finnegan, 1989, van Baar, 1990) and four reported Psychomotor Development Index (PDI)(Strauss et al., 1976, Chasnoff et al., Rosen and Johnson, 1985, van Baar, 1990), (Figure 7).

Methadone-exposed infants had poorer cognitive outcomes (MDI scores) than control infants (WMD of -1.56 (95% CI -4.98 to 1.87) between 127 methadone-exposed infants and 132 unexposed infants (Figure 7, Panel A), and also poorer motor outcomes (PDI scores: WMD of -2.46 (95% CI -6.75 to 1.82) between 100 methadone-exposed infants and 115 unexposed infants, (Figure 7, Panel B), but confidence intervals were wide and included zero.

Studies included in the 6 month meta-analysis were all of intermediate quality: attrition ranged from 31%(Rosen and Johnson, 1985) to 70%(Chasnoff et al.); two of the five studies gave no information about methadone dose and three described variable mean doses (range 15–42 mg per day)(Chasnoff et al., Kaltenbach and Finnegan, 1989, Rosen and Johnson, 1985). Two of the five studies excluded preterm infants(Chasnoff et al., van Baar, 1990); one included preterm infants(Rosen and Johnson, 1985); and two did not report gestational age of participants(Strauss et al., 1976, Kaltenbach and Finnegan, 1989).

Figure 7 : WMD in MDI (panel A) and PDI (panel B) at age 6 months between infants exposed to prenatal methadone and unexposed infants



3.5.2.2 Meta-analysis of neurodevelopmental scores at 2

years

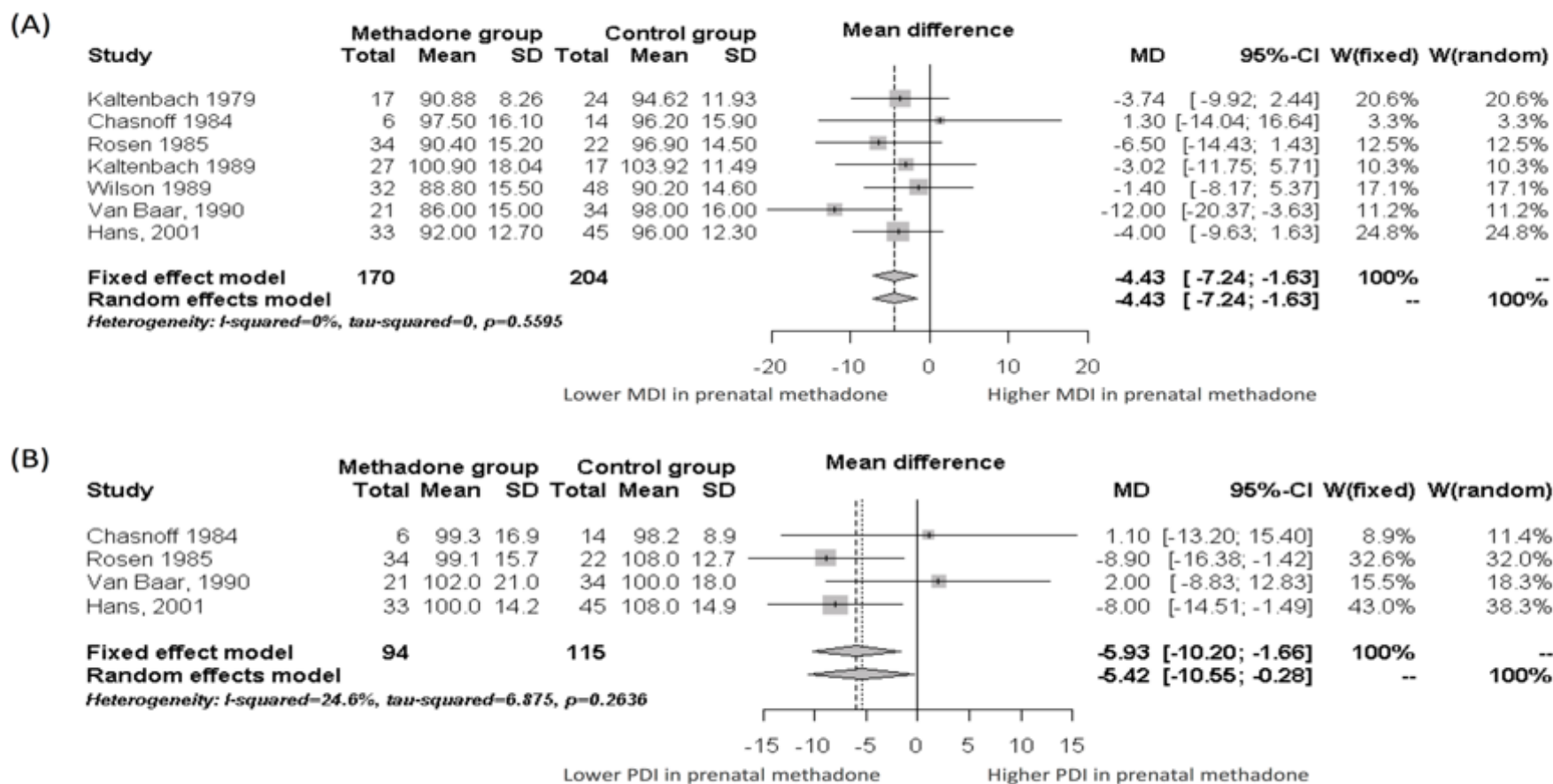
At 2 years of age, seven studies reported MDI(Kaltenbach et al., 1979, Chasnoff et al., Rosen and Johnson, 1985, Kaltenbach and Finnegan, 1989, van Baar, 1990, Hans and Jeremy, 2001, Wilson, 1989) and four reported PDI(Chasnoff et al., Rosen and Johnson, 1985, van Baar, 1990, Hans and Jeremy, 2001). Methadone-exposed children had poorer cognitive outcomes (MDI scores) than control children (WMD of -4.43 (95% CI -7.24 to -1.63) between 170 methadone-exposed children and 204 unexposed children, (Figure 8 Panel A), and also poorer motor outcomes (PDI scores: WMD of -5.42 (95% CI -10.55 to -0.28) between 94 methadone-exposed children and 115 unexposed children, (Figure 8, Panel B).

Studies included in the 2 year meta-analysis were also all of intermediate quality. Attrition rates ranged from 18%(Wilson, 1989) to 84%(Chasnoff et al.). Mean methadone dose was reported in five of seven studies and ranged from 15–42 mg per day(Chasnoff et al., Kaltenbach and Finnegan, 1987, Hans and Jeremy, 2001, Rosen and Johnson, 1985, Kaltenbach and Finnegan, 1989). Polydrug use was reported in five of seven studies and ranged from 56%(Rosen and Johnson, 1985) to >90% of mothers(Wilson, 1989, van Baar, 1990).

Two studies, describing 27 methadone-exposed children, included only term born infants(Chasnoff et al., van Baar, 1990); one included preterm infants(Rosen and Johnson, 1985) and the gestational age of participants was not stated in four

studies(Kaltenbach et al., 1979, Kaltenbach and Finnegan, 1989, Wilson, 1989, Hans and Jeremy, 2001). Five studies reported rates of treated NAS which ranged from 67%(Kaltenbach et al., 1979) to 92%(Kaltenbach and Finnegan, 1989); two studies provided no information about NAS(Chasnoff et al., Hans and Jeremy, 2001), and no study described the treatment received. For the MDI assessment, mean attrition from the original cohort escribed to the 2 year cohort assessed was 43% for the methadone group vs 27.7% for control; and for PDI assessment it was 45% versus 22.5%.

Figure 8 : WMD in MDI (panel A) and PDI (panel B) at age 2 years between children exposed to prenatal methadone and unexposed children



3.5.2.3 Qualitative analysis

Two studies reported outcomes using the Infant Behavior Record (IBR). Marcus *et al* reported poorer motor performance at 4 months in 15 methadone-exposed African-American infants compared with 23 matched unexposed infants (Marcus et al., 1982). Wilson *et al* compared 33 methadone-exposed infants with 55 unexposed infants, matched for maternal age, race, socioeconomic status and marital status, at 9 months of age and reported poorer fine motor co-ordination and less attentiveness and lower motor scores on the BSID, but no difference in cognitive scores was observed at this age (Wilson et al., 1981).

Five case series of methadone-exposed children used the BSID at varying ages. Suffet *et al* (Suffet and Brotman) found significant associations with gender at 1 year, with girls performing better than boys for both MDI (mean 108.8 versus 102.7, $p < 0.05$) and PDI (mean 102.3 versus 95.7, $p < 0.05$). This association persisted for cognition up to 2 years of age, when girls had higher mean MDI scores than boys (99.2 versus 82.0, $p < 0.01$). Bier *et al* (Bier et al., 2015) assessed cognition at 4 months of age using the BSID third edition (Bayley, 2006) in a large cohort of infants exposed prenatally to either low dose methadone (< 100 mg per day) or high dose methadone (≥ 100 mg per day) and reported MDI scores in the normal range.

Two studies used the Griffiths Scales of Mental Development; one at 6 months in 81 exposed versus 26 unexposed (McGlone and Mactier, 2015); and one at 1 year in 18

exposed versus 42 unexposed(Bunikowski et al.). McGlone *et al* reported reduced median scores across all domains, which persisted after adjustment for alcohol and maternal smoking, and noted that scores were lower for infants treated for NAS (n=39) compared with infants not requiring treatment (median General Quotient 95 vs 99, $p<0.008$). Bunikowski *et al* reported reductions in quotients for two subscales (hearing and speech and intellectual performance) at 1 year of age in a case series of prenatally exposed infants(Bunikowski et al.).

A cohort study reported no difference in ‘focus ratio’ (a measure of attention) between 2 year old methadone-exposed and unexposed children when observed during free play(Schneider and Hans, 1996). A case series of methadone-exposed children reported no difference in P2 amplitudes of auditory event-related potentials (ERP) in infants aged 4–15 days, but a difference was evident in older groups (16–32 days and 33–120 days)(Paul et al., 2014).

3.5.2.4 Children beyond 2 years of age

Twelve of the 29 neurodevelopmental studies evaluated children older than 2 years (323 methadone-exposed children compared to 321 unexposed children), using a wide range of assessment tools, see Table 3.

Of the 10 studies measuring cognitive outcomes, six reported no difference between methadone-exposed and unexposed children(Strauss et al., 1979, Lifschitz et al., 1985,

Rosen and Johnson, 1985, Kaltenbach and Finnegan, 1989, de Cubas and Field, 1993, Wilson, 1989). The remaining four studies reported poorer cognition in methadone-exposed children at 2.5 years(van Baar, 1990), 3 years(Hunt et al., 2008), 3.5 years(Van Baar and De Graaff, 1994), 4.5 years(Van Baar and De Graaff, 1994), 5.5 years(Van Baar and De Graaff, 1994) and 8.5 years(Davis and Templer, 1988). Two studies assessed language development using the Reynell Developmental Language Scales, and both report poorer performance in expressive and comprehensive language at 3 years (Hunt et al., 2008) and 4 years (Van Baar and De Graaff, 1994) in a total of 93 methadone-exposed children versus 76 unexposed children.

Of the seven studies assessing behavior, six reported more behavioral problems including more anxiety, more aggressive and withdrawn behavior and a higher rate of psychiatric referrals (16% vs 5%) in methadone-exposed children than in unexposed children(Wilson, 1989, Sandberg et al., 1990, de Cubas and Field, 1993, Van Baar and De Graaff, 1994, Hunt et al., 2008, Konijnenberg et al., 2015) (see Table 3).

Two cohort studies used care-giver questionnaires(Konijnenberg et al., 2015, Sandberg et al., 1990). Sandberg *et al* (Sandberg et al., 1990)observed ‘more feminine game play’ among methadone-exposed boys at 5–8 years and Konijnenberg and colleagues(Konijnenberg et al., 2015) report increased tendencies towards aggressive and withdrawn behavior at 4 years. The only study that reported no differences in childhood behavior evaluated hyperactivity in 19 methadone exposed and 34 comparison unexposed children at 2.5 years of age (van Baar, 1990). Details of all

studies reporting neurodevelopmental outcomes after prenatal methadone exposure are reported in Appendix II.

Table 3 : Summary of the twelve studies reporting neurodevelopmental outcomes in children beyond age 2 years

Author Year	Quality rating ^a	Meth	Un-exposed	Age ^b	Drug Info ^c	Assessment tool	Main findings ^d <i>Results appear as methadone vs unexposed</i>
Strauss 1979	B	31	27	5yr	No information	MSCA	86.8 (13.3) vs 86.2 (16.2), ns
Lifschitz 1985	B	26	41	3yr 5m	95% taking heroin or psychoactive drugs	MSCA	90.4 (13) vs 89.4 (10.8), ns
Rosen 1985	B	39	21	3yr	42 (mean of original cohort)	M-P	44.6 (2.1) vs 46.3 (2.3) ns
Davis 1988	C	12	28	8.5 yr	No information	WISC-R	89.58 (10.32) vs 96.32 (8.72) no p-value
Wilson 1989	B	26 12	41 12	3 – 5yr 6 – 11yr	No information	MSCA (GCI) Survey + school reports, IQ testing	GCI: 90.4 (13.0) vs 89.4 (10.8) ns IQ 1- 2 sd below norm 8% vs 5%, Language disability 8% vs 5%, Special education needs 16% vs 19%, Behavioural problems 75% vs 48%, Psychiatric referral 16% vs 5%
Kaltenbach 1989	B	27	17	3.5 – 4.5 yr	Mean dose 38.42	MSCA (GCI)	GCI: 106.5 (12.96) vs 106.05 (13.10), <i>t</i> =0.11
Sandberg 1990	B	30	16	5 – 8 yr	39.5 (boys), 38.7 (girls) Original cohort, 68% PD use; 15% moderate to heavy alcohol intake.	CGPQ ^e , CBAQ ^e (boys only)	Methadone-exposed boys showed more feminine game play than control boys (p<0.04).
Van Baar 1990	B	19	34	2.5 yr	No information	BSID (MDI, PDI, NDI) WWPA ^e	MDI 86 (15) vs 98 (16) p<0.05 PDI 102 (16) vs 100 (18) ns NDI 93 (16) vs 102 (22) WWPA 1.62 (1.03 – 2.66) vs 1.64 (1.28 – 2.52) ns

Author Year	Quality rating ^a	Meth	Un-exposed	Age ^b	Drug Info ^c	Assessment tool	Main findings ^d
De Cubas 1993	B	20	20	8.5 yr	No drug information. "moderate alcohol use"	SBIS, KABC-A, RATC, CBCL ^e	<i>Results appear as methadone vs unexposed</i> SBIS: 97.6 vs 98.1, ns KABC-A: 98.8 vs 102.4, <i>no p value</i> RATC: Methadone-exposed scored higher on anxiety, aggression, rejection, maladaptive outcome, p<0.01 for all. CBCL: More behaviour problems p<0.05
Van Baar 1994	B	23 26 23 22	32 32 31 30	3.5yr 4yr 4.5yr 5.5yr	PD use; 16/35 heroin and cocaine, only 2/35 solely methadone	SON IQ at 3.5 yr; RC and RE at 4 yr; RAKIT at 4.5 and 5.5 yr; IBR at 3.5 yr (n=22), 4.5 yr (n=23) 5.5 yr (n=22)	SON IQ: 99 (9) vs 109 (11), p<0.01 RC: 46 (6) vs 52 (6), p<0.01 RE: 46 (9) vs 50 (6), p<0.05 RAKIT 4.5 yr: 85 (11) vs 103 (15), p<0.01 RAKIT 5.5 yr: 90 (12) vs 102 (17), p<0.05 IBR: <i>Results median(range)</i> 3.5 yr: Free of fear: 9 (4-9) vs 6.5 (2-9), p<0.05; Activity level: 6 (3-9) vs 5 (2-9), p<0.05; Attention: 5 (1-7) vs 5.5 (1-9) p<0.05; Fine motor: 3 (1-5) vs 3 (1-5), p<0.05 4.5 yr: Co-operation: 6 (2-9) vs 7 (3-9), p<0.01; Endurance: 4 (2-9) vs 6 (1-9), p<0.01; Attention: 8 (2-9) vs 5 (2-8), ns 5.5 yr: Co-operation: 6 (1-9) vs 8 (4-9), p<0.01; Free of fear: 8 (2-9) vs 9 (5-9), ns; Attention: 5 (2-8) vs 5 (3-9), ns.
Hunt 2008	B	67	44	3yr	No information	SBIS; VL; MSCA; RC and RE	SBIS: 99.9 (15.1) vs 107.5 (13.4), p<0.01 VL: 38.4 (8.1) vs 46.1 (7.7), p<0.05 MSCA: 49.5 (8.7) vs 53.9 (8.3), p<0.05 RC: 42.4 (11.6) vs 49.2 (11.4), p<0.05 RE: 35.5 (7.9) vs 42.8 (12.8), p<0.05
Konijnenberg 2015	B	24	0	4 yr	Mean 85.96*; PD use in 40% (illegal drug use), 25% alcohol	CBCL ^e	Scores >55 on aggressive behaviour and withdrawn behaviour

* Mean methadone dose excludes outlier daily dose of 660mg methadone.

Explanatory notes relating to table 3:

^a Quality rating: A = good, B = intermediate, C = poor, based on modified GRADE criteria; ^b Age expressed in days (d), months (m) or years (yr); ^c Drug information includes mean daily methadone dose (in milligrams) and information on maternal polydrug (PD) use, where these are reported. Unless otherwise stated, all information in this column refers to methadone-exposed group only; ^d Scores are presented as mean values (standard deviation) unless otherwise stated; ^e Questionnaire completed by parent or care-giver

BSID = Bayley Scale of Infant Development (original version 1969), BW = birth weight, CBAQ = Child Behavior Attitude Questionnaire CBCL = Child Behavior Checklist, CGPQ = Child Game Participation Questionnaire, IBR = Infant behavior record, GCI = general cognitive index (used in the MSCA), MDI = mean developmental index (cognitive score), KABC-A = Kaufman Assessment Battery for Children, achievement component (tests the acquired knowledge of fact), M-P = Merrill-Palmer Scale, MSCA = McCarthy Scales of Childhood abilities, PD = polydrug (defined as methadone plus any other drug use during pregnancy, excluding tobacco), PDI = psychomotor developmental index (motor score), RA = Robert's Apperception RAKIT = Revision of the Amsterdam Children's Intelligence Test, RATC = Robert's Apperception Test for Children (tests the child's perception of common interpersonal situations), RC = Reynell Developmental Language Scales (Comprehensive), RE = Reynell Developmental Language Scales (Expressive), SBIS = Stanford-Binet Intellectual scale, SON-IQ = Snijders-Oomen Nonverbal Intelligence Test, VL = Vineland Social Maturity Scale, WISC-R = Wechsler Intelligence Scale for Children – Revised.

3.5.3 Visual development and function

Twelve studies reported visual outcomes (Table 4). Five of the twelve studies measured visual evoked potentials (VEPs; 143 methadone exposed and 103 unexposed), and four of these five reported more VEP abnormalities (absent; smaller, slower) in methadone-exposed children.

Two studies reported flash VEPs at 1–4 days after birth (McGlone et al., 2008, McGlone et al., 2013a) to be more frequently absent or immature, and to be smaller on average, in methadone-exposed infants than in comparison unexposed infants. Two studies reported pattern VEPs in infancy: at 4 months (Whitham et al., 2010) and at 6 months (McGlone et al., 2014), pattern-reversal and pattern-onset VEP peak times respectively were significantly slower (>10 milliseconds on average) in methadone-exposed infants than in the unexposed group. A follow up study of ten 3-year old children previously tested at 4 months found no group difference in pattern-reversal VEP peak times (Whitham et al., 2015).

A further six visual studies were case series detailing abnormal visual outcomes in a total of 108 methadone-exposed children (Nelson et al., 1987, Gaillard and Borruat, 2002, Hamilton et al., 2010, Gupta et al., 2012, Tinelli et al., 2013, Yoo et al., 2017). The most common abnormalities reported were nystagmus (50/108) and strabismus (51/108): 22/108 cases had both. The nystagmus direction in all described cases was horizontal and either jerk or pendular in waveform.

Abnormal visual outcomes at six months, reported as part of a cohort study (McGlone et al., 2014), were present in 40% (32/81) of methadone-exposed children (nystagmus 9/81 cases, strabismus 20/81 cases; both 5/81). One “negative priming” study estimated 4 year old methadone-exposed children’s ability to suppress attention to a non-relevant distractor, and found poorer visual selective attention in methadone-exposed children.

Table 4 : Twelve studies reporting childhood visual outcomes after prenatal methadone exposure

Author, year	Quality rating ^a	Meth	Un-exposed	Age ^b	Drug info ^c	Visual assessment	Main findings ^d <i>Results appear as meth vs unexposed</i>	Comments ^e
Nelson 1987	C	29	0	6 – 27 m	Mean 40.8; MUS; PD use reported	Strabismus	7/29 had strabismus. 4 esotropia diagnosed at a mean age of 10.5 months, 3 exotropia diagnosed at a mean age of 15 months. Paper compared the strabismus group with the non-strabismus group to look for associations.	Original cohort of 40 infants examined in neonatal period. No unexposed comparison group. 2/29 preterm. 21/29 treated for NAS, drug not stated
Gaillard 2002	C	5 cases	n/a	13 m 30 m 4 m 4 m 2 m	Case 1: BDZ, cannabis Case 2: no information Case 3: heroin and alcohol Case 4: heroin, BDZ, flupenthixol Case 5: BDZ	Nystagmus	Case 1, Female. Nystagmus until 3 yr. Development normal. Case 2: Male. Nystagmus presented at 30 m, still present at 38 m. Noted to have psychomotor retardation. Case 3: Male. Nystagmus still present at 8 m. Rest of examination normal. Case 4: Female. Nystagmus at 4 m, resolved by 10 m. Case 5: Male. Nystagmus noted at 2 m, resolved by 9 m.	Case series. 1. NAS, treatment not stated 2. No clarification regarding the developmental delay 3. Preterm (34 w). NAS but treatment not stated 4. NAS but treatment not stated 5. NAS but treatment not stated

Author, year	Quality rating ^a	Meth	Un-exposed	Age ^b	Drug info ^c	Visual assessment	Main findings ^d <i>Results appear as meth vs unexposed</i>	Comments ^e
McGlone 2008	B	21	20	1-4 d	No methadone information. “majority used BDZ” IUS; BDZ 8/13, cocaine 2/13, cannabinoids 1/13.	Flash VEP ^f ; Amplitude; Peak latency P2 and N3	Day 1 -4, methadone-exposed infants had fewer typical VEPs, more immature waveforms and non-detectable VEPs in 5/21 cases, p<0.01 Median amplitude 10.6mV vs 24.4mV, p<0.001. After 1 week, 14/21 methadone-exposed infants had repeat VEPs: remained low amplitude, median 11.3mV Peak latencies for P1 and N3 did not differ significantly between groups. 24% methadone-exposed had no detectable VEP but all unexposed infants had a detectable VEP.	Unmatched unexposed comparison group. All infants ≥37 weeks GA. VEP assessors blinded to group. VEP measured at 7 days if methadone-exposed infant still in hospital. 7/21 infants treated for NAS with morphine.
Hamilton 2010	C	20	n/a	3 m – 7 yr	No methadone information. 11/20 additionally exposed to BDZ, 8/20 to heroin.	Full age-appropriate visual assessment including electrophysiology; CVI	95% had reduced visual acuity 70% nystagmus (horizontal, mostly pendular or jerk) 50% had delayed visual maturation 35% had strabismus 30% had refractive errors. 25% (n=5) had significant neurodevelopmental problems (4 = developmental delay, 1 = Cerebral palsy). Incidence of CVI was 25%	2 preterm infants included 12 treated for NAS, drug not stated. 3 children with neurodevelopmental problems had CVI 4/20 had MRI scans, 1 reported abnormal white matter signal.

Author, year	Quality rating ^a	Meth	Un-exposed	Age ^b	Drug information ^c	Visual assessment	Main findings ^d <i>Results appear as methadone vs unexposed</i>	Comments ^e
Whitham 2010	B	22	33	4 m	Mean methadone 45.4 (range 15–100) MUS; 77% +ve in methadone mothers, 33% +ve control mothers (opioids = 3, cannabis = 8, BDZ = 2)	Pattern reversal VEP (P1 latency) Binocular viewing	P1 latencies (ms) to 48' 136.25 (18.02) vs 124.34 (12.35) – 12ms (95%CI 4-20ms, p=0.0052) P1 latencies (ms) to 69' 134.99 (33.46) vs 119.92 (11.74) – 15ms (95%CI 2.4-28ms, p=0.021) Multiple regression analysis revealed that prenatal methadone exposure is a significant predictor of prolonged P1 latency in response to checks of 48' retinal arc (Regression coefficient - 0.30, p<0.05) after correcting for confounders.	Part of a buprenorphine vs methadone vs unexposed study. Original cohort 72 opioid mothers and 35 mothers not taking opioids (unexposed group matched for maternal age, parity, gravida, self-reported alcohol use and smoking). Mean GA at birth 38.09 vs 38.85, no range GA. VEP assessors were blinded. 11/22 treated for NAS with morphine.
Gupta 2012	C	22	n/a	Mean 18.5 m (range 4-56 m)	No methadone information. 6/22 heroin 12/22 BDZ 2/25 significant alcohol	Nystagmus (± compensatory head posture) Strabismus Binocular best corrected visual acuity (LogMAR) Other visual abnormalities	All children had horizontal nystagmus: 9/22 jerk nystagmus, 8/22 pendular, 5/22 type nystagmus not stated. 16/22 strabismus, 15/16 esotropia, 1/16 exotropia, 6 no strabismus. Median VA 0.5 logMAR (range 0.2 – 1) in 18 children where VA testing possible. 1 child not fixing and 2 fixing and following when tested at 4m and 6m. 2/22 bilateral optic nerve hypoplasia, 8/22 delayed visual maturation	Case series 25 children, 2 exposed to opiates other than methadone and 1 exposed only to BDZ GA at birth not stated. 12/25 demonstrated signs of NAS, information about treatment for NAS not stated.

Author, year	Quality rating ^a	Meth	Un-exposed	Age ^b	Drug information ^c	Visual assessment	Main findings ^d <i>Results appear as methadone vs unexposed</i>	Comments ^e
McGlone 2013	B	100	50	1 -3 d	Maternal history (n=100), MUS (n=84); IUS (n=70), and meconium, n=74. 30 control infants had meconium samples	Flash VEP ^f : When present, amplitude and implicit times of peaks and troughs measured (P1, P2, N3, P3)	Methadone-exposed were less likely to demonstrate P1 components of VEP (21% vs 48%, p=0.001) and N2 components of the VEP (38% vs 60%, p = 0.011) Methadone-exposed infants had smaller amplitude VEPs median 27 μ V vs 39 μ V, p<0.001 and had more immature or atypical VEPs, p=0.001. All differences persisted after correcting for confounders (HC, cigarette smoking, excess alcohol) No association between maternal methadone dose and VEP abnormalities. NAS did not impact on VEP amplitude, morphology or implicit times.	Unexposed group matched for GA (completed weeks), BW (\pm 250g), Carstairs deprivation index (\pm 1) Excluded infants <36 weeks GA. 2 VEP assessors, 1 blinded to group. All VEPs recorded prior to any NAS treatment. 48/100 treated for NAS with morphine, 22/48 required 2 drugs (morphine and phenobarbital)
Tinelli 2013 ³	C	2	n/a	Case 1: 3 m – 12 m Case 2: 2.5 m – 8 m	Case 1: 30 Case 2: 35; MUS	Pendular horizontal nystagmus	Case 1: Preterm (33 w). Nystagmus noted at 2 m, still present at 1yr Reduced VA (1.3 logMAR) at 3 m, gradually improved (0.47 logMAR). Case 2: Term (40 w). Nystagmus at 5 m with associated reduced VA (0.87 logMAR). 8 m nystagmus still present but able to follow target. VA improving (0.47logMAR)	Case 1: No NAS. Normal CrUSS and fundi at 2 m. At 1 yr development normal Case 2: NAS treated with phenobarbital for 15 days. Flash VEP prolonged latency and reduced amplitude right eye. Neurological examination at 8 m normal

^fFlash VEP categorized as typical, atypical, immature or not detectable

Author, year	Quality rating ^a	Meth	Un-exposed	Age ^b	Drug information ^c	Visual assessment	Main findings ^d <i>Results appear as methadone vs unexposed</i>	Comments ^e
McGlone 2014	A	81 ^g	26 ^g	6 m	No methadone dosing information; PD use in 90%: 75% opioids; 67% BDZ; 64% cannabis; 26% stimulants. 20/46 tested for FFAE had elevated levels. 3/18 control infants had elevated FFAE and 2 tested positive for cannabinoids.	Modified Atkinson Battery ^h Pattern onset VEP: present or absent. C2 latency C2 amplitude	32/81 vs 2/26 infants failed the visual assessment ⁱ . Abnormalities in methadone-exposed infants: Strabismus (25%) - 12 exotropia, 8 esotropia, reduced VA >0.9 logMar (22%), horizontal nystagmus (11%) Methadone-exposed infants had a five-fold higher chance of failing their visual assessment after correcting for alcohol exposure ⁱ (p=0.007) Relative risk of abnormal visual assessment in methadone-exposed infants was 5.1 (95%CI 1.3 – 20), p=0.02. C2 latency (ms) at 120' 115 vs 99, p=0.019, at 60' 115 vs 106, p=0.036, at 15' 128 vs 108, p=0.0002 C2 amplitude (µV) at 120' 24 vs 26, p=0.091, at 60' 24 vs 34, p=0.003, at 15' 10 vs 17, p=0.003 Methadone-exposed infants significantly less likely to have a VEP in response to the smallest check size of 15' (51/70 vs 24/24, p=0.006). Overall, 70% methadone-exposed infants had one or more abnormal VEP parameter.	Initial cohort 100 methadone exposed vs 50 unexposed.. Infants <36 weeks GA excluded. 1 pediatrician and 1 optometrist assessed vision. Optometrist blinded to group. Corrected for confounding effect of excess prenatal alcohol exposure 55/81 treated NAS with morphine. No infant had a clinical diagnosis of fetal alcohol syndrome.

^g Same cohort as McGlone 2013, examined at 6 months; ^h Atkinson Battery is a full visual assessment and includes pupil response to light, observation for nystagmus, convergence of eyes to approaching object, defensive blink, visual following of falling toy, batting and reaching, near retinoscopy, dynamic retinoscopy, Cardiff acuity card; ⁱ Fail defined as presence of strabismus, nystagmus, reduced VA or refractive error >3 dioptres;

Author, year	Quality rating ^a	Meth	Un-exposed	Age ^b	Drug information ^c	Visual assessment	Main findings ^d <i>Results appear as methadone vs unexposed</i>	Comments ^e
Konijnenberg 2015	B	22	25	Mean age 4 yr	Mean dose 86.19 ^j PD common: 45% BDZ, 22% IV drug use, 18% AMP	Saccade latency SNP difference score (Assessed using eye tracking)	Mean saccade latency(ms) 376.3 (74.6) vs 331.2 (45.50) ^k SNP difference score -12 (85.93) vs 23.5 (45.50) ^l . Less random distractor trials (121 vs 315) and unexposed trials (115 vs 336) included in the analysis ^m .	Part of a study comparing buprenorphine exposed and methadone exposed infants with an unexposed group, which was not matched for maternal or infant characteristics. Mean GA at birth 38.7 vs 39.8 weeks, range not stated. Assessor blinding not stated. 13/22 treated for NAS, drug not stated.
Whitham 2015	B	10 ⁿ	15 ⁿ	3 yr	No methadone information PD use 5/10 BDZ; 7/10 positive urine screen	Binocular pattern reverse VEP latencies	P100 latencies: 48' retinal arc: 103.7 vs 104.7, p=0.47, 69' retinal arc: 101.1 vs 102.7, p=0.65 Multiple regression analysis showed that HC at time of testing was a significant predictor of P100 latency for checks of 69', p=0.04.	Follow up study of Whitham 2010- Inclusion of preterm infants not known as GA not stated. 11 methadone-exposed infants tested; one had significantly prolonged P100 latencies, and was excluded from the analysis as an 'outlier'. Blinding of assessor not stated. 6/10 treated for NAS.

^j Mean methadone dose excludes outlier daily dose of 660mg methadone; ^k Proportion of infants affected per group is not stated; ^l no p-value stated; ^m Trials excluded if child looks at distractor instead of target, failed to fixate or anticipated the position of the target before it appeared; ⁿ Same cohort as Whitham 2010, examined at 3 years

Author, year	Quality rating ^a	Meth	Un-exposed	Age ^b	Drug information ^c	Visual assessment	Main findings ^d <i>Results appear as methadone vs unexposed</i>	Comments ^e
Yoo 2017	C	32	0	Infancy; ages not stated; follow up at various ages	Dose available for 24/32; maximum dose <100 n = 14, ≥100 n = 9. PD available for 24/32, MUS; psychotropic meds 75%, heroin or opioid pain med 71%, cocaine 67%, BDZ 37%, marijuana 37%, alcohol 29%, buprenorphine 4%, barbiturates 4%	Full, age-appropriate ophthalmic exam. Strabismus, refraction, nystagmus, amblyopia, ocular alignment	21/32 with strabismus (16 exodeviations, 5 esodeviations), median age of onset 12 months. 5/21 with strabismus had additional nystagmus. Presence of strabismus not associated with methadone dose, exposure by trimester, polydrug exposure, history of NAS, prematurity or small for gestational age.	9 preterm and 5 small for gestational age infants included 27 NAS, 25/27 treated, drug not stated. 3 with perinatal intracranial disease. Blinding of assessor not stated. Likely underestimate as sample limited by insurance cover and incomplete follow-up.

Explanatory notes relating to table 4:

^a Quality rating: A = good, B = intermediate, C = poor, based on modified GRADE criteria;

^b Age expressed in days (d), months (m) or years (yr);

^c Drug information includes mean daily methadone dose (in milligrams), maternal urine screening (MUS) and/or infant urine screening (IUS) for drug exposure and information on maternal polydrug (PD) use, where these are reported. Unless otherwise stated, all information in this column refers to methadone-exposed group only;

^d Scores are presented as mean values (standard deviation) unless otherwise stated;

^e Comments include information on attrition, matching, gestation, blinding, proportion of infants treated for NAS and pharmacological treatment for NAS, where provided in the original study;

3.5.4 Neuroimaging

There were two small cohort studies reported neuroimaging using different modalities so synthesis was not possible in 35 methadone-exposed and 22 unexposed infants (Table 5). Pasto *et al* observed ‘slit-like ventricles’ in the newborn period using cranial ultrasound(Pasto et al., 1985), and Walhovd *et al* observed a regional difference in a measure of white matter microstructure (MD from dMRI) at around 3 weeks of age(Walhovd et al., 2012). Neither study was blinded and the majority of infants in both studies were treated for NAS.

Table 5 : Two studies reporting childhood neuroimaging outcomes after prenatal methadone exposure

Author Year	Quality Rating ^a	Meth	Un-exposed	Age ^b	Drug info ^c	Imaging modality, techniques and measurements	Main findings <i>Results appear as methadone vs unexposed</i>	Comments ^d
Pasto 1985	B	22 22 22	15 15 15	24 h 72 h 7d	Mean dose 40. No screening information. “Almost all mothers used illicit drugs during pregnancy”	Ultrasound for qualitative assessment of ventricular configuration, 2D measurements including ICHD	Significantly more of the methadone-exposed infants had slit-like ventricular configurations during the 24-73 hours following birth, and at 1 month after birth compared with control infants, $p < 0.05^c$. Methadone-exposed infants had smaller ICHD at 1 month ^{e,f} .	Initial cohort 52 methadone exposed and 38 unexposed infants (matched for maternal age and SES). All infants born at term. Blinding was not stated. 17/22 were treated for NAS with morphine (n=7), phenobarbital (n=4) or a combination of 2 of either morphine, phenobarbital and diazepam (n=6).
Walhovd 2012	B	13	7	Mean age at MRI = 21 d (13 – 44 d) in meth infants vs 23 d (14 – 32 d) in unexposed infants	Mean dose 57.9 (range 23.97 – 126.67). No screening or PD information	3T MRI Tract-based spatial statistics (MD) Whole tract analysis (probabilistic)	MD of left SLF was 0.00138326 (S.D., 0.00003818) for methadone-exposed and 0.00133076 (S.D., 0.00002958) for comparison infants, after co-varying for age at scan, $p = 0.046$	Initial cohort 15 methadone exposed and 9 unexposed infants (matching not stated). Mean GA at birth 38.1 (range 36-42) vs 40.0 (38 – 41) weeks. Blinding not stated. 11/13 treated NAS with morphine.

2D = 2 dimensional, 3T = 3 Tesla, ICHD = intracerebral hemi-diameter, MD = Mean diffusivity, MRI = magnetic resonance imaging, NAS = Neonatal Abstinence Syndrome, SES = socio-economic status, SLF = superior longitudinal fasciculus, TBSS = tract-based spatial statistics

Explanatory notes relating to Table 5:

^a Quality rating: A=good quality, B= intermediate quality, C= poor quality, based on modified GRADE criteria;

^b Age at time of imaging assessment expressed in hours (hr) or days (d);

^c Drug information includes mean daily methadone dose (in milligrams), maternal urine screening (MUS) for drugs and infant urine screening (IUS) for drugs, and information on maternal polydrug use (PD) where reported. Unless otherwise stated, all information in this column refers to methadone-exposed group only;

^d Comments include information on attrition, matching, gestation, blinding, proportion of infants treated for NAS and pharmacological treatment for NAS, is stated where provided in the original study;

^e Proportion of infants affected per group is not stated;

^f no p-value stated.

3.6 Discussion

This systematic review of long-term neurodevelopmental, visual and neuroimaging outcomes of children with prenatal methadone exposure has collated detailed outcomes from 43 studies describing a total of 1476 methadone-exposed children and 864 unexposed comparison children. The data describe poorer neurodevelopment and visual development in children with prenatal methadone exposure compared with unexposed comparison children. Eight studies reported BSID data at 6 months and/or at 2 years of age and were amenable for meta-analysis: meta-analyses point estimates of cognitive and motor indices in children exposed to prenatal methadone are reduced at 6 months of age, and by 2 years the WMD in both indices are increased with 95% confidence intervals that make the possibility of no difference unlikely. The emergence of difficulties as children grow older is well-recognized after complications in the perinatal period and reflects the ontogeny of higher-order function through childhood. The finding of behavioural problems in six out of seven studies that measured this domain, and lower cognitive performance in four out of 10 studies that reported outcome after 2 years, suggests that exposed children may be at increased risk of long term problems.

The association between prenatal methadone exposure and atypical visual development is striking, with four out of five studies reporting significant differences in VEPs in infancy and childhood, reflecting altered visual pathways (McGlone et al., 2008, Whitham et al., 2010). McGlone *et al* (McGlone et al., 2014) in their cohort of 81 methadone-exposed and 29 unexposed infants at 6 months of age, describe a methadone-attributable risk of abnormal visual assessment of 80%, after correcting for

excess prenatal alcohol exposure. The prevalence of childhood strabismus and nystagmus in the methadone-exposed population is considerably higher than expected, which suggests that disorders of childhood visual function, as well as altered electrophysiological measures, are associated with prenatal methadone exposure.

3.7 Study strengths

A strength of this work is its pragmatic and systematic approach to summarizing childhood outcome following prenatal methadone exposure across three important domains chosen to reflect a broad perspective of the developing brain: neurodevelopment, visual development and function, and neuroimaging. We excluded studies of neonatal neurodevelopment in order to prevent confounding by NAS, and we excluded studies of alternative opioid substitutes in order to derive maximum inference about methadone, the single most commonly used pharmacological agent in the management of pregnant women with opioid use disorder.

3.8 Study limitations

Opioid dependent women prescribed methadone commonly use other drugs including alcohol (McGlone et al., 2012, McGlone et al., 2013b). Investigation and reporting of additional substance use in the studies reviewed was variable, and only one study had examined prenatal exposure in all infants in detail using extensive toxicology (McGlone et al., 2013a). Where additional drug use was investigated, the majority of infants were found to be polydrug exposed. Thus, a significant limitation of the meta-analysis is that its finding of poorer cognitive and motor indices at 2 years

of age cannot be attributed to methadone *per se*. However, the findings may be used for comparison with childhood outcome studies of alternative opioid substitutes in pregnancy.

The review was also limited by intermediate to poor quality of included studies which introduces a risk of bias due to small study populations, high attrition, potential confounding by preterm birth, use of historical neurodevelopmental assessments, lack of assessor blinding and poor validity of comparison groups, with inadequate control for socioeconomic status, quality of care or environmental factors. However, meta-analysis showed low statistical heterogeneity, although this could reflect restricted sample sizes. Attrition of methadone-exposed children at 2 years of age included in the meta-analysis was twice that of comparison children, increasing the chance of a type 2 error given that attrition is often explained by impairment in pediatric neurodevelopmental outcome studies. For the same reason, it is possible that the extent of neurodevelopmental and visual impairment associated with prenatal methadone exposure may be under-estimated.

A further limitation is the historical nature of the majority of included studies, with only 15 of the 43 studies were published in the past decade. The original literature search included published literature from 1975 onwards, as this represents the time that methadone came into clinical use and it was important to capture all studies investigating potential effects of prenatal exposure, accepting that they may not represent our current population, in order to fully assimilate all the available evidence in this area. The eight studies assessing childhood development included in the meta-

analysis were all of the original BSID era, which has now been superseded. Seven were published between 1979 and 1990 and one article contained combined data derived from several earlier studies (Hans and Jeremy, 2001) so although it was published in 2001 when Bayley-II was established, the article contained original data using BSID, therefore met inclusion criteria.

The dated nature of the included literature might affect application of results to contemporary populations because patterns of drug misuse change over time and strategies for MAT of opioid use disorder in pregnancy have evolved. For example, the dose of methadone prescribed for MAT in current practice is typically higher than that reported in historical studies. A final limitation is that only nineteen of 43 studies provided information about maternal methadone dose, so it was not possible to explore dose response relationships.

3.9 Clinical implications of this study

Infants of methadone-maintained women with OUD are at increased risk for neurodevelopmental and visual impairment in early childhood, so may benefit from increased surveillance to aid early detection and timely intervention. This is particularly relevant for visual difficulties that are treatable, but which may be undiagnosed, or misdiagnosed as behavioural or learning difficulty. A history of opioid exposure during pregnancy should be sought if children present to services with disorders of visual function. The data indicate that follow-up to at least 2 years is required to detect neurodevelopmental difficulties, and carers should be made aware that impairment might emerge in later childhood.

Improved understanding of the prenatal effects of OUD treatments, including the use of alternative substitutes, have been identified as research priorities (World Health Organisation, 2014). Buprenorphine is a partial mu-opioid agonist and kappa-antagonist that binds to opioid receptors with higher affinity but lower activity than full agonists such as heroin and methadone (Walsh et al., 1995). Buprenorphine has a ceiling effect due to its partial antagonist activity, meaning that higher doses do not increase its effect due to opioid receptor saturation. This reduces the risk of overdose, but also means that buprenorphine can only be considered with daily doses of methadone of 30mg or less, as higher doses of methadone are unable to be matched by buprenorphine.

Buprenorphine has been evaluated as an opioid substitute in pregnancy. The MOTHER trial, one of the largest randomised controlled trials comparing buprenorphine to methadone in a total of 175 opioid dependent mothers, assessed short term infant outcomes relating to NAS and its treatment (Jones et al., 2010), appeared to produce encouraging results suggesting less severe NAS and shorter hospital stays with buprenorphine. These findings, along with improved growth and longer gestation have been replicated by other studies (Metz et al., 2011, Pritham et al., 2012, Lacroix et al., 2011). However, there was a large drop out in the MOTHER trial in the buprenorphine group (33% vs 18%) suggesting maternal dissatisfaction with the drug. If buprenorphine was rolled out as a replacement for methadone, this could result in a paradoxical increase in adverse fetal and/or neonatal outcomes if the mother then uses other illicit opioids such as heroin to control symptoms of withdrawal.

The concerns relating to buprenorphine replacing methadone have also been raised in a recent Cochrane review which concluded that there are insufficient data to establish whether buprenorphine is equivalent for all maternal outcomes, particularly adherence to treatment(Minozzi et al., 2013). Furthermore, confounding by indication could explain improved neonatal outcomes in buprenorphine groups(Brogly et al., 2014) due to the significant drop out in the buprenorphine groups secondary to maternal dissatisfaction with the treatment. It is therefore plausible that the improved outcomes reported in trials such as the MOTHER trial reflect intrinsically different populations of women with OUD, who are less severely affected and therefore able to tolerate the proportionally lower doses of opioid thus find buprenorphine an acceptable alternative. Therefore, although buprenorphine appears an attractive option when compared to methadone, the issues relating to confounding by indication result in clinical equipoise about the safest opioid substitute for mother and child.

3.10 Future research

Future research should aim to test methadone-exposed against buprenorphine-exposed infants and children, with well-matched un-exposed groups across a range of cognitive and behavioral domains with childhood follow-up extending beyond 2 years. Quantitative MRI is a powerful non-invasive technique that is sensitive to detecting alterations in neonatal brain structure and can predict functional impairment in childhood(Boardman et al., 2010, Ball et al., 2010) with relatively low sample size(Ball et al., 2013); its use could be informative in future research designed to

investigate the safety and harm of management strategies for opioid use disorder in pregnancy.

This chapter of the thesis has explored the existing literature reporting childhood outcomes after prenatal methadone exposure. The data presented highlight that being born to an opioid-dependent mother who has been prescribed maintenance methadone in pregnancy is associated with adverse visual and neurodevelopmental outcomes in infancy and early childhood, but deficiencies in the existing literature limit causal inference about harm and factors other than methadone *per se* could account for these observations. The need for further contemporaneous research into optimal management of pregnant women with opioid use disorder is identified and future studies should be designed to evaluate neonatal brain development, as well as long term neurocognitive, visual and behavioural outcomes. The next chapter will describe the effect prenatal methadone exposure on brain development, as investigated by MRI.

CHAPTER 4: PRENATAL METHADONE EXPOSURE AND BRAIN DEVELOPMENT

4.1 General introduction

In Chapter One, the advantages of using MRI to understand disease in neonates was explored, in particular, the use of dMRI to investigate underlying brain microstructure, and advanced segmentation techniques to allow calculation of brain compartment volumes, all specific to the neonatal brain. The limited literature using MRI to investigate the effects of prenatal drug exposure was also reviewed in Chapter One. Comparisons were made with the adult literature on white matter changes associated with opioid use, which supports the hypothesis that opioid use contributes to a myelin-related pathology resulting in disruption of the white matter microstructure. Chapter Three further explored the childhood neurodevelopmental and visual sequelae associated with prenatal methadone exposure. However, many of the studies in this area are unable to isolate the effects specifically of the prenatal drug exposure and are confounded by the potential influence of postnatal factors closely linked with parental substance misuse.

In this chapter, the results of the MRI study undertaken in term born neonates exposed prenatally to methadone and those not exposed to any opioids during pregnancy will be presented. The recruitment of methadone-exposed study participants, obtaining informed consent from mothers prescribed methadone, preparation of the infant for MRI scan, transportation to MRI scanning area, supervising MR image acquisition, co-ordinating reporting of structural images, organising follow-up imaging in the

event of incidental findings and involvement in defining the TBSS covariates was performed by Victoria Monnelly. The final processing of the dMRI data and the TBSS analysis was performed by Dr Devasuda Anblagan. The control group of un-exposed infants were selected based on age matching from a previously described group of healthy term neonates recruited and scanned as part of a study of typical brain development. The use of previously acquired MRI data negated the need to recruit and scan healthy control term born babies as part of this study.

The first part of this chapter is a formatted version of a first author paper published in *Neuroimage Clinical* (Monnelly et al., 2018) which tests the hypothesis that white matter development is altered in infants with prenatal methadone exposure, manifest by (1) reduced FA across the white matter skeleton (2) increased RD (3) increased MD across the white matter skeleton when compared with healthy neonates not exposed to opioids during pregnancy. The paper has been reformatted for the purpose of this thesis.

The second part of this chapter assesses the brain volumes in a sub group of neonates with prenatal methadone exposure and compares them to unexposed control neonates to test the hypothesis that brain volumes will be smaller in prenatal methadone exposure compared with healthy neonates not exposed to opioids. The volumetric analysis included in this section of Chapter 4 was undertaken by Dr Ahmed Serag and the meconium analysis was undertaken by Dr Donata Favoretto.

4.2 Background

Globally, in 2015 there were estimated to be 17.7million past-year users of heroin or opium, and increased heroin use is a major driver of the current opioid epidemic (United Nations Office on Drugs and Crime). Pregnant women with OUD due to heroin are recommended MAT with an alternative opioid, usually methadone or buprenorphine, because treatment is associated with improved use of antenatal services, reduced use of heroin during pregnancy and reduced preterm delivery. Fetal benefits of MAT include improved growth and lower risk of intrauterine death(Laslo et al., 2017).

Methadone is a synthetic long acting mu-opioid agonist, which crosses the placenta freely, thereby exposing the developing fetus to exogenous opioid at a critical period of brain development. Pre-clinical studies suggest that prenatal methadone exposure may modify developing dopaminergic, cholinergic and serotonergic systems, and alter myelination. Antenatal exposure to the drug has behavioural consequences including depression, anxiety, and impaired learning, memory and social function(Wong et al., 2014, Robinson et al., 1996, Vestal-Laborde et al., 2014, Chen et al., 2015). In humans, prenatal methadone exposure is associated with increased incidence and severity of NAS (Zelson et al., 1973, Wilson et al., 1981) compared with heroin exposure, and with altered visual maturation in childhood (McGlone et al., 2008, McGlone et al., 2013a, Whitham et al., 2010). These observations raise the possibility that prenatal methadone exposure may modify early brain development; however, the possible role of confounding by postnatal events, including pharmacotherapy with opioid for NAS

and environmental factors, leaves uncertainty about the impact of prenatal methadone exposure on the developing brain.

dMRI is an established technique for studying brain development in early life. It provides objective measures of white matter microstructure that are sensitive to atypical developmental and injurious processes in the perinatal period, and which correlate with neurodevelopmental outcome in childhood (Counsell et al., 2014). Specifically, FA is a voxel-wise measure of the directional dependence of water molecule diffusion in tissue which is influenced by fiber density, axonal diameter and myelination, thereby enabling inference about underlying tissue microstructure. TBSS enables unbiased group-wise analysis of FA volumes derived from dMRI data (Smith et al., 2006, Ball et al., 2010). It has been applied to neonatal dMRI to map microstructural change in white matter tracts of preterm infants at term equivalent age (Anjari et al., 2007), to identify clinical risk factors for altered brain development (Ball et al., 2010, Boardman et al., 2014, Anblagan et al., 2016), and to investigate neuroprotective treatment strategies in randomized clinical trials (Porter et al., 2010, O'Gorman et al., 2014, Azzopardi et al.)

4.3 Hypothesis

Based on the harmful effects of prenatal methadone exposure on neural systems and abnormal behavioural outcomes in pre-clinical models; and on human studies which suggest a modifying effect of prenatal methadone on postnatal behaviour and development, we hypothesized that white matter development of neonates would be altered in neonates exposed to methadone *in utero*. We used TBSS to examine risks

associated with prenatal methadone exposure, while minimizing the role of confounding by postnatal events and drug exposures.

4.4 Methods and Materials

4.4.1 Participants

The study was conducted according to the principles of the Declaration of Helsinki and ethical approval was obtained from the UK National Ethics Service (South East Scotland Research Ethics Committee 02, 14/SS/1106). Written informed parental consent was obtained for all participants. The study group consisted of infants >37 weeks' postmenstrual age (PMA) whose mothers had been prescribed methadone during pregnancy for the treatment of OUD (cases) and a comparator group of healthy infants born at >37 weeks' PMA whose mothers did not use opioids (controls).

Mothers of cases were identified through a specialist antenatal clinic for pregnant women with substance misuse. All cases were born at the Royal Infirmary of Edinburgh between February 2015 and April 2017 and underwent MRI brain scanning at the Clinical Research Imaging Centre, University of Edinburgh. The controls were selected, based on age matching, from a previously described group of healthy term neonates recruited as part of a study of typical brain development (Blesa et al., 2016) (South East Scotland Research Ethics Committee 02, 13/SS/0143). For cases and controls, exclusion criteria were congenital infection or chromosomal abnormalities, or any implanted medical device.

Clinical and demographic information was extracted from the mother and infant clinical records. Birth weight and head circumference (HC) were described in terms of z-score for week of gestational age, calculated using INTERGROWTH-21st reference standards (Cheikh Ismail et al., 2013). The Scottish Index of Multiple Deprivation (SIMD) was used to characterize deprivation. The SIMD is the official Government tool used to identify areas of deprivation: it divides Scotland into around 6,505 areas each containing around 350 households and assigns an index to each area based on multiple measures of deprivation. The data are ranked from most to least deprived and are presented as deciles.

Details of methadone use, tobacco smoking, alcohol intake, and use of non-prescribed drugs were ascertained from medical records, including prescription charts, biological screening samples when these were performed as part of clinical care, and maternal interview at the time of delivery.

The baby was prepared for MRI scan (VM) and this process involved checking with mother that consent for participating was still valid, ensuring the baby had been fed milk (as the MRI scan was performed as a 'feed and swaddle' scan), a full change of clothes to ensure the baby was free of any metal as most baby clothes contain metallic poppers which are not MRI compatible. Following this, the baby had acoustic protection applied as flexible earplugs and neonatal earmuffs (MiniMuffs, Nat's Medical Inc., CA), an open-topped hat applied to ensure the ear protection was secure but to avoid the head becoming too warm as MRI generates heat energy and were swaddled in a thin cotton swaddle blanket. The baby was then escorted in a pram (with

a full box of emergency drugs and equipment under the pram as a precaution) to the research MRI scanner, which was located approximately a ten minute walk from the maternity building. Often the baby was walked for longer than ten minutes in an endeavour to get them to sleep prior to arriving at the MRI scanner so he / she could be transferred during sleep into the scanner. With mother's permission, a bottle of formula milk or maternal milk was also taken to MRI, along with a pacifier (dummy) if the baby had one to soothe the baby if they were unsettled at any point during the scan. This process took between 60 and 90 minutes for each baby.

4.4.2 MRI acquisition

MRI was performed on a Siemens Magnetom Verio 3T system (Siemens Healthcare GmbH, Erlangen, Germany) using a 12-channel matrix phased array head coil. All infants were scanned axially to acquire: 3D T1-weighted MPRAGE volume (1 mm³ resolution), T2-weighted STIR (0.9 mm³ resolution), T2-weighted FLAIR (1 mm³ resolution), and dMRI (11 T2- and 64 diffusion encoding direction ($b = 750 \text{ s/mm}^2$) single-shot spin-echo echo planar imaging (EPI) volumes with 2 mm isotropic voxels, TE = 106 ms and TR = 7300 ms. Images were reported by a pediatric radiologist with experience in neonatal MRI (AQ).

MRI was performed in the neonatal period during natural sleep, without sedation. A neonatologist (VM) was present for the duration of each MRI scan, and the infant had continuous oxygen saturation and heart rate monitoring.

4.4.2.1 TBSS

DMRI data were preprocessed using FSL tools (FMRIB, Oxford, UK; <http://www.ndcn.ox.ac.uk/divisions/fmrib>). This included brain extraction, and removal of bulk infant motion and eddy current induced artefacts by registering the diffusion-weighted volumes to the first T2-weighted EPI volume for each subject. Using DTIFIT, FA volumes were generated for every subject. Diffusion volumes were assessed visually and were excluded if there was motion corruption.

TBSS analysis was performed using a pipeline optimized for neonatal dMRI data (Ball et al., 2010). An average FA volume and mean FA skeleton (thresholded at $FA > 0.15$) were created from the aligned data. Statistical comparison between groups with and without exposure to methadone during pregnancy was performed with FSL's Randomise using a general linear univariate model, with GA at image acquisition and HC z-score at image acquisition listed as covariates. All FA data were subject to family-wise error correction for multiple comparisons following threshold-free cluster enhancement (TFCE) and are shown at $p < 0.05$ (Smith and Nichols, 2009).

4.4.3 Statistics

Student's t-test or the Mann-Whitney test was used to investigate differences in clinical and demographic variables between infants exposed to methadone ($n = 20$) and those not exposed ($n = 20$) and chi-squared or Fisher's exact test was used to compare proportions. Statistical analysis was performed using SPSS v22.0 (SPSS Inc, Chicago, IL).

4.5 Results

4.5.1 All participants

Between February 2015 and March 2017, 55 women prescribed methadone received obstetric care at the Royal Infirmary of Edinburgh. A total of 25 methadone-exposed infants were recruited and underwent MRI scanning as part of the research study investigating brain development. See Figure 9 for participant flow.

Conventional structural (sMRI) and dMRI data amenable to TBSS analysis were acquired from 40 neonates: 20 cases (10 female), who were exposed to prenatal methadone, and 20 unexposed controls (7 female). Tables 6 and 7 summarize maternal and infant characteristics, respectively.

Figure 9 : Details of participant flow through the study

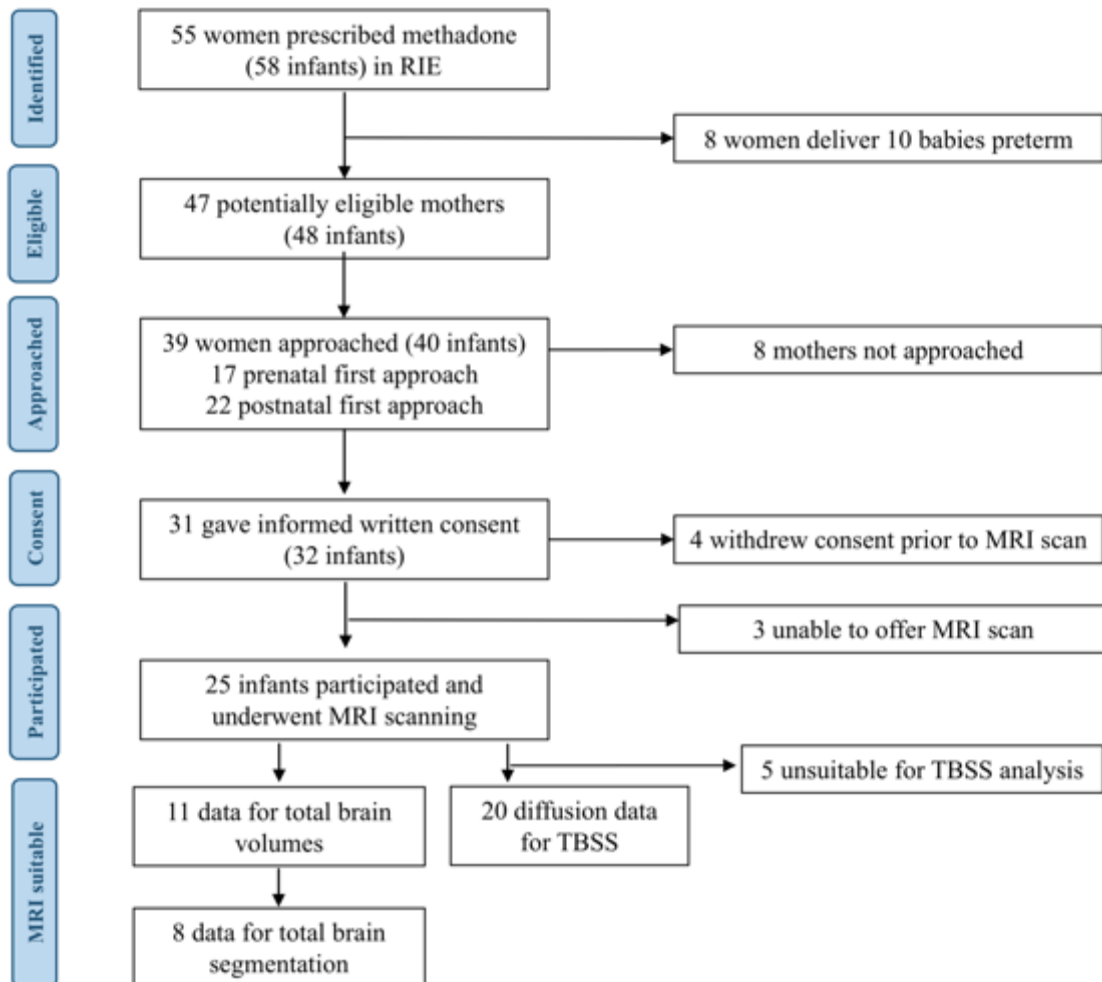
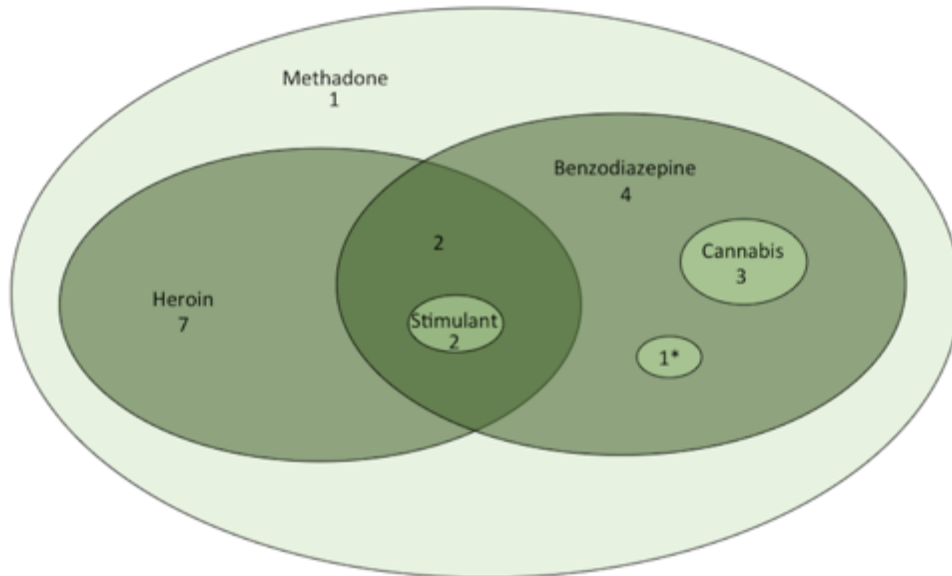


Table 6 :Maternal characteristics of participants with dMRI

	Methadone n = 20	Control n = 20	p value
Mean age (range) / years	30.0 (23-41)	30.9 (19-39)	0.56
Mean BMI (range)	25.8 (21-41)	23.2 (19-39)	0.08
Median SIMD decile (Interquartile range)	3 (2-5)	8 (6-10)	0.012

BMI, body mass index; SIMD, Scottish Index of Multiple Deprivation.

Figure 10 : Euler diagram indicating prenatal drug exposures



Stimulant includes cocaine and amphetamine; *codeine phosphate and tramadol.

The mean methadone dose prescribed at pregnancy booking was 55 mg/day (range 0-160) and the mean dose at delivery was 70 mg/day (range 8-160). Nineteen (95%) of the women prescribed methadone smoked tobacco, one reported drinking excessive alcohol (4 units/day at booking), and nineteen women had illicit or prescribed polydrug use (Fig 10). Additional prescribed medications included paroxetine (n=1), mirtazapine (n=1), gabapentin (n=2), and pregabalin (n=1).

Table 7: Infant characteristics of participants with dMRI

	Metadone n = 20	Control n = 20	p value
Mean PMA at birth (range) / weeks	38 ⁺⁵ (37 ⁺¹ – 41 ⁺⁰)	39 ⁺¹ (37 ⁺² – 41 ⁺³)	0.32
Gender (M:F)	10:10	13:7	0.52
Mean birth weight (range) / g	2721 (2150 – 3440)	3349 (2346 – 4550)	<0.01
Mean birth weight z-score (sd)	-1.062 (0.68)	0.443 (0.86)	<0.01
Median (range) postnatal age at scan / days	3 (1 to 21)	13 (5 to 29)	0.005
Mean PMA at scan (range) / weeks	39 ⁺² (37 ⁺² – 41 ⁺⁴)	41 ⁺¹ (39 ⁺⁰ – 42 ⁺²)	0.004
Mean HC at scan (range) / cm	33.1 (31.2 - 35.0)	35.9 (32.6 – 37.4)	<0.01
Mean HC z-score (sd)	-0.523 (0.986)	1.146 (0.837)	<0.01

PMA, postmenstrual age; HC, head circumference.

None of the cases had neonatal encephalopathy, seizures or hypoglycemia. The mean arterial cord pH of the group was 7.26 (range 7.16 – 7.36). There were three minor congenital anomalies (1 hypospadias, 1 cleft lip, 1 fixed bilateral talipes). One methadone exposed infant required admission to the Neonatal Unit for treatment of transient tachypnoea of the newborn.

No infant had received pharmacological treatment for NAS at the time of image acquisition and none of the control group was exposed prenatally to opioid drugs.

4.5.2 MRI features

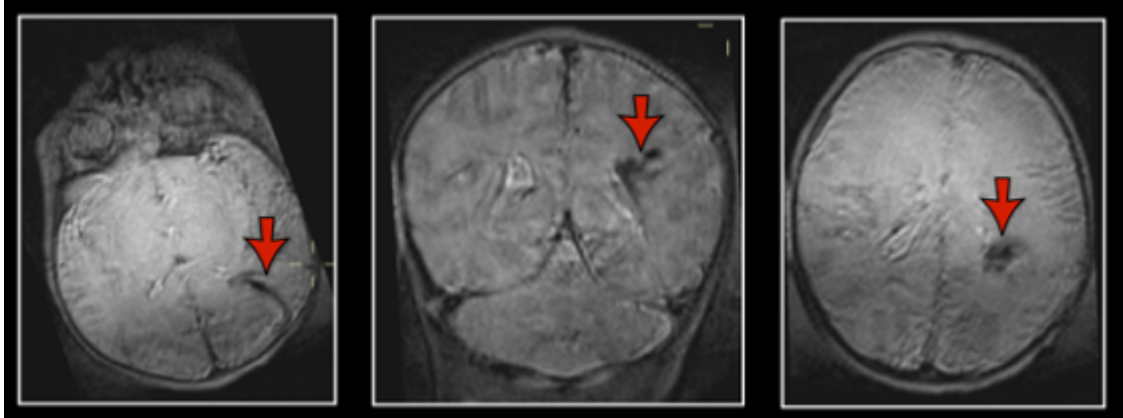
None of the cases or controls had features consistent with injury to white matter or grey matter on conventional structural T1- or T2-weighted structural MRI. Four cases had mild enlargement of the lateral ventricular system; 1 case had asymmetric myelination of the posterior limb of the internal capsule (but had developed symmetric myelination on repeat MRI four weeks later); and no case had abnormalities in brainstem, cerebellum, deep or cortical grey matter, or extracerebral space.

4.5.3. Incidental MRI findings

Two (10%) methadone exposed infants had developmental venous anomalies (DVA): one consisted of an area of low T2-weighted signal in the left peritrigonal white matter (most likely haemosiderin) with a curvilinear vessel extending peripherally and draining into the superior anastomotic vein of Troland, which continues up to the superior sagittal sinus (see Figure 11). The second DVA was characterized by an area

of low T2-weighted signal in the right peritrigonal white matter with a low T2-weighted signal vessel that drains toward the choroid plexus.

Figure 11 : Left developmental venous anomaly with associated cavernoma



T1 weighted images in transverse (image on left and right) and coronal (middle image) planes. Red arrow depicts left sided developmental venous anomaly in the left peritrigonal white matter with a curvilinear vessel extending peripherally (image on left) up to the superior sagittal sinus.

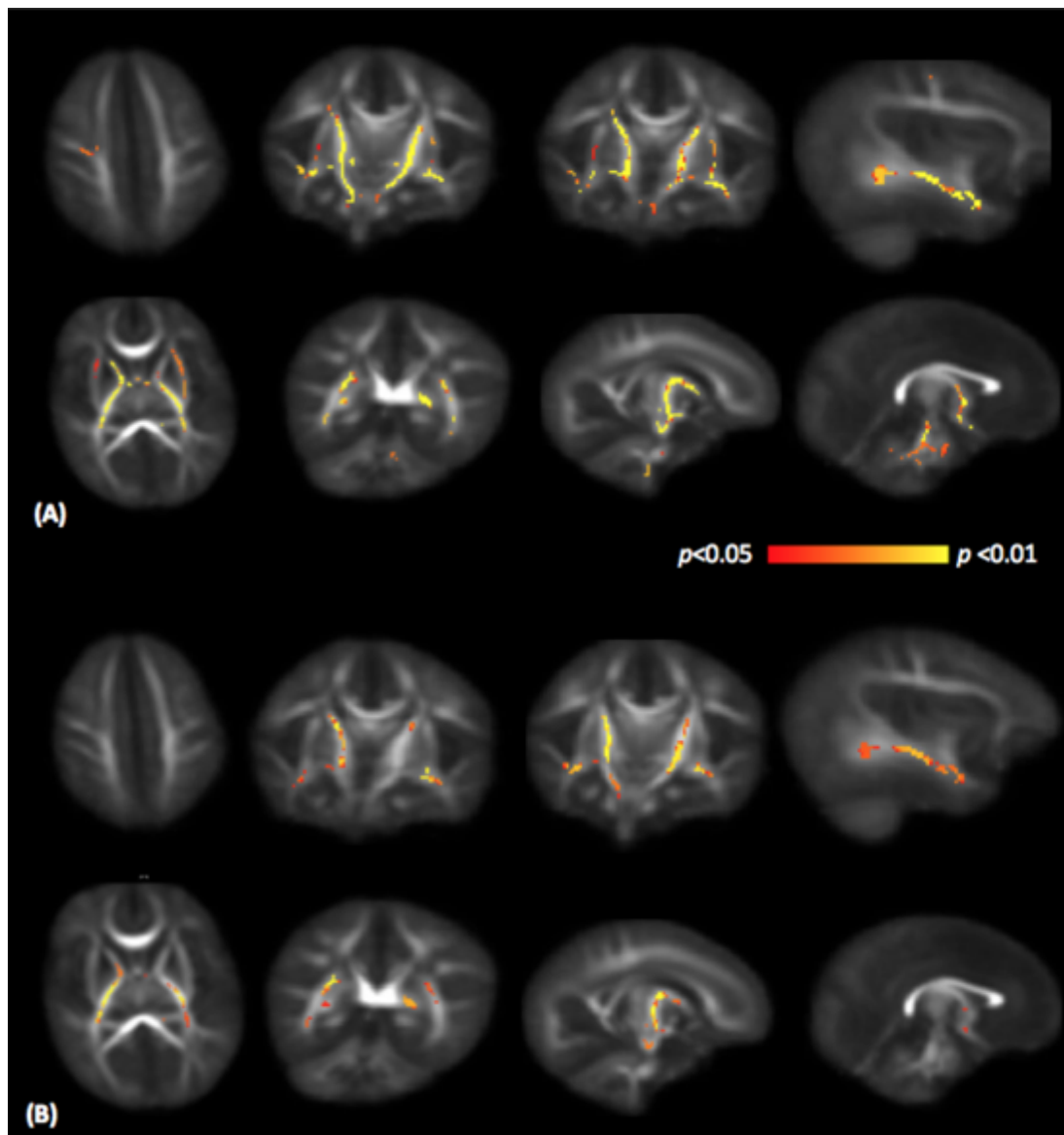
Another infant had asymmetrical myelination of the PLIC on their original research MRI. The left PLIC was starting to myelinate although there was no myelination of the right PLIC. There was normal myelination noted of the anterolateral thalami, brainstem and cerebellum and no cystic white matter change or evidence to suggest underlying volume loss in this case. The baby underwent a repeat MRI 4 weeks later which showed age and gestation appropriate symmetrical myelination in both PLICs. The mother was reassured, and the infant discharged from further follow up.

4.5.4 White matter correlates of prenatal methadone

exposure

Methadone-exposed neonates had decreased FA within the centrum semiovale, ILF, and the internal and external capsules after adjustment for PMA at MRI ($p < 0.05$, TCFE corrected) (Figure 12, panel A). Mean HC z-scores were lower in the methadone exposed group (-0.52 (0.99) vs 1.15 (0.84), $p < 0.001$). After adjustment for HC z-scores, differences in FA remained in the anterior and posterior limbs of the internal capsule and the ILF (Figure 12, panel B).

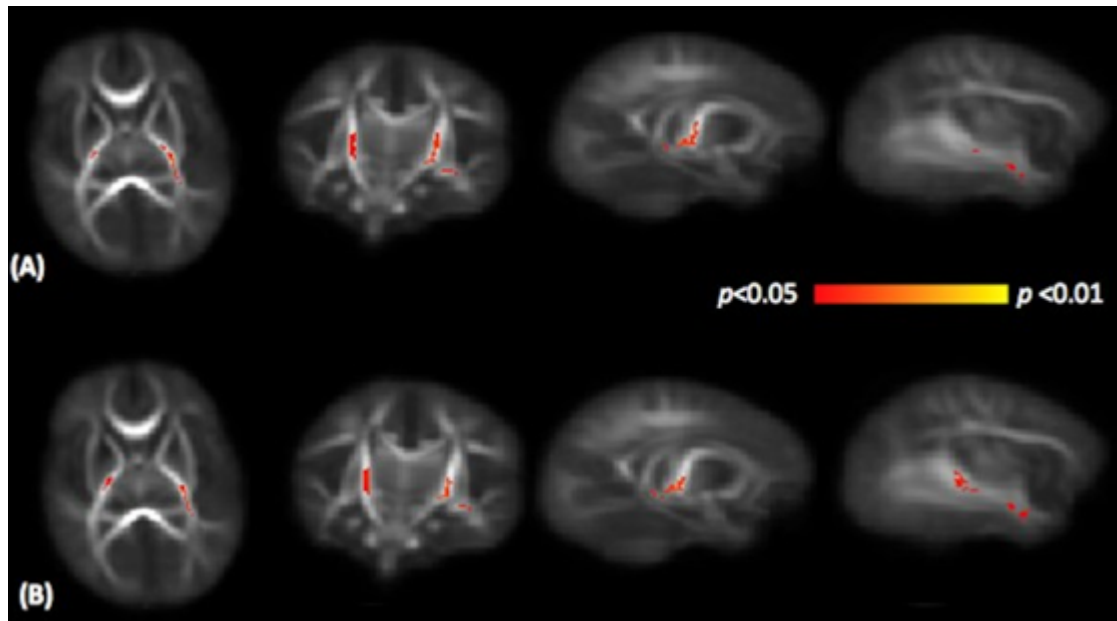
Figure 12 : Mean FA map of the subjects in transverse, coronal and sagittal planes



Panel A adjusted for PMA at scan and Panel B adjusted for PMA at scan and HC z-score. Voxels with significantly lower FA in neonates with prenatal methadone exposure are shown in yellow-red colour scale.

RD was increased in internal capsule and ILF in neonates with prenatal methadone exposure (Figure 13). There were no differences in MD or AD between groups.

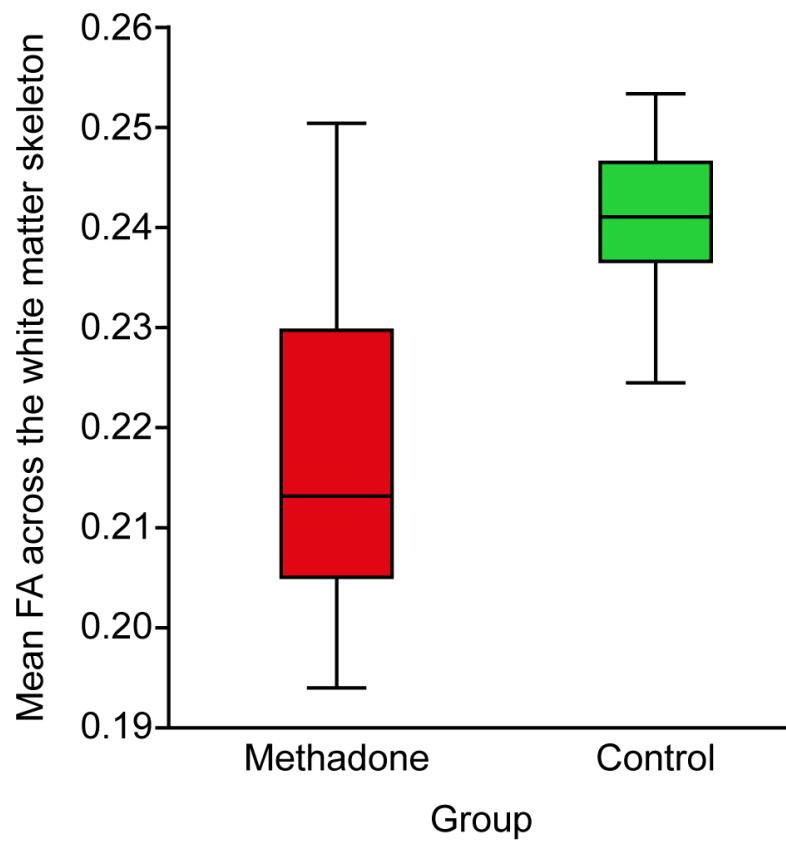
Figure 13 : Mean RD map of the subjects in transverse, coronal and sagittal planes



Panel A adjusted for PMA at scan and panel B adjusted for PMA at scan and HC z-score. Voxels with significantly higher RD in neonates with prenatal methadone exposure are shown in yellow-red colour scale.

The median FA across the white matter skeleton was 12% lower among methadone-exposed infants (Figure 14).

Figure 14 : Mean FA across the white matter skeleton of neonates with prenatal methadone exposure compared with unexposed controls



4.6 Discussion

These data show that prenatal exposure to methadone is associated with altered microstructure in major white matter tracts of the newborn brain, independent of head growth. Children whose mothers take methadone during pregnancy are at increased risk of neurodevelopmental impairment, behavioural difficulties, and visual problems, but study designs have left uncertainty about the role of confounding by prematurity, postnatal opioid exposure for treatment of NAS, and environmental factors, in mediating adverse outcomes (Rosen and Johnson, 1985, Wilson, 1989, van Baar, 1990, Hunt et al., 2008, McGlone and Mactier, 2015, Konijnenberg, 2015, Hans and Jeremy, 2001). An association between prenatal methadone exposure and reduced somatic and head growth is documented (Mactier et al., 2014) but to our knowledge, this is first study to demonstrate brain tissue effects present around the time of birth after methadone exposure *in utero*.

We used TBSS to investigate brain development because of its sensitivity to group-wise differences in FA when used to survey the entire white matter skeleton (Ball et al., 2013). FA is a robust marker of tract microstructure that reflects fibre density, axonal diameter, wrapping by pre-myelinating oligodendrocytes and myelination. Therefore, these data suggest that neonates exposed to methadone *in utero* have less coherently organized and more immature fibre tracts compared to controls. Furthermore, correspondent increases in radial diffusivity without changes in axial diffusivity imply that abnormal myelination may contribute to altered FA among the cases. Since neonatal FA values in major white matter tracts correlate with later

neurodevelopment, the findings may explain the prevalence of neurobehavioral problems seen in children with prenatal methadone exposure.

Quantitative MRI techniques have identified specific vulnerabilities of the developing brain to psychoactive drugs. Functional connectivity of the amygdala–frontal and thalamic networks is altered in neonates with prenatal cocaine exposure (Salzwedel et al., 2015) and in a preliminary study, Walhovd and colleagues reported higher mean diffusivity in the superior longitudinal fasciculus. However, this study was small, with 13 methadone-exposed cases compared to 7 controls, infants were scanned at mean age of 3 weeks after birth and 85% of the cases had been treated with morphine for NAS, which limits inference about the effects of prenatal opioid exposure (Walhovd et al., 2012).

Two (10%) cases had DVA, which was higher than expected based on estimated prevalence of 1.5% in neonates (Brinjikji et al., 2017). These did not occur in the cases exposed to cocaine, which is known to be associated with central nervous system vascular anomalies (Frank 1999); therefore, the possibility that prenatal methadone exposure is associated with CNS vascular malformation warrants further study.

4.7 Study strengths

The strengths of the study are that: dMRI acquisition took place soon after birth before exposure to postnatal opioids or other pharmacological treatment for NAS; preterm birth, which is an important source of confounding for neurodevelopmental outcome was excluded; detailed information about methadone dose and exposure to other drugs

was available; research resources were preserved as use of existing term MRI data negated the need to recruit a new cohort of term un-exposed infants .

4.8 Study limitations

A limitation of the study was the high rate of polydrug use, both illicit and prescribed, among the women prescribed methadone, and therefore evaluating causation was not possible. Polydrug use is consistently observed in other cohorts of methadone using pregnant women (McGlone et al., 2013b, van Baar, 1990, Rosen and Johnson, 1985). In our study population, heroin and oral benzodiazepines were used by 11 (55%) and 12 (60%) of cases respectively, so it is possible that either drug could have confounded the observed association. No other drug class (anti-depressant, anti-epileptic, stimulant) was taken by more than 15% of the cases so it is unlikely that exposure to these classes of drug explained the findings.

Furthermore, all of our cases had been exposed to once daily dosing with methadone, and we cannot exclude potential mediating or interacting factors such timing and dose effects of methadone. However, our data support pre-clinical studies, objective studies of visuo-cortical function in the newborn period and later infancy, and neurodevelopmental and behavioural studies, which strongly suggest an adverse effect of prenatal methadone upon the developing fetal brain and upon long-term childhood outcomes.

Our study was also slightly limited by the use of historical term controls, as it was not possible to obtain detailed history of pregnancy exposures and there was a reliance on

maternal report for such exposures; however, none of the control participants were prescribed opioids during pregnancy, and the likelihood of undisclosed heroin use or non-prescription opioids was low.

4.9 Clinical implications and future research

Prenatal methadone exposure is associated with altered white matter microstructure that is apparent soon after birth. The data focus research attention on determining optimal management of pregnant women with OUD, including a pressing need to evaluate methadone dose regimens and alternative substitutes; future study designs should evaluate fetal or neonatal brain development and long-term neurocognitive outcome.

The research implication of highly prevalent polydrug use in the target population is that future studies into optimal management of OUD in pregnancy are likely to require pragmatic designs that define case definition based on prescribed substitute, with *post hoc* adjustment for other drug exposures if they are shown to differ between groups. It is essential to obtain a clear prenatal exposure profile, and the gold standard should be to obtain meconium samples from all infants enrolled in studies investigating the effects of prenatal drug exposure.

4.10 Meconium analysis

4.10.1 Background

As discussed in Chapter One, substance misuse and alcohol misuse often co-exist, and in view of the well described neurological and developmental consequences of prenatal alcohol exposure, an attempt was made to ascertain prenatal alcohol exposure, to ensure results and interpretation of findings were not confounded by fetal alcohol spectrum disorder (FASD) by obtaining samples of meconium from the methadone-exposed infants shortly after birth once informed consent for study participation had been obtained.

As previously discussed in Chapter One, meconium is a stable matrix and is able to detect both drug and alcohol metabolites indicated prenatal exposure reflecting the midtrimester and last trimester of pregnancy. Free fatty acyl esters (FFAE and Ethyl Glucuronide (EtG) can be measured in meconium and concentrations of FFAE >600nanogram/gram indicate regular alcohol consumption during pregnancy (Chan et al., 2003), with a high sensitivity of 82% - 100% but variable specificity ranging from 13 – 98% (McQuire et al., 2016). Thus a FFAE concentration >600ng/g translates into a positive predictive value of only 55% of excessive prenatal alcohol consumption (Abernethy et al., 2017). Concentrations of EtG >30nanogram/gram have a relatively high sensitivity and specificity for regular alcohol consumption during pregnancy(Himes et al., 2015). Measuring both FFAE and EtG results is more likely to detect true cases of high prenatal alcohol exposure.

4.10.2 Methods

Where feasible, meconium samples from the methadone-exposed infant were obtained once informed consent had been gained. Since FAEEs are unstable at room temperature, samples were anonymized, frozen at -20°C and transported in batches on dry ice to the University of Firenze and Padova in Italy for analysis. A fully validated method was used for the detection of FAEEs and EtG by liquid chromatography-tandem mass spectrometry (Vaiano et al., 2016). As historical controls were used as part of the MRI study it was not possible to have a comparison group of meconium samples.

4.10.3 Results

Meconium samples were successfully obtained and analysed in 12 out of 25 infants who took part in the study, see Table 8. Eleven of the infants participated in the TBSS study and 7 infants also had sMRI data amenable to brain volume analysis. There were 5 cases of raised FFAE, but only one case with FFAE $>600\text{ng/g}$ and EtG $>30\text{ng/g}$, indicating that only 1/12 infants had been exposed to excessive prenatal alcohol exposure.

Table 8 : Results of meconium samples

Cases*	FFAE total ng/g	EtG ng/g	Indicative of excessive alcohol?
1	204	ND	No
2	416	ND	No
3	441	ND	No
4	502	ND	No
5	533	ND	No
6	555	ND	No
7	575	ND	No
8	615	<10	No
9	644	<10	No
10	709	12	No
11	791	21	No
12	904	36	Yes

*cases randomly assigned number

Case 12* first sought antenatal care at approximately 29⁺⁵ weeks gestation, classifying her as a ‘late booker’. She was using heroin and codeine at booking and was commenced on a methadone programme during her pregnancy. Her reported weekly units of alcohol at her booking appointment was zero. Regarding the cases of raised FFAE with EtG below the threshold (cases 8 – 11), one mother was taking heroin and started methadone during pregnancy, two mothers successfully reduced their methadone dose during pregnancy, both of whom were also taking benzodiazepines,

and saliva testing in the remaining mother showed evidence of continued heroin and crack cocaine use during the pregnancy.

4.10.4 Interpretation

Based on interpreting both raised FFAE and EtG concentrations, there was only one case (8%) of prenatal excessive alcohol exposure in a high-risk population. Although fetal rates of alcohol exposure in a non-drug taking population in Canada have been estimated at 2.5%(Gareri et al., 2008), population data from Scotland reports that with almost half infants with prenatal methadone exposure were also exposed to excess alcohol when their meconium was tested (McGlone et al., 2012). Therefore, this number is significantly lower than expected. If the cases with the raised FFAE were included, this figure rises to 5/12 (42%). However, the positive predictive value of a raised FFAE is only 55% (Abernethy et al., 2017) and so when the two biomarkers are interpreted together this results in increased accuracy relating to prenatal alcohol exposure (Bakdash et al., 2010). In addition, data from 235 babies selected at random every 8 days from a large maternity unit in the West of Scotland suggests that approximately 15% of pregnant women are consuming significant amounts of alcohol during the later stages of pregnancy(Abernethy et al., 2017). Further work funded by the Scottish Government is being undertaken to further our understanding of the scale of this problem, but results are not yet published.

Importantly only 12 / 25 infants had meconium samples taken and analysed. It is likely that some or all of the mothers who did not provide a sample represent those who were using multiple drugs and / or alcohol, and so these results may under-represent the true

extent of prenatal alcohol exposure within a population of opioid dependent pregnant women prescribed methadone. The resultant sampling bias limits the interpretation of the meconium results in this study.

4.11 Brain volume analysis

4.11.1 Background

As outlined in Chapter One, Section 1.4.5, HC is a surrogate for total brain volume. Reduced HC is well described in prenatal methadone exposure, even when confounders such as smoking and socioeconomic status are adjusted for (Mactier et al., 2014). Brain volumes also correlate with neurodevelopmental outcomes in childhood, as explored in Chapter One. Two small studies investigating the effects of prenatal opioids on brain volumes have reported reduced regional volumes, particularly in the basal ganglia, but these studies used manual segmentation (Yuan et al., 2014, Walhovd et al., 2007). Another study reported brain volumes in later childhood when other postnatal environmental factors could confound the results (Sirnes et al., 2017). All three studies are limited by incomplete prenatal drug exposure profiling and small sample size.

We aimed to explore whether total brain volumes and regional volumes are reduced in neonates with prenatal methadone exposure.

4.11.2 Methods

A sub-group of the originally recruited methadone-exposed infants with T2 weighted images amenable to volumetric analysis generating whole brain volumes were compared with a group of term born healthy unexposed control infants, matched as closely for GA at scan as feasible, who had been recruited for a previous study (Anblagan et al., 2016, Telford et al., 2017). There was a prioritization of MR sequence acquisition, which meant that not all infants had T2 weighted images that were suitable for volumetric analysis, resulting in a smaller subgroup analysis for the volume analysis. To estimate whole brain volumes, we used a method for brain extraction named ALFA (Accurate Learning with Few Atlases) which proved to provide robust and accurate volume measurements for neonatal brain MR images (Serag et al., 2016). The method uses a sparsity-based atlas selection strategy that requires a very limited number of atlases (here $k=5$) 'uniformly' distributed in the low-dimensional data space, combined with a machine learning based label fusion technique.

A further subgroup of these infants ($n= 8$) had sMRI data amenable for full segmentation. To generate tissue segmentations, we used SEGMA (Serag et al., 2017) which is an automatic SEGmentation Approach for human brain MRI based on machine learning. SEGMA was proved to provide accurate segmentations across the life course (including the neonatal period) and was used to classify brain MRI scan into cortical grey matter, deep grey matter, white matter cerebellum, brainstem and CSF.

4.11.3 Results

Ten methadone-exposed infants and eleven un-exposed infants had sMRI data amenable to calculating whole brain volumes, see Tables 9 and 10 for maternal and infant characteristics respectively.

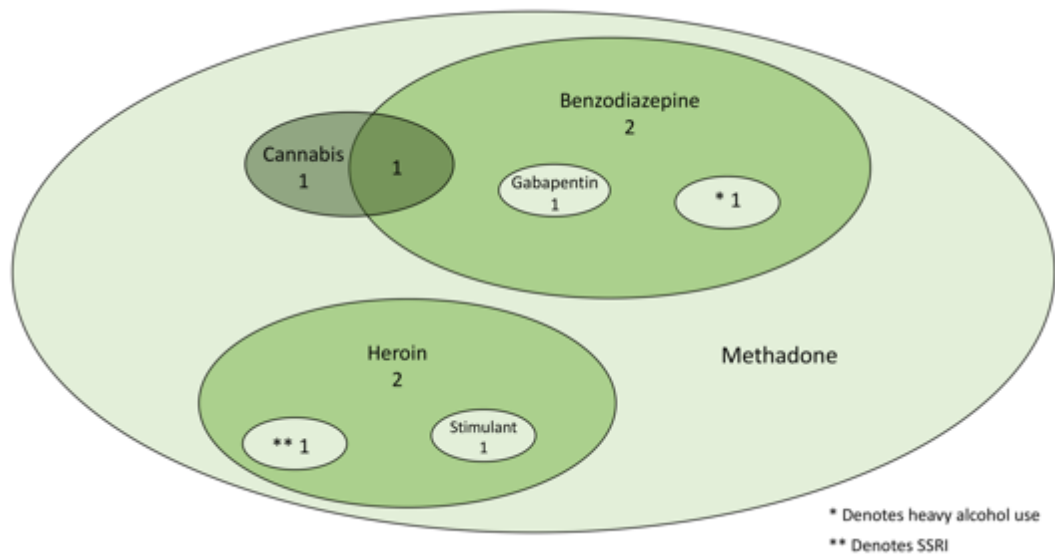
4.11.3.1 Whole brain volume

Table 9 : Maternal characteristics of participants with sMRI data used for whole brain volumes

	Methadone n = 10	Control n = 11	p-value
Mean age (range) / years	30.0 (22 – 35)	32.4 (27 -37)	0.27
Mean BMI (range)	25 (20 – 33.3)*	23 (18.5 – 27.6)	0.34
Median SIMD decile (Interquartile range)	4.5 (3 – 6)	8.0 (7 – 9)	0.007**

*n=9. BMI not done in one case.

Figure 15 : Euler diagram illustrating the drug exposure profiles for the 10 methadone-exposed participants with sMRI data used for whole brain volumes



The mean daily methadone dose at booking was 61.5mg and this increased slightly to a mean dose at delivery of 63.2mg. All mothers were polydrug users, and one mother reported heavy alcohol use during parts of her pregnancy, see Figure 15. All methadone-maintained mothers were smokers.

Table 10 : Infant characteristics of participants with sMRI data used for whole brain volumes

	Methadone n = 10	Control n = 11	p value
Mean PMA at birth (range) / weeks	39 ⁺⁴ (37 ⁺⁵ – 40 ⁺⁶)	39 ⁺¹ (37 ⁺² – 40 ⁺⁵)	0.289
Gender (M:F)	5 : 5	9 : 1	0.182
Mean birth weight (range) / g	3001 (2370 – 4140)	3369 (3020– 4550)	0.07
Mean BW z-score (sd)	-0.72 (0.94)	0.24 (1.09)	0.02
Median (range) postnatal age at scan / days	4 (1 to 21)	16 (5 to 49)	0.001
Mean PMA at scan (range) / weeks	40 ⁺² (38 ⁺⁵ – 41 ⁺⁴)	41 ⁺⁵ (39 ⁺⁶ – 47 ⁺¹)	0.026
Mean HC at scan (range) / cm	33.86 (31.3 - 35.0)	36.50 (34.8 – 39.5)	0.0002
Mean HC z-score (sd)	-0.23 (0.96)	1.40 (0.58)	0.0013
Mean intracranial volume (sd) / ml	408 (36)	511 (52)	<0.01

Methadone-exposed infants have significantly lower intracranial volumes compared with unexposed infants, 408ml (sd 36ml) vs 511ml (sd 52ml), $p < 0.001$, see Figure 16. Although GA at birth was similar, the mean gestational age at MRI scan differed by 10 days, mediated by a significant difference in age at MRI scan ($p = 0.001$).

Figure 16 : Box plot showing median intracranial volumes in methadone-exposed (green) and unexposed controls (yellow)

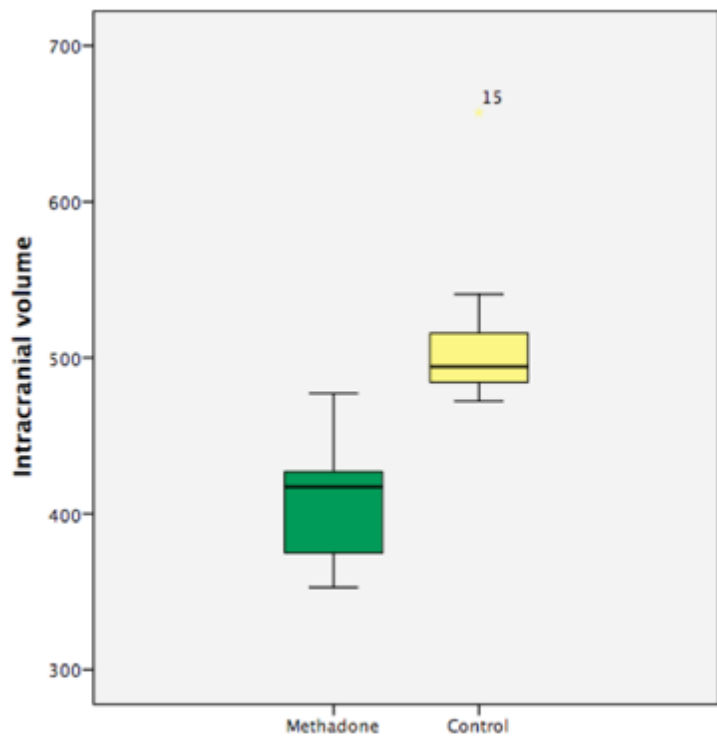
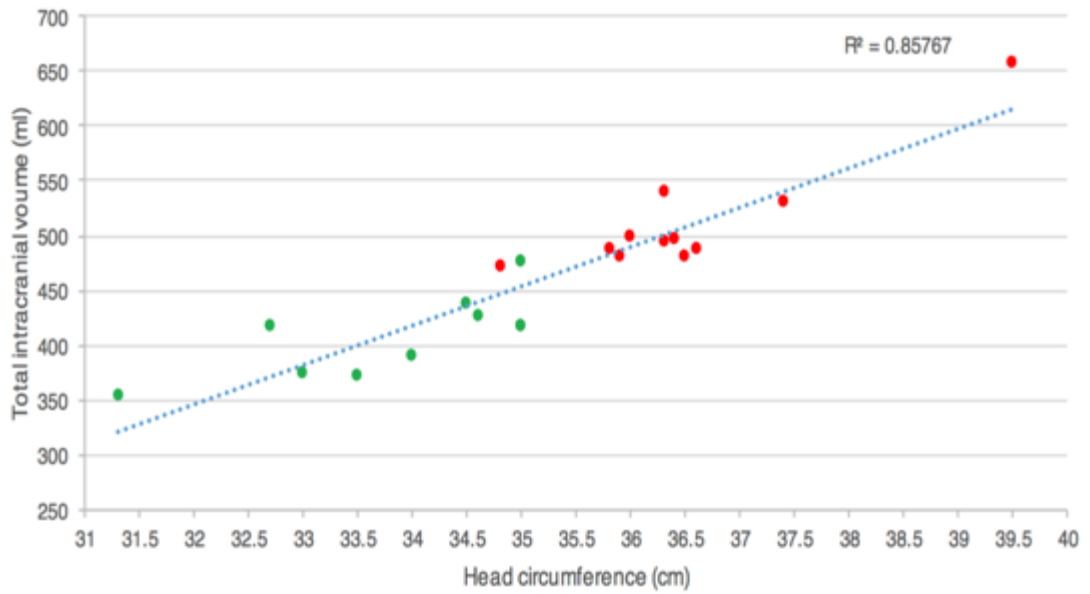


Figure 17 : Correlation between head circumference and total intracranial volume



Green dots indicate prenatal methadone exposed infants, red dots indicate unexposed control infants

There is a positive correlation between HC on the day on MRI acquisition and total intracranial volume, ($R^2 = 0.86$), see Figure 17. These measurements do not account for gestational age at birth or at imaging as they are unadjusted. The outlier is a baby who was imaged at 47⁺¹ weeks corrected gestation.

4.11.3.2 Intracranial segmentation volume analysis

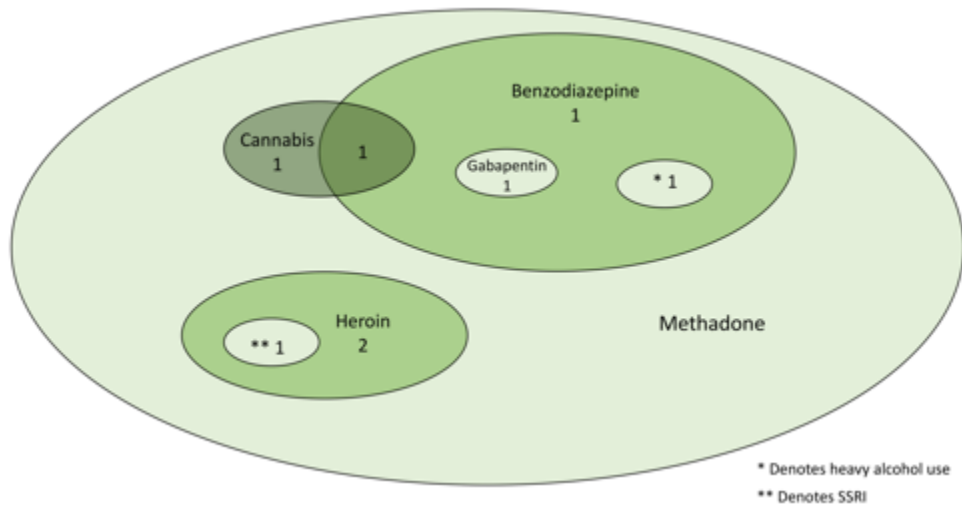
Of the 10 methadone-exposed, 8 were also suitable for segmentation analysis, and of the 11 control infants, 7 were amenable to segmentation, see Tables 11 and 12 for maternal and infant characteristics of the group amenable to full brain segmentation.

Maternal age and BMI were similar, but methadone group were from more deprived parts of the city than the control mothers ($p = 0.048$).

Table 11 : Maternal characteristics of participants with sMRI data amenable to full segmentation

	Methadone n = 8	Control n = 7	p-value
Mean age (range) / years	30.7 (25 – 35)	32.0 (28 – 36)	0.522
Mean BMI (range)	24.8 (20 – 33.3)	21.8 (18.5 – 26)	0.183
Median SIMD decile (Interquartile range)	4 (2 – 6)	7 (4 – 10)	0.048*

Figure 18 : Euler diagram illustrating the drug exposure profiles for the subgroup with sMRI data used for brain segmentation



All mothers maintained on methadone were polydrug users. 7/8 were cigarette smokers. See Figure 18 for drug exposure profile of included infants.

Table 12 : Infant characteristics of those with sMRI data amenable to full segmentation

	Methadone n = 8	Control n = 7	P value
Mean PMA at birth (range) / weeks	39 ⁺⁴ (37 ⁺⁵ – 40 ⁺⁶)	38 ⁺⁶ (37 ⁺⁶ – 40 ⁺⁵)	0.516
Gender (M : F)	5 : 3	0 : 7	0.026
Mean birth weight (range) / g	2814 (2370 – 3252)	3254 (3070 – 3800)	0.017*
Mean BW z-score (sd)	-1.06 (0.53)	0.06 (0.70)	0.006**
Median (range) postnatal age at scan / days	4 (1 to 21)	18 (13 to 49)	0.007**
Mean PMA at scan (range) / weeks	40 ⁺¹ (38 ⁺⁶ – 41 ⁺⁴)	41 ⁺⁵ (40 ⁺⁴ – 47 ⁺¹)	0.023*
Mean HC at scan (range) / cm	33.75 (31.3 - 35.0)	36.85 (35.9 – 39.5)	0.001**
Mean HC z-score (sd)	-0.21 (1.09)	1.38* (0.44)	0.008**

*n=6, not possible to calculate HC z-score from one patient with PMA 47⁺¹

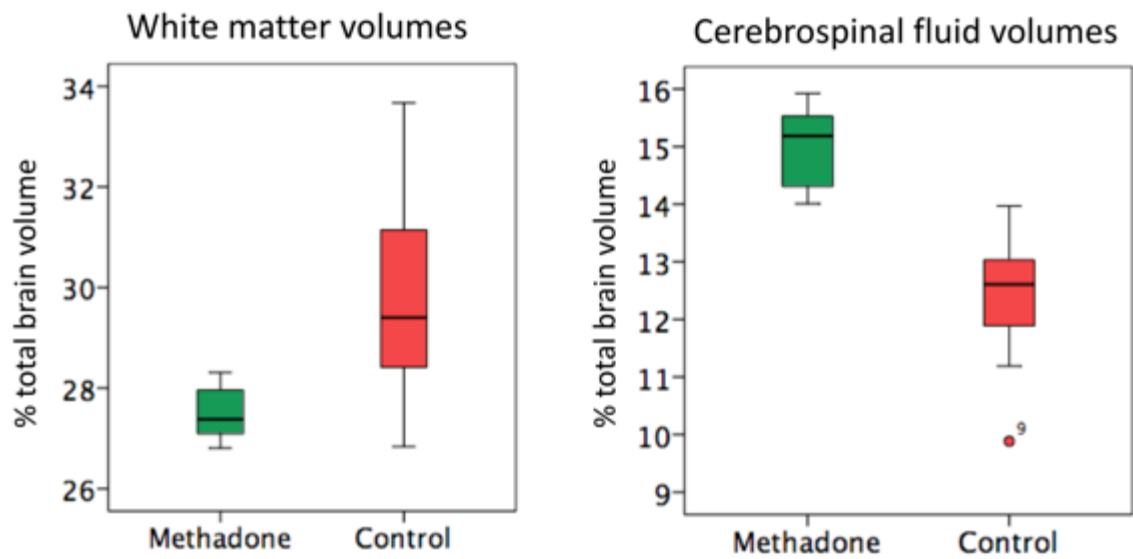
Methadone-exposed infants were smaller, despite being older at birth, see Table 13. There was a significant difference in their HC, with methadone exposed having smaller HC (p = 0.001) and lower HC z-scores (p=0.008). The significant difference in HC and whole brain volume was accounted for by normalising the segmented brain volumes to the total intracranial volume, and the relative brain proportions of each group for the 6 segmented areas are presented in table 13.

Table 13 : Table showing relative brain proportions in the 15 infants with full intracranial segmentation

	Relative brain proportions (%)		p-value
	Methadone-exposed n = 8	Control n = 7	
White matter	27.5	29.9	0.049
Deep gray matter	5.9	6.1	0.24
Cortical gray matter	37.9	39.2	0.13
Cerebellum	5.1	5.6	0.13
Brainstem	1.2	1.2	0.81
Cerebrospinal fluid	15.0	12.3	0.001

The relative volume of white matter was significantly lower in methadone-exposed infants than in unexposed control infants ($p = 0.049$). There was a concurrent increase in total cerebrospinal fluid in methadone exposed infants, which also reached statistical significance ($p = 0.001$). These results are illustrated in Figure 19.

Figure 19 : Box plot showing the relative volume of cerebral white matter and CSF in participants with full intracranial segmentation in methadone-exposed (green) and un-exposed control infants (red)



4.11.4 Interpretation

These data showed reduced total brain volumes and a positive correlation between HC and intracranial volume in methadone-exposed neonates, consistent with previous research. In a smaller subgroup, segmentation analysis demonstrated reduced white matter volumes with presumed compensatory increased CSF volumes within the ventricles, normalized for HC. Although this differs from the limited published work describing lower basal ganglia volumes in association with prenatal opioid use, the potential biological mechanism relating to oligodendrocyte precursor vulnerability to exogenous opioids provides a plausible explanation for the observed results in this subgroup. It is also consistent with the novel finding described earlier in this Chapter that white matter microstructure is less coherently organised shortly after birth in prenatal methadone exposure before postnatal events confound interpretation.

Diffuse white matter injury, resulting in reduced white matter volume is part of the observed preterm phenotype, and is associated with altered neurodevelopmental outcome (Boardman et al., 2010). Reduced white matter volume has also been shown to be predictive of delayed language in a cohort of infants with congenital heart disease (Rollins et al., 2017). Increased ventricular volumes are associated with worse neurodevelopmental outcome at 2 years, 3.5 years, and reduced processing speed at 5.5 years in preterm infants (Keunen et al., 2016).

A strength of the data is the use of contemporary automated validated technology to calculate the brain volumes and comparing to a group of healthy un-exposed neonates. Due to the prioritization of acquiring diffusion MR data many infants did not have T2

weighted images acquired or that were suitable for volumetric analysis, resulting in small numbers of participants for the volume analysis. Small sample size has been a challenge for other studies reporting brain volumes in the context of prenatal drug exposure, as has polydrug use. Benzodiazepine use was common in the mothers in this study, and midazolam exposure is known to result in alterations in hippocampal growth in preterm infants (Duerden et al., 2016), so it is possible that some of the differences demonstrated in this small group reflect other prenatal exposures, such as benzodiazepines, alcohol or other unknown substances. Although these findings are hypothesis generating and are pilot data, they warrant further investigation, as they are contrary to previously published volume studies, with biological mechanistic plausibility.

4.12 Conclusion

In this chapter of the thesis, age-optimized TBSS has been used to examine risks to the developing brain associated with prenatal methadone exposure, while minimizing the potential role of confounding by postnatal socioeconomic disadvantage, drugs used to treat NAS, and preterm birth. The association between prenatal methadone exposure and abnormal white matter development is described in term infants, compared with non-exposed controls, apparent at the time of birth. Pilot data from the volumetric analysis of a subgroup of participants showed reduced white matter with associated increased ventricular volume. Further research is still required to optimise management of OUD in pregnancy for the mother-infant dyad. The implications of this work are further explored in the next chapter of the thesis.

CHAPTER 5: DISCUSSION

This thesis has systematically reviewed the published literature and meta-analysed the results and investigated the possible effects of prenatal methadone exposure on early brain development using neonatal MRI. The key findings are summarised below and will be further explored in this chapter.

5.1 Summary of key study findings

- Systematic review of long term neurodevelopment, vision and neuroimaging in 1467 children with prenatal methadone exposure and 864 unexposed children shows that prenatal methadone exposed children are at increased risk of abnormal development (Chapter 3)
- Meta-analysis reveals lower scores for both motor and cognitive outcomes at developmental testing age 6 months and 2 years in children with prenatal methadone exposure compared to non-exposed control children (Chapter 3)
- Childhood behavioural problems are more likely in children with prenatal methadone-exposure (Chapter 3)
- Visual development and function is adversely affected in children with prenatal methadone exposure, with strabismus and nystagmus being considerably higher than expected in children with prenatal methadone exposure (Chapter 3)
- Prenatal methadone exposure is associated with decreased FA within the centrum semiovale, ILF, and the internal and external capsules after adjustment for age at MRI (Chapter 4)

- Mean HC z-scores were lower in the methadone exposed group, but after adjustment for this, differences in FA persisted in the anterior and posterior limbs of the internal capsule and the ILF (Chapter 4)
- Overall FA is 12% lower in prenatal methadone exposure than in un-exposed control infants (Chapter 4)
- Head circumference positively correlates with overall brain volume, and so methadone-exposed infants have both smaller head circumferences and lower total brain volumes than non-exposed infants at similar gestations (Chapter 4)
- When examining the relative brain proportions in a smaller subgroup, there is an increase in CSF, explained by a concurrent decrease in white matter volume in methadone-exposed infants (Chapter 4)
- It was not possible to assess causation due to multiple confounders including polydrug use (Chapter 3, 4)
- There was a higher than expected rate of incidental MRI findings of DVA, and the significance of this is unknown (Chapter 4)
- There was a higher than expected rate of congenital anomalies, consistent with previous literature but in a small sample size the significance remains uncertain (Chapter 4)

These data support pre-clinical studies, objective studies of visuo-cortical function in the newborn period and later infancy, and neurodevelopmental and behavioural studies, which strongly suggest an adverse effect of prenatal methadone upon the developing brain and upon long-term childhood outcomes, and importantly it shows

that prenatal methadone exposure is associated with atypical brain development which is apparent at birth.

5.2 Challenges of research in OUD

The challenges of undertaking research in OUD have been briefly discussed in Chapter One but will be further expanded upon in this chapter where relevant, based upon the experience from this study.

5.2.1 Polydrug use

The high rates of polydrug use observed during this study are consistent with the published literature(Lind et al., 2017).

Higher rates of mental health disorders, particularly anxiety disorders treated with benzodiazepines, may in part account for the over-representation of benzodiazepine use in this population. There are adverse effects associated with postnatal midazolam exposure in preterm infants, including reduced hippocampal growth, which correlates to later adverse neurodevelopmental outcome(Duerden et al., 2016), and this may influence results from developmental outcome studies with high rates of polydrug use. The effects of other psychoactive substances on brain development have been discussed in Chapter Four.

There were also high rates of cannabis use amongst pregnant women in this study. A systematic review of the MRI effects of cannabis use in adolescents reports abnormal

white matter microstructure in several major white matter tracts, and a dose-dependent relationship to these changes (Baker et al., 2013). Although this review included studies assessing alcohol as well as cannabis use, it again highlights the possibility that some of the observed changes of reduced FA and increased RD might be partly attributable to cannabis rather than methadone *per se*, a conclusion already drawn from this work.

Pregnant women who only take opioids appear to be in a minority (McGlone et al., 2013b) and this makes undertaking studies in a population of infants exposed to only one opioid extremely difficult. Although such studies would help to avoid the confounders of polydrug use and co-existent mental health disorders which currently limit the literature in this area, their usefulness on a pragmatic basis is questionable. Since polydrug use is an almost inevitable part of OUD, future studies in this field should have a pragmatic design to address this ongoing issue.

5.2.2 Concomitant alcohol use

Prenatal alcohol exposure results in a well described constellation of physical and behavioural problems, termed fetal alcohol spectrum disorder (FASD). Alcohol use is under-estimated in both pregnancy (Abernethy et al., 2017) and in some populations of opioid dependent women prescribed methadone (McGlone et al., 2012), and therefore is a potential confounder.

Self-reported alcohol use within this study was low, with only one mother reporting alcohol use at booking. Of 12 samples obtained and analysed in the study, only one

sample (not the mother who admitted to alcohol use at booking) had both FFAE and EtG above the thresholds, indicating significant prenatal alcohol exposure. The details of this case have been discussed in Chapter Four (section 4.11.4).

The low number of meconium samples obtained and amenable to analysis was disappointing. Possible reasons were multiple; Firstly, I could not obtain meconium until the mothers had signed consent. The majority of mothers were approached postnatally, and in order to allow enough time for them to consider participation, the consent process was at least one day after the initial approach, by which time most of the infants had already passed meconium. Secondly, I was reliant on the mothers to alert myself or midwifery staff once the baby had passed meconium to allow me to obtain and store the sample, and therefore those that did not want to provide a sample were able to discard the meconium. This is well described in a previous study relating to prenatal alcohol exposure (Abernethy et al., 2017).

Finally, there was a considerable degree of apprehension from the mothers about the meconium sample and what information might be yielded from it and as a result less than half of the mothers (12/25) provided a meconium sample. It is interesting that of the 39 women initially approached, 31 gave informed consent for MRI scan (79%) although some were not eligible due to prematurity and some logistically could not be offered scans. This is a much higher uptake for MRI scans than the uptake for meconium sampling (79% vs 48%) and implies that MRI is an acceptable research modality for women with OUD.

Furthermore, despite reassurance that the samples were all anonymised, and the information was to quality assure the study to ensure that correct conclusions were drawn about methadone effects, many mothers remained sceptical and ultimately many did not provide samples. The majority of the mothers involved in the study had social services input as part of a comprehensive package of care, and in cases where the decision regarding discharge home or to foster care had not been finalised, the meconium sample seemed to place additional pressure on the mothers. It is possible that the mothers who did not provide a sample are more likely to be those who were polydrug using or using alcohol, and so these results may reflect an under-representation of the true extent of prenatal alcohol exposure. This resultant sampling bias limits the interpretation of the meconium results in this study.

5.2.3 Inherent population challenges

I intended to first meet with potential participants when they attended antenatal appointments at the Royal Infirmary of Edinburgh Maternity department, before their baby was born to discuss the study and leave them with an information leaflet. It was standard practice for all women prescribed methadone to be regularly reviewed in a high risk obstetric clinic, by a consultant obstetrician with a specialist interest in maternal substance misuse. I would then aim to see them again as soon after delivery of their baby as was feasible to discuss study participation again, with the opportunity for questions. However, as mothers frequently did not attend their antenatal appointments, antenatal discussions about research participation were only possible in 17 cases. The remaining 22 cases were approached postnatally.

Full informed written consent was obtained prior to being enrolled. This involved at least two detailed conversations about what study enrolment meant for them and their baby. Particular details covered were (1) what the MRI scan would involve: feed and swaddle, no medication (2) timeline: up to an hour scan time (3) safety issues: mothers still hospital inpatients, therefore not able to go in the MRI scanner for safety reasons (4) possibility of incidental findings (5) meconium sample (6) voluntary nature of involvement, with emphasis on this point.

Gaining the trust of the study participants was a challenge, requiring significantly more time than initially anticipated. One potential explanation for this is that rates of abuse, in particular early sexual abuse, amongst women with OUD, range between 30 and 50% among women with OUD (Hans, 1999), and this may explain the apparent distrust of people in authority, or those with perceived 'power'.

There were four cases where maternal written consent was obtained, and this was withdrawn prior to the MRI scan. The most common reason for withdrawal was that the partner was not happy with research participation, even though the mothers were interested and had given consent. This illustrated the vulnerability of the women prescribed methadone; all the mothers who gave consent did so voluntarily. Although there is the possibility that they felt unable to say no to participating, they always had at least an overnight period to consider participating. Additionally, the overwhelming reaction of the mothers towards the study was positive, and many were grateful that a research study was interested in the outcomes of their children. Many also voiced concerns about previous children they had whilst also on methadone and were

extremely willing to participate. I was very careful to reiterate that participation was entirely voluntary, and care would be in no way compromised as I was not part of the primary care-giving team. Therefore, it seems more likely that their withdrawal of consent represented, at least in part, a dominance of their partner.

A further challenge of performing research relating to prenatal drug exposure lay with the inherent socioeconomic risk factors such as mental health problems, domestic violence, poor physical health, low education, poverty, unemployment, crime and social isolation (Konijnenberg, 2015), all of which can also affect child development. These have been explored extensively in Chapter One, as have studies which have tried to disentangle the ‘nature vs nurture’ aspect.

To the best of my knowledge this is the first study to demonstrate differences in the structure of the brain white matter within days of birth in infants prenatally exposed to methadone before postnatal factors can confound the results. This means that the later outcomes of children with prenatal methadone exposure are not solely attributable to postnatal events.

The significance of adequate levels of environmental stimulation for the maturation of central nervous system has been identified in a rat model, where exposure to an impoverished environment results in delayed maturation of the central nervous system, demonstrated by reduced amplitude VEP and delayed myelination (Narducci et al., 2018). There is significant alteration of developmental trajectories under such

environmental conditions, and should the same be true in humans, any study which imaged children later in childhood would be potentially confounded by this.

5.3 MRI to investigate potential effects of prenatal drug exposure

Most of the existing literature using TBSS in neonates has been either in preterm infants or in term asphyxiated infants, and its use to investigate effects of prenatal drug exposure is relatively new.

5.3.1 The advantages

All of the aforementioned benefits of MRI are applicable to the infant prenatally exposed to drugs. MRI is safe, non-invasive, there is no ionising radiation, no need for sedation, and it allows detailed exploration of underlying brain microstructure. Water diffusion metrics such as FA map to later developmental outcomes, and other metrics such as RD give further information as to possible underlying pathophysiology underlying the findings.

A particular advantage was the ability to perform an MRI very shortly after birth. It is common practice within the UK and throughout Europe and the US for infants with prenatal drug exposure to have an obligatory hospital stay after birth for a pre-defined period, usually 3-7 days. This is primarily to observe and score for NAS, and if appropriate, to commence pharmacological treatment. Thus, infants were already in

hospital and this facilitated the early acquisition of MRI data, before postnatal events could impact brain development and potentially confound results.

Another major advantage was the small sample size of only 20 per group required to detect a difference between groups. Ball and colleagues have shown that TBSS is sensitive to detect change in groups of this size (Ball et al., 2013). They also noted that TBSS sensitivity is most stable in larger treatment groups or when a larger treatment effect is simulated. The effect observed within my study population was a 12% difference in FA, demonstrated amongst 20 methadone exposed and 20 unexposed infants, whilst the effect size Ball et al used to demonstrate a difference was 5% increase in FA. Smaller sample sizes have also been used; Porter and colleagues demonstrated significant and widespread changes in FA between asphyxiated and non-asphyxiated term infants with only 10 infants per group (Porter et al., 2010), so the sample sized used, although seems small, is large enough using TBSS to detect significant differences using TBSS.

Finally, the use of a standardised MRI protocol might enable the investigation of potential effects of prenatal drug exposures to be tested and potentially allow comparison of different drug effects.

5.3.2 The challenges

There were several challenges encountered during the organisation and running of the study, related to the MRI scan. Many mothers were surprised that the MRI scan would take so long, and some were put off by the length of time they would be away from

their baby for. For the mothers whose babies were going straight from hospital into foster care, their reluctance to participate was understandable, as their time with their baby was limited. Of the 25 methadone-exposed infants who participated, 11 went into foster care.

Motion artefact was a problem, particularly with T2 weighted images. Of the 25 infants scanned, 20 had dMRI data amenable to analysis. Of note, a considerable amount of time was spent preparing the infants and optimising their comfort to try to obtain adequate conventional and diffusion imaging data. At times this involved long walks with the infant in the pram prior to the MRI scan, bottle feeding the infant prior to, or on occasion removing the infant from the MRI scanner and offering a further feed and then returning them into the scanner. All infants were swaddled to try to reduce their jitteriness, as they were all in varying stages of drug withdrawal. This required a significant amount of time and effort and flexibility from the whole research MRI team, including the radiographers. For a neonatal diffusion MRI study, a 20% rate of motion degraded images can be considered quite low when compared to other reported rates(O'Gorman et al., 2014) and is testament to the efforts made to try to obtain high quality imaging data.

The occurrence of movement artefact was also likely influenced by the timing of the MRI scan relative to the infant's clinical condition. The median day of scan was day 2, with day 0 representing the day of birth. Thus, infants were usually between 24 and 48 hours old at MRI, which correlated with the time that NAS starts to manifest. Although none of the babies were undergoing treatment for NAS at the time of MRI

scanning, this is not the same as reporting that none of the infants were starting to show features of NAS. One of the cardinal features of NAS is tremors and jitters. Whilst the swaddling contained some of their tremors, these fine tremors were still occurring in many of the babies, and this accounted for the distortion of images found in many subjects. dMRI is slightly less affected by movement artefact compared with conventional T1-weighted and T2-weighted images, which may be another reason why there were more subjects with diffusion than structural T2-weighted data. The movement degradation in one case was so bad that it was not possible even to provide a clinical interpretation of the conventional images and in this case the diffusion data was not usable either.

Unfortunately, in spite of these measures, only 10 out of 25 subjects (40%) had T2 weighted images that were amenable to calculating intracranial volumes, and only 8 out of 25 (32%) had images where the intracranial segmentation could be performed. This likely reflects the sequence acquisition prioritisation, with the diffusion sequences being performed first, and repeated immediately if there appeared to be motion artefact, and T2-weighted sequences being performed later in the acquisition sequence.

There were three cases of incidental findings (IF) out of 25 patients scanned, representing 12% of the study population; two cases had DVA and one case had asymmetrical myelination of the PLIC. IF are defined as the finding of a brain lesion

on neuroimaging that clinicians would not have predicted, and as such are asymptomatic. The significance of most IF are unknown, and they can generate significant anxiety for parents(Gupta et al., 2016). IF were specifically discussed during the consenting process for the MRI study, and most mothers were accepting of the risk.

The reported incidence of IF is variable, with little neonatal data. A meta-analysis in adults reports a prevalence of 0.7% (95% confidence intervals of 0.47% - 0.98%), with an increasing prevalence with increasing age(Morris et al., 2009). In a large paediatric population, there were IF in 25% children undergoing neuroimaging(Jansen et al., 2017), however, this has selected out a population not entirely representative of normal healthy children and therefore could be an overestimate. Another paediatric study reported a 12.5% of children taking part in a research study had an IF discovered on neuroimaging (Kaiser et al., 2015). A study in very low birthweight infants estimated the incidence to be around 10% (Malova et al., 2017). The true incidence of IF in otherwise well babies is unknown and given the consistent reports of increasing prevalence with age, it is likely to be lower than the 10% or 25% quoted here.

All three IF were managed as per the research protocol: parents were informed initially by telephone, repeat MRI scan was organised and referral was made to the appropriate healthcare team after the repeat MRI scan. For the two patients with DVA, the repeat MRI scans were discussed at a neuroradiology multidisciplinary meeting, and both are under follow up with a paediatric neurologist but being expectantly managed. The case with asymmetric myelination of the PLIC underwent a repeat MRI 4 weeks later, and

the asymmetry had resolved and the PLIC was fully myelinated. Mother was informed by telephone and there was no need for further follow up of the child.

The importance of having the research MRI scans reported by a qualified radiologist was highlighted through these findings of IF. A considerable amount of time was spent by a consultant paediatric neuro-radiologist reporting the MRI scans, and for the 3 cases of incidental findings, arranging repeat scans, and discussing the cases at relevant neuro-radiology multidisciplinary meetings to decide on appropriate follow up.

An interesting observation was that many of the mothers felt a significant amount of guilt about the potential impact of their substance misuse on the health and wellbeing of their child, and this has been reported previously (Williams, 1985). It was important not to exacerbate this pre-existing guilt. Many mothers worried that the MRI scan would 'find' something relating to their methadone, or other drug / alcohol ingestion. Careful and simple explanation of the concept of groupwise MRI scan analysis (ie, not comparing individual scans, but comparing the group of babies exposed to methadone and babies not exposed) helped alleviate this.

A final issue relating to the use of MRI to assess potential effects of prenatal drug exposure related to the inherent characteristics of the population, including their mistrust of the medical profession. These have been explored in detail earlier in this chapter.

5.4 Prenatal methadone exposure and developmental venous anomalies

There were 2 cases out of 25 of DVA, previously termed venous angioma. DVA are reported to be the most frequently occurring venous malformation, although as they are often identified as an incidental finding on MRI scans, the true incidence in neonates is unknown. Aetiologically DVA result from arrest in the parenchymal venous development. Although DVAs are usually considered benign, they often co-exist with cavernous haemangioma (Aoki and Srivatanakul, 2016) and this can contribute to potential vascular morbidity and risk of spontaneous haemorrhage.

The rate of 8% DVA in my study is higher than the previously reported 1.5% (Brinjikji et al., 2017). The prevalence appears to increase with age, with a reported prevalence in under 1 year olds of 1.5% compared with 7.6% in pre-school age children. A recently published large study in 3966 children reports that DVA is in the top 3 most common incidental brain findings on neuroimaging, with 63 DVA out of 940 children with IF (Jansen et al., 2017).

DVA has previously been reported in association with prenatal methadone exposure (Brinjikji et al., 2017). Tinelli and colleagues published two case reports of children with prenatal methadone exposure and nystagmus (Tinelli et al., 2013), where one child had an MRI brain as part of their investigations, which showed an incidental finding of a DVA (termed venous angioma).

DVA can also be accompanied by signal intense MRI lesions, which are felt to represent delayed myelination(Linscott et al., 2014). DVA has been reported in the context of acute demyelination in an adult (Jung et al., 1997), and recently in adults with multiple sclerosis there are case reports of DVA with demyelination occurring adjacent to the DVA(Ma et al., 2017, Rogers et al., 2018), suggesting that myelination or demyelination is linked to the aetiological process in some way.

If DVA has an incidence of 1.5%, one would not expect to identify 2 cases in a cohort of 25 patients. This may be a chance observation, and its significance is unknown. It is nonetheless an interesting observation that warrants further consideration.

5.5 VEP and MRI findings from this study

In Chapter Three, the findings of abnormal or immature VEPs in infants and children with prenatal methadone exposure was discussed. Visual evoked potentials reflect the integrity and maturity of the cortico-visual system. More specifically, VEPs represent optic nerve myelination (Whitham et al., 2010); the more well myelinated the optic nerve, the faster the visual evoked potentials are conducted. Conversely, the less well myelinated, the more likely the VEP will have an increased latency or reduced amplitude or be absent altogether.

We have demonstrated in our study that major white matter fibre tracts are less coherently organised in neonates with prenatal methadone exposure, reflected by lower FA values. This is likely to be mediated by impaired myelination, reflected by lower RD values. The reduced integrity of the white matter tracts offers a potential

pathophysiological explanation for the abnormal or absent VEPs demonstrated in several recent studies. The white matter abnormalities described in this study are evident in the same timeframe as a large VEP study of neonates demonstrated abnormal or atypical VEP (McGlone et al., 2013a).

VEP findings correlate with dMRI measures in a mouse model of multiple sclerosis. FA decreased, and RD increased in the optic nerve, with decreases in VEP latency and reductions in amplitude (Nishioka et al., 2017). VEPs are also known to reflect the maturation pattern of the brain, with stepwise decreases in the VEP latency being demonstrated in preterm babies over time, reflecting a synchronized progress in myelination in several parts of the visual pathway (Tsuneishi and Casaer, 1997). In addition, using multiple sclerosis as a model of demyelination, there is human evidence of significant correlation between white matter microstructure alterations on dMRI and VEP latency using TBSS (Lobsien et al., 2014).

5.6 Prenatal opioid exposure and congenital abnormalities

There were 3 congenital abnormalities noted in the infants who had undergone TBSS analysis. These consisted of a cleft lip, a hypospadias and bilateral fixed talipes. There was also an additional case of an infant whose images were significantly degraded due to movement artefact and so was not included in the TBSS analysis, who had a hypospadias and a facial nerve palsy. In total, there were 4 out of 25 infants with congenital anomalies. There were no congenital abnormalities in the control group.

Whilst the sample size is small, the number of congenital abnormalities appears to be over-represented in the methadone-exposed group.

A recent systematic review assessing congenital malformations and opioid use in pregnancy reported the most common malformations as oral clefts in case-control studies and talipes in cohort studies(Lind et al., 2017). Our study finding, although in a small sample size, would be consistent with this finding. Another observational study reported the rate of congenital anomalies to be 8.3% in opioid-exposed compared to 4.2% in unexposed infants(Norgaard et al., 2015), and a recent retrospective review reported a higher incidence of congenital abnormalities in methadone-exposed infants compared to unexposed infants, odds ratio 2.57 (95% confidence intervals 1.4 – 4.7)(Kelty and Hulse, 2017).

The occurrence of two cases of hypospadias in 25 methadone-exposed infants gives an incidence of 8%, which is also much higher than the reported 1 in 200 – 1 in 300 usually reported(Cunha et al., 2015). The underlying mechanism for hypospadias is thought to be related to reduced exposure to testosterone during early fetal life, either via reduced levels or reduced sensitivity to testosterone(Baskin, 2000). Animal models of prenatal morphine exposure in males have shown opiate-induced suppression of testosterone in the fetal testes(Singh et al., 1980). A further study by Sandberg and colleagues postulated that if there was reduced fetal testosterone in prenatal opioid exposure, such children may show gender role differences in play and subsequently reported more feminised play behaviours in boys. Prenatal opioid exposure might result in sex hormone alterations *in utero* (Sandberg et al., 1990) and this mechanism

could underlie increased incidence of hypospadias, although the numbers in this study are very small.

It remains unclear whether methadone exerts a directly teratogenic effect on the developing fetus. Most congenital anomalies occur at a rate of less than 1 per 1000 births, and so although the small sample sizes of many of the included studies limit their ability to identify rare conditions, the fact that an association has been described between prenatal opioid exposure and congenital anomalies, suggests an increased incidence and warrants further study to prove this.

5.7 Clinical implications of this study

This study raises serious concerns about the effects of the most commonly used drug for OUD during pregnancy, methadone. It has also highlighted the increased risk of neurodevelopmental, behavioural and visual problems in children with prenatal methadone exposure, and this raises the question of how this information be taken forward into clinical practice. The thesis will now explore potential alternative options for the management of OUD in pregnancy, and the follow up options for at risk infants and children.

5.7.1 Management of OUD in pregnancy

Current management of OUD in pregnancy is opioid replacement therapy(2012). However, in light of the study findings of altered brain microstructure associated with prenatal methadone exposure, alternative treatment strategies for OUD in pregnancy should be considered. This is of such importance that the US congress have passed the

Protecting our Infants Act 2015, which calls for a review of treatment options for prenatal opioid abuse (Laslo et al., 2017).

5.7.1.1 Methadone dosing regimens

Although the benefits of MAT with an opioid substitute during pregnancy are unequivocal (2012), there is no consensus regarding the optimal dosing regimen for methadone. A single daily dose of methadone is commonly prescribed, due to the long acting nature of methadone. Some authors suggest that accelerated metabolism of methadone during pregnancy might predispose women and fetuses to daily withdrawal stress and risk of relapse to illicit drugs (McCarthy et al., 2015, McCarthy et al., 2017, Bogen et al., 2013). Studies that support the use of divided dosing to minimize fluctuations in serum concentration report favourable effects on maternal symptoms of withdrawal, and on fetal neurobehaviour and reduced NAS prevalence (McCarthy, 2015, Wittmann and Segal, 1991, Jansson et al., 2009), but no study has evaluated the impact of dosing regimen on brain development either fetal or neonatal, or long-term outcome. Monitoring of maternal plasma methadone concentration during the peripartum period with dose titration to keep levels within the maternal therapeutic range has been suggested, but this is not practiced widely (McCarthy et al., 2017).

5.7.1.2 Alternative opioid substitutes: Buprenorphine

Buprenorphine is a partial mu-opioid agonist and kappa-opioid antagonist. It appears to be an acceptable substitute to some pregnant women. As discussed in chapter One, there is less fetal neurobehavioural suppression with buprenorphine compared to methadone (Jansson et al., 2012). Buprenorphine is also associated with lower risk of

preterm birth, improved growth parameters at birth and less NAS, without apparent harm, when compared with methadone (Wurst et al., 2016, Jones et al., 2010). These neonatal outcomes suggest that buprenorphine may have a more favourable safety profile for the child in the short term although some more recent studies have shown a small and statistically non-significant difference in neonatal outcomes with buprenorphine compared with methadone(Nechanska et al., 2018).

However, buprenorphine has also been shown to have an effect on oligodendrocytes(Eschenroeder et al., 2012), with subsequent effects on myelination(Sanchez et al., 2008). In addition, an increased rate of gastrointestinal birth defects with 3.2% occurring in buprenorphine compared to 0.6% in control groups has been reported(Kelty and Hulse, 2017). Studies of development and behaviour that include both buprenorphine-exposed and methadone-exposed infants to form an ‘opioid exposed’ group also report poorer outcomes in the opioid-exposed group(Norgaard et al., 2015, Konijnenberg and Melinder, 2015). However, ‘opioid-exposure’ does not represent a homogenous group and should not therefore be interpreted as one. Furthermore, it is possible that more of the adversity is derived from the methadone-exposed group, as has been previously demonstrated(Whitham et al., 2010, Konijnenberg and Melinder, 2015). It is imperative that more long-term data is acquired before another opiate substitute is widely adopted into practice without the evidence to support its use.

5.7.1.3 Medically assisted opioid detoxification

One alternative treatment strategy for OUD in pregnancy increasingly being used is opioid detoxification, with parts of Europe and the US reporting its success. Detoxification has the benefit of avoiding the complications of addiction and limiting the prenatal exposure of the fetus to opioids.

Current international guidance advises against detoxification during pregnancy, stating that there are increased fetal risks and a high rate of relapse(ACOG, 2012) . This is largely based around two case reports from the 1970s(Rementeria and Nunag, 1973, Zuspan et al., 1975). As was the case with the introduction of methadone as a treatment for OUD in pregnancy, the clinical practice of avoiding opiate detoxification in pregnancy is also not supported by compelling evidence, rather by two case reports fifty years ago.

There are several published studies attempting to provide evidence to support detoxification during pregnancy. However, the multitude of issues surrounding detoxification are complex. Firstly; Is it possible to detoxify during pregnancy? Secondly; is it safe for mother and fetus? Thirdly; does it matter how and when you detoxify? And finally; what are the long-term benefits of opioid detoxification during pregnancy?

There are reports of over 600 patients in the literature who have been reported to detoxify from opiates during pregnancy with no apparent fetal harm(Bell et al., 2016, Jones et al., 2008, Luty et al., 2003), thus we could conclude that detoxification from opiates during pregnancy is possible. One study reporting successful detoxification in

21 women in Norway without adverse effects provides a very clear message that their results occurred in the context of a supervised and residential tapered detoxification programme, with full psychological support available and intensive fetal monitoring to guide the taper of opioid agonist therapy (Haabrekke et al., 2014). This may be integral to the success or failure of detoxification as a means of addressing perinatal opiate exposure.

However, in many studies there is lack of denominator data about the proportion of mothers where detoxification was attempted but unsuccessful, ie relapse. Successful detoxification rates vary from 29% (17/58) (Maas et al., 1990), 41% (42/101) (Luty et al., 2003), 56% (53/95) (Stewart et al., 2013) and 58% (20/34) (Dashe et al., 1998). Thus, detoxification may not be a viable treatment option for some pregnant women.

The issue of relapse following detoxification is an important consideration. Rates of relapse varied from 17% where intensive support was provided as part of a comprehensive detoxification programme to as high as 73% when detoxification was unsupported (Bell et al., 2016) suggesting that it can be modified and improved when delivered as part of a comprehensive care package. The variable but high risk of relapse and subsequent overdose poses a significant risk to both mother and fetus (Jones et al., 2017). A Norwegian study of non-pregnant addicts who underwent detoxification reported significant over-representation of recently detoxified patients in the deaths by overdose in the first four weeks following detoxification (Ravndal and Amundsen, 2010), suggesting that such a dramatic finding should not be ignored.

In relation to the safety of detoxification during pregnancy, the main reported fetal outcomes are miscarriage or fetal demise, gestation at delivery and rate of preterm delivery, growth parameters and rates of NAS. All of the studies report longer gestation at delivery and less preterm birth, increased weight and head circumference and most report significantly lower rates of NAS, as would be expected.

With regards to timing of detoxification, one study reported a single case of first trimester miscarriage out of five women who underwent detoxification in the first trimester of their pregnancy(Luty et al., 2003) and conclude that the trend to increased miscarriages observed supports the view that it is not safe in the first trimester. However, the baseline risk for miscarriage is significantly higher in the first trimester than later in pregnancy and this may account for the observed association. Furthermore, it is difficult to draw any firm conclusions from a study with a subgroup of 5 patients where one has an event. The largest study included 301 pregnant women of whom only 28 underwent detoxification during the first trimester, but this series reported no adverse fetal events or cases of miscarriage(Bell et al., 2016), suggesting that if Luty and colleagues 20% miscarriage rate had been true, there should have been 8 cases of first trimester miscarriage, and there were none.

The current studies are not powered to detect rare events such as stillbirth, SIDS and maternal overdose. These rare but important events may become more apparent if the practice of detoxification during pregnancy is widely adopted without further studies(Gleeson et al., 2017). In addition, at present there are no long-term studies assessing childhood outcome after opiate detoxification during pregnancy(Bell et al.,

2016). The only study that attempted to address longer term outcomes was a small Norwegian study which compared 11 children aged 4.5 years prenatally exposed to opioids but whose mother underwent detoxification during pregnancy with 12 matched control children(Walhovd et al., 2015). There was no difference between HC at birth, intelligent quotient (IQ), neuroanatomical volumes quantified by MRI and there were no cases of NAS. However, there was a significant difference in vision between groups, with opiate-exposed children exhibiting reduced visual acuity compared to control children. As explored in detail in chapter Four, visual problems have been repeatedly associated with prenatal opioid exposure, and this study suggests that the onset of visual interference is early, as these children had visual problems despite all mothers undergoing detoxified during pregnancy.

Whilst there is inconclusive evidence at present to support widespread adoption of opiate detoxification during pregnancy, there continue to be ongoing and growing concerns surrounding the lasting adverse effects of prenatal methadone exposure on the developing brain, albeit the long-term effects remain unquantified. Current limited evidence suggests in the right population of pregnant women, and under the right circumstances, with appropriate fetal monitoring, that supervised residential detoxification, and intensive psychological support to prevent relapse may be the key to safe and successful opiate withdrawal in pregnancy and may be a viable option to optimise the outcomes for this vulnerable group of children. Further large studies, including assessment of timing of detoxification, and fetal, neonatal and childhood outcomes are required.

5.7.2 Neurodevelopmental follow up for all infants with prenatal methadone exposure

Children with prenatal methadone exposure are an unequivocally vulnerable group of patients(Dryden et al., 2009). There is emerging evidence that these children have worse neurodevelopmental outcomes, more behavioural problems and more visual problems, and that early intervention would be beneficial (Moe, 2002). Yet despite this, there is no recommendation for increased surveillance to identify these problems at an early stage and support caregivers with early intervention strategies. This need has been previously identified to try to offset some of the risk conferred by prenatal drug exposure(Ornoy et al., 2010) but has not been widely implemented.

Reduced FA correlates to worse neurodevelopmental outcome, as discussed in Chapter One. Should all infants with a history of prenatal methadone exposure, or indeed prenatal opioid exposure, be enrolled in a developmental follow up programme? A similar programme is well established for preterm infants, another vulnerable group of patients, and has been shown to improve outcome(Spittle et al., 2015).

Instituting a follow up programme would have considerable impact on neonatal and paediatric services in terms of additional workload and is likely to face resistance in the short term at least, especially in the context of current NHS pressures. However, the additional work and cost are likely to be outweighed by the additional benefits gained by early identification of deficits, allowing early intervention- known to improve longer term outcomes(Brooks-Gunn et al., 1994), and may also result in improved school performance with longer benefits for health, education and society.

However, one must acknowledge the difficulties in engaging with families where there is substance misuse. Attrition rates in studies following up children at 2 years ranged from 18% (Wilson, 1989) to 84% (Chasnoff et al.), and one study reported that over half the infants given appointments for outpatient clinic defaulted on two or more occasions (Dryden et al., 2009), reflecting the complex and chaotic lifestyles often associated with OUD. It is therefore very likely that should a developmental screening programme be instigated for these children, a significant proportion would not attend their appointments, resulting in a waste of valuable NHS resources, and a balance of benefit versus drain on resources would have to be judged.

5.8 Study strengths

Regarding the systematic review and meta-analysis (Chapter 3), a strength of this work is its pragmatic and systematic approach to summarizing childhood outcome following prenatal methadone exposure across three important domains chosen to reflect a broad perspective of the developing brain: neurodevelopment, visual development and function, and neuroimaging. Studies of neonatal neurodevelopment were excluded in order to prevent confounding by NAS. Studies often incorrectly consider 'opioids' as a homogenous group, and this limits causal inference about specific opioid medications. Therefore, studies reporting outcomes following exposure to alternative opioid substitutes were excluded, in order to derive maximum inference about a single pharmacological agent, methadone, commonly used in the management of pregnant women with OUD.

Regarding the MRI study detailed in Chapter Four, the acquisition of dMRI took place soon after birth, before exposure to postnatal opioids or other pharmacological treatment for NAS could potentially confound the interpretation. Preterm birth, which is an important source of confounding for neurodevelopmental outcome, was excluded. The MRI analysis was quantitative, used well established processing pipelines specific for the neonatal brain and was adequately powered according to previous research in MRI studies (Ball et al., 2013). We adjusted for OFC and showed that the findings were robust to differences in global brain growth. Importantly, this is the first study that shows alterations in newborn brain structure associated with prenatal methadone exposure shortly after birth.

5.9 Study limitations

The major limitations relate to the interpretability of the systematic review and meta-analysis and the limited inferences about harm. Firstly, estimates of impairment may be biased by intermediate to low quality evidence; most were intermediate, and some were low, with a mean modified GRADE score of 3.5/8. Secondly, comparison groups were often poorly described beyond the definition of ‘non-opioid exposed’, with inadequate control for socioeconomic status or environmental status or polydrug or alcohol use. Finally, many of the included studies were published prior to the millennium, making them now nearly 20 years out of date. This has potentially important implications, as patterns of substance misuse are changing. Where methadone dose was stated in the studies included in the meta-analysis, the mean dose was generally between 15mg and 42mg per day which is much lower than the mean dose of methadone in this MRI study, 69mg per day. Therefore, research performed in the 1970s and 1980s may not

be representative or applicable to the current situation we are in. Although no study to date has demonstrated a dose-dependent relationship, mostly due to inability to derive such information due to methodological limitations, there is biological plausibility in a dose-dependent relationship existing and contributing to later adverse effects. The differences in doses then becomes more important, as the earlier studies may further under-represent any developmental under-performance that modern children exposed to higher doses of methadone may experience.

Other sources of potential bias in the systematic review included high attrition, and likely confounding by preterm birth and postnatal opioid exposure. Attrition of methadone-exposed children at 2 years of age included in the meta-analysis was twice that of comparison children, increasing the chance of a type 2 error given that attrition is often explained by impairment in pediatric neurodevelopmental outcome studies. For the same reason, it is possible that the extent of neurodevelopmental and visual impairment associated with prenatal methadone exposure may be under-estimated.

Polydrug use was seldom disclosed and infrequently reported in the included studies. Where additional drug use was investigated, the majority of infants were found to be polydrug exposed, consistent with previous observations that opioid dependent women prescribed methadone commonly use other drugs including alcohol (McGlone et al., 2013b, McGlone et al., 2012) Investigation and reporting of additional substance use in the studies reviewed was variable, and only one study had examined prenatal exposure in all infants in detail using extensive toxicology (McGlone et al., 2013a).

These data highlight that being born to an opioid-dependent mother who has been prescribed methadone during pregnancy is associated with adverse visual and neurodevelopmental outcomes in infancy and early childhood, however, deficiencies in the existing literature limit causal inference about harm, and factors other than methadone *per se* could account for these observations. These findings may be used for comparison with childhood outcome studies of alternative opioid substitutes in pregnancy.

Regarding the MRI study, polydrug use among cases was also a major limitation, as it prevented causal inference. Because evaluating causation was not possible, it was also not possible to exclude potential mediating or interacting factors such timing and dose effects of methadone or the role of other prenatal drug exposures. Whilst polydrug use is not a problem unique to our study, it continues to be a significant limitation within the field. This study was also limited by reliance on maternal report for drug exposures among control women. However, none of the control participants were prescribed opioids during pregnancy, and the likelihood of undisclosed heroin use or non-prescription opioids was low.

Finally, the inability to obtain meconium samples for all participants involved, which precluded an accurate assessment of prenatal drug and alcohol exposure was a limitation. Studies assessing impact of prenatal drug exposure need sensitive and reliable measures of drug exposure, for both cases and control groups, and in order to achieve this a combination of maternal self-reporting and biological samples, such as meconium, should be used (Konijnenberg, 2015).

5.10 The future

5.10.1 The impact of prescription opioids during pregnancy

Opioid use during pregnancy is not solely seen in OUD secondary to illicit addiction. There has been a dramatic increase in prescription opioids over the last decade, contributing to the so called ‘opioid crisis’. Unlike previous opioid epidemics which predominantly affected poor urban minority populations, the current opioid crisis disproportionately affects white middle-class people covered by private health insurance(McCarthy 2016). This has been reflected in the subsequent increase in opioid-dependence related claims, which have risen by a staggering 3203% to 7 million in 2014. Patients prescribed opioids for pain are at risk of opioid abuse and misuse, particularly if they have other co-morbid factors such as mental health issues or previous substance misuse disorders(Kaye et al., 2017). Sadly, the surge in prescription opioid medications has been mirrored by a surge in fatalities related to overdoses, with 16,651 overdose deaths from prescription opioids versus only 3036 heroin related deaths in 2010 (Volkow et al., 2014).

The prescription opioid epidemic extends to pregnant women and their unborn children, who may be exposed to opioid during pregnancy, with one study estimating that 14.4% pregnant women had at least one opioid prescription dispensed during their pregnancy(Bateman et al., 2014). Although the majority of prescription opioids used during pregnancy in the US appear to be prescribed for acute pain(Yazdy et al., 2015), the increased use of prescribed opioids has been temporally associated with an increase in opioid use disorder among pregnant women in the US (Patrick et al., 2012), particularly in rural areas(Villapiano et al., 2017).

There are few studies reporting neonatal outcomes for prescription opioids (Lester and Lagasse, 2010) but their use is more commonly associated with neonatal complications (Patrick et al., 2015b), such as preterm birth, known to be associated with oxycodone (Kelly et al., 2011) and tramadol use (Kallen et al., 2013). NAS (Patrick et al., 2015b) is also more common, as prescription opioids also cross the placenta, exposing the developing fetus to exogenous opioid during pregnancy, which abruptly ceases after birth. However, as previously discussed in Chapter 1, NAS is a short-term description of behaviour and the long-term consequences of NAS *per se* remain unknown. Similarly, the long-term consequences of prenatal prescription opioid exposure rather than illicit opioid exposure remain unknown.

There is the potential to study the effects of prenatal exposure to prescription opioids, as this population, especially if women are using a single opioid agent for analgesia as this minimises the effect of polydrug use, and this population may not have the same environmental and social confounders frequently encountered in those with OUD. Interestingly, a retrospective study investigating neonatal outcomes in two groups of methadone-exposed infants, those taking methadone for pain and those with OUD, found that outcomes (growth and NAS) were better in the pain group than the OUD group (Sharpe and Kuschel, 2004), although this could reflect confounding by indication.

The American pain society suggest that counselling pregnant women about the risks versus the benefits of chronic opioid therapy and recommend no use or minimal use (Chou et al., 2009). However, it is extremely difficult to counsel women accurately

when there is very little data regarding prescription opioid use and neonatal outcomes, and no data on longer-term outcomes. In addition, prescription opioids encompass a variety of medications, and it cannot be assumed that the potential effects on the developing fetus will be the same for all medications within a class.

The ideal solution is a primary prevention policy to curb inappropriate prescribing of opioids(Volkow et al., 2014) and doctors have been urged to limit opioid prescribing(McCarthy, 2016). However, the situation is more complex during pregnancy, and pain management in particular poses significant challenges, as many alternative analgesic medications are contraindicated. The reality is that numbers of pregnant women prescribed an opioid medication continue to rise, with nearly 27% of pregnant women having at least one prescription in a large data linkage study in the US(Patrick et al., 2015b), the vast majority being long term prescriptions. The assumption that opioids are ‘safer’ to use in pregnancy than other medications is unfounded, and possibly untrue. If there are no feasible alternatives, and opioid medications have to be prescribed during pregnancy for medical reasons, the prenatally exposed children could be entered into a longitudinal study to assess infant and childhood outcomes, to advance understanding of the consequences of prescription opioid use during pregnancy, accepting the potential limiting factor of confounding by indication.

5.10.2 Future directions

Before concluding, this thesis will now highlight how this study should shape the direction of future studies. Firstly, there is an urgent need for studies which assess long-term childhood neurodevelopmental outcomes after prenatal opioid exposure. The research implication of highly prevalent polydrug use in the target population is that future studies into optimal management of OUD in pregnancy are likely to require pragmatic designs that define cases based on prescribed substitute, with *post hoc* adjustment for other drug exposures if they are shown to differ between groups. In order to do this, studies will need to quantify prenatal drug and alcohol exposure, using a validated biological sample analysis such as infant meconium. Many of the discussion points in Chapters Three, Four and Five would be addressed by a longitudinal pragmatic study design, which accurately profiles prenatal exposures using infant biological matrices such as meconium and is limited to one specific opioid substitute. Grouping together several opioids as a combined ‘opioid exposure’ is not useful, as it is not a homogenous exposure, and different opioids are likely to have different effects on the fetus. Additionally, future studies should follow children into adolescence as the emergence of subtle developmental sequelae, such as problems with executive dysfunction often become more apparent in later childhood.

Furthermore, future studies should use emerging technologies including advanced imaging techniques, faster acquisition times, motion correction, and further evaluate early infant brain development before postnatal environmental factors influence brain development and neurodevelopment. There remains a pressing need to evaluate alternative opioid substitutes, to allow comparison of outcomes, and improve

understanding about the optimal management of OUD in pregnancy. As part of this, alternative strategies for managing OUD in pregnancy should also be explored. The ultimate goal would be for pregnant women to not expose their unborn child to any opioid, or to as little as possible to minimise adverse effects. The potential harm associated with detoxification during pregnancy has not been adequately demonstrated, and future research in this area is vital, and again, it is vital that study designs include long term childhood outcomes. Medically assisted detoxification could revolutionise the management of OUD in pregnancy but warrants further investigation.

5.11 Conclusions

This thesis has furthered the understanding of the detrimental effects of prenatal methadone exposure, firstly through systematic review and meta-analysis of existing published literature describing adverse neurodevelopmental and visual outcomes and secondly, using advanced MRI to study a challenging population and demonstrate altered white matter microstructure which is apparent soon after birth. This new information about timing of brain changes is critical, as it indicates that prenatal methadone exposure has early demonstrable effects on brain connectivity, which may in turn account for differences observed in the developmental trajectories of such exposed infants. In the context of an opioid epidemic this data strongly supports the pressing need to evaluate methadone dose regimens in pregnancy, alternative opioid substitutes and alternative treatment strategies for OUD. Future study designs should evaluate fetal and neonatal brain development and long-term neurocognitive outcome,

with the aim of optimising the outcome for both the mother and infant affected by OUD.

BIBLIOGRAPHY

- United Nations Office on Drugs and Crime, World Drug Report 2017. (ISBN:978-92-1-14829-1, eISBN:978-92-1-060623-3, United Nations publication Sales No. E.17.XI.6).
2012. ACOG Committee Opinion No. 524: Opioid abuse, dependence, and addiction in pregnancy. *Obstet Gynecol*, 119, 1070-6.
2014. Guidelines for the identification and management of substance use and substance use disorders in pregnancy. Geneva: World Health Organisation. Publications Office of the European Union, Luxembourg. The European Monitoring Centre for Drugs and Drug Addiction (2017). *European Drug Report 2017: Trends and Developments*.
- United Nations Office on Drugs and Crime. World Drug Report 2016. (United Nations publication, Sales No E.16.XI.7).
- ABERNETHY, C., MCCALL, K. E., COOPER, G., FAVRETTO, D., VAIANO, F., BERTOL, E. & MACTIER, H. 2017. Determining the pattern and prevalence of alcohol consumption in pregnancy by measuring biomarkers in meconium. *Arch Dis Child Fetal Neonatal Ed*.
- ACOG 2012. ACOG Committee Opinion No. 524: Opioid abuse, dependence, and addiction in pregnancy. *Obstet Gynecol*, 119, 1070-6.
- ALAEI, A., ZARGHAMI, M., FARNIA, S., KHADEMLOO, M. & KHODDAD, T. 2014. Comparison of brain white matter hyperintensities in methamphetamine and methadone dependent patients and healthy controls. *Iranian journal of radiology : a quarterly journal published by the Iranian Radiological Society*, 11, e14275.
- ALI, K., ROSSER, T., BHAT, R., WOLFF, K., HANNAM, S., RAFFERTY, G. F. & GREENOUGH, A. 2017. Antenatal smoking and substance-misuse, infant and newborn response to hypoxia. *Pediatr Pulmonol*, 52, 650-655.
- ALI, K., ROSSOR, T., BHAT, R., WOLFF, K., HANNAM, S., RAFFERTY, G. F., PEACOCK, J. L. & GREENOUGH, A. 2016. Antenatal substance misuse and smoking and newborn hypoxic challenge response. *Arch Dis Child Fetal Neonatal Ed*, 101, F143-8.
- ANBLAGAN, D., PATAKY, R., EVANS, M. J., TELFORD, E. J., SERAG, A., SPARROW, S., PIYASENA, C., SEMPLE, S. I., WILKINSON, A. G., BASTIN, M. E. & BOARDMAN, J. P. 2016. Association between preterm brain injury and exposure to chorioamnionitis during fetal life. *Sci Rep*, 6, 37932.
- ANJARI, M., SRINIVASAN, L., ALLSOP, J. M., HAJNAL, J. V., RUTHERFORD, M. A., EDWARDS, A. D. & COUNSELL, S. J. 2007. Diffusion tensor imaging with tract-based spatial statistics reveals local white matter abnormalities in preterm infants. *NeuroImage*, 35, 1021-1027.
- AOKI, R. & SRIVATANAKUL, K. 2016. Developmental Venous Anomaly: Benign or Not Benign. *Neurol Med Chir (Tokyo)*, 56, 534-43.

- AZZOPARDI, D., ROBERTSON, N. J., BAINBRIDGE, A., CADY, E., CHARLES-EDWARDS, G., DEIERL, A., FAGIOLO, G., FRANKS, N. P., GRIFFITHS, J., HAJNAL, J., JUSZCZAK, E., KAPETANAKIS, B., LINSELL, L., MAZE, M., OMAR, O., STROHM, B., TUSOR, N. & EDWARDS, A. D. Moderate hypothermia within 6 h of birth plus inhaled xenon versus moderate hypothermia alone after birth asphyxia (TOBY-Xe): a proof-of-concept, open-label, randomised controlled trial. *The Lancet Neurology*, 15, 145-153.
- BAKDASH, A., BURGER, P., GOECKE, T. W., FASCHING, P. A., REULBACH, U., BLEICH, S., HASTEDT, M., ROTHE, M., BECKMANN, M. W., PRAGST, F. & KORNHUBER, J. 2010. Quantification of fatty acid ethyl esters (FAEE) and ethyl glucuronide (EtG) in meconium from newborns for detection of alcohol abuse in a maternal health evaluation study. *Anal Bioanal Chem*, 396, 2469-77.
- BAKER, S. T., YUCEL, M., FORNITO, A., ALLEN, N. B. & LUBMAN, D. I. 2013. A systematic review of diffusion weighted MRI studies of white matter microstructure in adolescent substance users. *Neurosci Biobehav Rev*, 37, 1713-23.
- BALDACCHINO, A., ARBUCKLE, K., PETRIE, D. J. & MCCOWAN, C. 2015. Erratum: neurobehavioral consequences of chronic intrauterine opioid exposure in infants and preschool children: a systematic review and meta-analysis. *BMC Psychiatry*, 15, 134.
- BALL, G., BOARDMAN, J. P., ARICHI, T., MERCHANT, N., RUECKERT, D., EDWARDS, A. D. & COUNSELL, S. J. 2013. Testing the sensitivity of Tract-Based Spatial Statistics to simulated treatment effects in preterm neonates. *PLoS One*, 8, e67706.
- BALL, G., COUNSELL, S. J., ANJARI, M., MERCHANT, N., ARICHI, T., DORIA, V., RUTHERFORD, M. A., EDWARDS, A. D., RUECKERT, D. & BOARDMAN, J. P. 2010. An optimised tract-based spatial statistics protocol for neonates: Applications to prematurity and chronic lung disease. *Neuroimage*, 53, 94-102.
- BALSHEM, H., HELFAND, M., SCHUNEMANN, H. J., OXMAN, A. D., KUNZ, R., BROZEK, J., VIST, G. E., FALCK-YTTER, Y., MEERPOHL, J., NORRIS, S. & GUYATT, G. H. 2011. GRADE guidelines: 3. Rating the quality of evidence. *J Clin Epidemiol*, 64, 401-6.
- BASKIN, L. S. 2000. Hypospadias and urethral development. *J Urol*, 163, 951-6.
- BASSI, L., CHEW, A., MERCHANT, N., BALL, G., RAMENGGHI, L., BOARDMAN, J., ALLSOP, J. M., DORIA, V., ARICHI, T., MOSCA, F., EDWARDS, A. D., COWAN, F. M., RUTHERFORD, M. A. & COUNSELL, S. J. 2011. Diffusion tensor imaging in preterm infants with punctate white matter lesions. *Pediatr Res*, 69, 561-6.
- BASSI, L., RICCI, D., VOLZONE, A., ALLSOP, J. M., SRINIVASAN, L., PAI, A., RIBES, C., RAMENGGHI, L. A., MERCURI, E. & MOSCA, F. 2008. Probabilistic diffusion tractography of the optic radiations and visual function in preterm infants at term equivalent age. *Brain*, 131, 573-582.
- BATEMAN, B. T., HERNANDEZ-DIAZ, S., RATHMELL, J. P., SEEGER, J. D., DOHERTY, M., FISCHER, M. A. & HUYBRECHTS, K. F. 2014. Patterns of opioid utilization in

- pregnancy in a large cohort of commercial insurance beneficiaries in the United States. *Anesthesiology*, 120, 1216-24.
- BAYLEY, N. 1969. Bayley scales of infant development: Birth to two years Psychological Corporation. *New York*.
- BAYLEY, N. 1993. *Bayley scales of infant development: Manual*, Psychological Corporation.
- BAYLEY, N. 2006. *Bayley scales of infant and toddler development: Bayley-III*, Harcourt Assessment, Psych. Corporation.
- BELCHEVA, M. M., BOHN, L. M., HO, M. T., JOHNSON, F. E., YANAI, J., BARRON, S. & COSCIA, C. J. 1998. Brain opioid receptor adaptation and expression after prenatal exposure to buprenorphine. *Developmental Brain Research*, 111, 35-42.
- BELL, J., TOWERS, C. V., HENNESSY, M. D., HEITZMAN, C., SMITH, B. & CHATTIN, K. 2016. Detoxification from opiate drugs during pregnancy. *American Journal of Obstetrics and Gynecology*, 215, 374.e1-374.e6.
- BERNS, G. S., MOORE, S. & CAPRA, C. M. 2009. Adolescent engagement in dangerous behaviors is associated with increased white matter maturity of frontal cortex. *PLoS One*, 4, e6773.
- BESCHNER, G. M., BROTMAN, R. & ABUSE, N. I. O. D. 1977. *Symposium on Comprehensive Health Care for Addicted Families and Their Children: May 20 & 21, 1976, New York, New York : [proceedings]*, National Institute on Drug Abuse.
- BIER, J. B., FINGER, A. S., BIER, B. A., JOHNSON, T. A. & COYLE, M. G. 2015. Growth and developmental outcome of infants with in-utero exposure to methadone vs buprenorphine. *Journal of Perinatology*, 35, 656-659.
- BLAIR, P. S., SIDEBOTHAM, P., BERRY, P. J., EVANS, M. & FLEMING, P. J. 2006. Major epidemiological changes in sudden infant death syndrome: a 20-year population-based study in the UK. *Lancet*, 367, 314-9.
- BLESA, M., SERAG, A., WILKINSON, A. G., ANBLAGAN, D., TELFORD, E. J., PATAKY, R., SPARROW, S. A., MACNAUGHT, G., SEMPLE, S. I., BASTIN, M. E. & BOARDMAN, J. P. 2016. Parcellation of the Healthy Neonatal Brain into 107 Regions Using Atlas Propagation through Intermediate Time Points in Childhood. *Front Neurosci*, 10, 220.
- BOARDMAN, J. P., CRAVEN, C., VALAPPIL, S., COUNSELL, S. J., DYET, L. E., RUECKERT, D., ALJABAR, P., RUTHERFORD, M. A., CHEW, A. T. M., ALLSOP, J. M., COWAN, F. & EDWARDS, A. D. 2010. A common neonatal image phenotype predicts adverse neurodevelopmental outcome in children born preterm. *NeuroImage*, 52, 409-414.
- BOARDMAN, J. P., WALLEY, A., BALL, G., TAKOUSIS, P., KRISHNAN, M. L., HUGHES-CARRE, L., ALJABAR, P., SERAG, A., KING, C., MERCHANT, N., SRINIVASAN, L., FROGUEL, P., HAJNAL, J., RUECKERT, D., COUNSELL, S. & EDWARDS, A. D. 2014. Common Genetic Variants and Risk of Brain Injury After Preterm Birth. *Pediatrics*, 133, e1655-e1663.
- BOGEN, D. L., PEREL, J. M., HELSEL, J. C., HANUSA, B. H., ROMKES, M., NUKUI, T., FRIEDMAN, C. R. & WISNER, K. L. 2013. Pharmacologic evidence to support

- clinical decision making for peripartum methadone treatment. *Psychopharmacology (Berl)*, 225, 441-51.
- BORA, E., YUCEL, M., FORNITO, A., PANTELIS, C., HARRISON, B. J., COCCHI, L., PELL, G. & LUBMAN, D. I. 2012. White matter microstructure in opiate addiction. *Addict Biol*, 17, 141-8.
- BRINJIKJI, W., EL-RIDA EL-MASRI, A., WALD, J. T. & LANZINO, G. 2017. Prevalence of Developmental Venous Anomalies Increases With Age. *Stroke*, 48, 1997-1999.
- BRITISH ASSOCIATION OF PERINATAL MEDICINE 2016. Fetal and Neonatal Brain Magnetic Resonance Imaging: Clinical Indications, Acquisitions and Reporting. A Framework for Practice.
- BROGLY, S. B., SAIA, K. A., WALLEY, A. Y., DU, H. M. & SEBASTIANI, P. 2014. Prenatal buprenorphine versus methadone exposure and neonatal outcomes: systematic review and meta-analysis. *Am J Epidemiol*, 180, 673-86.
- BROOKS, D. & BARTH, R. P. 1998. Characteristics and outcomes of drug-exposed and non drug-exposed children in kinship and non-relative foster care. *Children and Youth Services Review*, 20, 475-501.
- BROOKS-GUNN, J., MCCARTON, C. M., CASEY, P. H., MCCORMICK, M. C., BAUER, C. R., BERNBAUM, J. C., TYSON, J., SWANSON, M., BENNETT, F. C., SCOTT, D. T. & ET AL. 1994. Early intervention in low-birth-weight premature infants. Results through age 5 years from the Infant Health and Development Program. *JAMA*, 272, 1257-62.
- BUNIKOWSKI, R., GRIMMER, I., HEISER, A., METZE, B., SCHAFFER, A. & OBLADEN, M. Neurodevelopmental outcome after prenatal exposure to opiates. *European Journal of Pediatrics*, 157, 724-30.
- BURNS, L., CONROY, E. & MATTICK, R. P. 2010. Infant mortality among women on a methadone program during pregnancy. *Drug and Alcohol Review*, 29, 551-556.
- BURNS, L., MATTICK, R. P., LIM, K. & WALLACE, C. 2007. Methadone in pregnancy: treatment retention and neonatal outcomes. *Addiction*, 102, 264-70.
- BYRNES, E. M. & VASSOLER, F. M. 2017. Modeling prenatal opioid exposure in animals: Current findings and future directions. *Frontiers in Neuroendocrinology*.
- CANTWELL, R., CLUTTON-BROCK, T., COOPER, G., DAWSON, A., DRIFE, J., GARROD, D., HARPER, A., HULBERT, D., LUCAS, S., MCCLURE, J., MILLWARD-SADLER, H., NEILSON, J., NELSON-PIERCY, C., NORMAN, J., O'HERLIHY, C., OATES, M., SHAKESPEARE, J., DE SWIET, M., WILLIAMSON, C., BEALE, V., KNIGHT, M., LENNOX, C., MILLER, A., PARMAR, D., ROGERS, J. & SPRINGETT, A. 2011. Saving Mothers' Lives: Reviewing maternal deaths to make motherhood safer: 2006-2008. The Eighth Report of the Confidential Enquiries into Maternal Deaths in the United Kingdom. *Bjog*, 118 Suppl 1, 1-203.
- CARREY, N. J., BUTTER, H. J., PERSINGER, M. A. & BIALIK, R. J. 1995. Physiological and cognitive correlates of child abuse. *J Am Acad Child Adolesc Psychiatry*, 34, 1067-75.

- CHAN, D., BAR-OZ, B., PELLERIN, B., PACIOREK, C., KLEIN, J., KAPUR, B., FARINE, D. & KOREN, G. 2003. Population baseline of meconium fatty acid ethyl esters among infants of nondrinking women in Jerusalem and Toronto. *Ther Drug Monit*, 25, 271-8.
- CHASNOFF, I. J., SCHNOLL, S. H., BURNS, W. J. & BURNS, K. Maternal nonnarcotic substance abuse during pregnancy: effects on infant development. *Neurobehavioral Toxicology & Teratology*, 6, 277-80.
- CHAVEZ, C. J., OSTREA, E. M., JR., STRYKER, J. C. & SMIALEK, Z. 1979. Sudden infant death syndrome among infants of drug-dependent mothers. *J Pediatr*, 95, 407-9.
- CHE, Y., SUN, H., TAN, H., PENG, Y., ZENG, T. & MA, Y. 2005. The effect of prenatal morphine exposure on memory consolidation in the chick. *Neuroscience Letters*, 380, 300-304.
- CHEIKH ISMAIL, L., KNIGHT, H. E., OHUMA, E. O., HOCH, L. & CHUMLEA, W. C. 2013. Anthropometric standardisation and quality control protocols for the construction of new, international, fetal and newborn growth standards: the INTERGROWTH-21st Project. *Bjog*, 120 Suppl 2, 48-55, v.
- CHEN, H. H., CHIANG, Y. C., YUAN, Z. F., KUO, C. C., LAI, M. D., HUNG, T. W., HO, I. K. & CHEN, S. T. 2015. Buprenorphine, methadone, and morphine treatment during pregnancy: behavioral effects on the offspring in rats. *Neuropsychiatr Dis Treat*, 11, 609-18.
- CHEONG, J. L., HUNT, R. W., ANDERSON, P. J., HOWARD, K., THOMPSON, D. K., WANG, H. X., BEAR, M. J., INDER, T. E. & DOYLE, L. W. 2008. Head growth in preterm infants: correlation with magnetic resonance imaging and neurodevelopmental outcome. *Pediatrics*, 121, e1534-40.
- CHOU, R., FANCIULLO, G. J., FINE, P. G., ADLER, J. A., BALLANTYNE, J. C., DAVIES, P., DONOVAN, M. I., FISHBAIN, D. A., FOLEY, K. M., FUDIN, J., GILSON, A. M., KELTER, A., MAUSKOP, A., O'CONNOR, P. G., PASSIK, S. D., PASTERNAK, G. W., PORTENOY, R. K., RICH, B. A., ROBERTS, R. G., TODD, K. H. & MIASKOWSKI, C. 2009. Clinical guidelines for the use of chronic opioid therapy in chronic noncancer pain. *J Pain*, 10, 113-30.
- COHEN, M. C., MORLEY, S. R. & COOMBS, R. C. 2015. Maternal use of methadone and risk of sudden neonatal death. *Acta Paediatrica*, 104, 883-887.
- CONRADT, E., LAGASSE, L. L., SHANKARAN, S., BADA, H., BAUER, C. R., WHITAKER, T. M., HAMMOND, J. A. & LESTER, B. M. 2014. Physiological correlates of neurobehavioral disinhibition that relate to drug use and risky sexual behavior in adolescents with prenatal substance exposure. *Dev Neurosci*, 36, 306-15.
- CONWAY, K. P., COMPTON, W., STINSON, F. S. & GRANT, B. F. 2006. Lifetime comorbidity of DSM-IV mood and anxiety disorders and specific drug use disorders: results from the National Epidemiologic Survey on Alcohol and Related Conditions. *J Clin Psychiatry*, 67, 247-57.
- COUNSELL, S. J., BALL, G. & EDWARDS, A. D. 2014. New imaging approaches to evaluate newborn brain injury and their role in predicting developmental disorders. *Curr Opin Neurol*, 27, 168-75.

- CRIME, U. N. O. O. D. A. 2017. United Nations Office on Drugs and Crime, World Drug Report 2017. (ISBN:978-92-1-14829-1, eISBN:978-92-1-060623-3, United Nations publication Sales No. E.17.XI.6).
- CUNHA, G. R., SINCLAIR, A., RISBRIDGER, G., HUTSON, J. & BASKIN, L. S. 2015. Current understanding of hypospadias: relevance of animal models. *Nat Rev Urol*, 12, 271-80.
- DASHE, J. S., JACKSON, G. L., OLSCHER, D. A., ZANE, E. H. & WENDEL, G. D., JR. 1998. Opioid detoxification in pregnancy. *Obstet Gynecol*, 92, 854-8.
- DAVIDSON WARD, S. L., BAUTISTA, D., CHAN, L., DERRY, M., LISBIN, A., DURFEE, M. J., MILLS, K. S. C. & KEENS, T. G. 1990. Sudden infant death syndrome in infants of substance-abusing mothers. *The Journal of Pediatrics*, 117, 876-881.
- DAVIS, C. P., FRANKLIN, L. M., JOHNSON, G. S. & SCHROTT, L. M. 2010. Prenatal oxycodone exposure impairs spatial learning and/or memory in rats. *Behav Brain Res*. Netherlands: 2010 Elsevier B.V.
- DAVIS, D. D. & TEMPLER, D. I. 1988. Neurobehavioral functioning in children exposed to narcotics in utero. *Addictive Behaviors*, 13, 275-283.
- DE CUBAS, M. M. & FIELD, T. 1993. Children of methadone-dependent women: developmental outcomes. *American Journal of Orthopsychiatry*, 63, 266-76.
- DE VRIES, T. J., VAN VLIET, B. J., HOGENBOOM, F., WARDEH, G., VAN DER LAAN, J. W., MULDER, A. H. & SCHOFFELMEER, A. N. 1991. Effect of chronic prenatal morphine treatment of mu-opioid receptor-regulated adenylate cyclase activity and neurotransmitter release in rat brain slices. *European journal of pharmacology*, 208, 97.
- DELANO, K., GARERI, J. & KOREN, G. 2013. Rates of fetal polydrug exposures in methadone-maintained pregnancies from a high-risk population. *PLoS ONE [Electronic Resource]*, 8, e82647.
- DELGADO, J., TORO, R., RASCOVSKY, S., ARANGO, A., ANGEL, G. J., CALVO, V. & DELGADO, J. A. 2015. Chloral hydrate in pediatric magnetic resonance imaging: evaluation of a 10-year sedation experience administered by radiologists. *Pediatr Radiol*, 45, 108-14.
- DINIERI, J. A., WANG, X., SZUTORISZ, H., SPANO, S. M., KAUR, J., CASACCIA, P., DOW-EDWARDS, D. & HURD, Y. L. 2011. Maternal Cannabis Use Alters Ventral Striatal Dopamine D2 Gene Regulation in the Offspring. *Biological Psychiatry*, 70, 763-769.
- DOBERCZAK, T. M., SHANZER, S., CUTLER, R., SENIE, R. T., LOUCOPOULOS, J. A. & KANDALL, S. R. 1988. One-year follow-up of infants with abstinence-associated seizures. *Arch Neurol*, 45, 649-53.
- DROBLENKOV, A. V., KARELINA, N. R. & SHABANOV, P. D. 2010. Changes in neurons and gliocytes in the mesoaccumbocingulate system on perinatal exposure to morphine in rats. *Neurosci Behav Physiol*, 40, 848-51.
- DRUGS, A. C. O. T. M. O. 2003. Hidden Harm - responding to the needs of children of problem drug users. Advisory Council on the Misuse of Drugs.
- DRYDEN, C., YOUNG, D., HEPBURN, M. & MACTIER, H. 2009. Maternal methadone use in pregnancy: factors associated with the development of neonatal

- abstinence syndrome and implications for healthcare resources. *BJOG: An International Journal of Obstetrics & Gynaecology*, 116, 665-671.
- DUBOIS, J., DEHAENE-LAMBERTZ, G., KULIKOVA, S., POUPON, C., HUPPI, P. S. & HERTZ-PANNIER, L. 2014. The early development of brain white matter: A review of imaging studies in fetuses, newborns and infants. *Neuroscience*, 276, 48.
- DUERDEN, E. G., FOONG, J., CHAU, V., BRANSON, H., POSKITT, K. J., GRUNAU, R. E., SYNNEs, A., ZWICKER, J. G. & MILLER, S. P. 2015. Tract-Based Spatial Statistics in Preterm-Born Neonates Predicts Cognitive and Motor Outcomes at 18 Months. *AJNR Am J Neuroradiol*, 36, 1565-71.
- DUERDEN, E. G., GUO, T., DODBIBA, L., CHAKRAVARTY, M. M., CHAU, V., POSKITT, K. J., SYNNEs, A., GRUNAU, R. E. & MILLER, S. P. 2016. Midazolam dose correlates with abnormal hippocampal growth and neurodevelopmental outcome in preterm infants. *Ann Neurol*, 79, 548-59.
- DUTRIEZ-CASTELOOT, I., BERNET, F., DEDIEU, J. F., CROIX, D., LABORIE, C., MONTEL, V., LESAGE, J., BEAUVILLAIN, J. C. & DUPOUY, J. P. 1999. Hypothalamic-pituitary-adrenocortical and gonadal axes and sympathoadrenal activity of adult male rats prenatally exposed to morphine. *Neurosci Lett*, 263, 1-4.
- EISCH, A. J., BARROT, M., SCHAD, C. A., SELF, D. W. & NESTLER, E. J. 2000. Opiates Inhibit Neurogenesis in the Adult Rat Hippocampus. *Proceedings of the National Academy of Sciences of the United States of America*, 97, 7579-7584.
- ENTERS, E. K., GUO, H. Z., PANDEY, U., KO, D. J. & ROBINSON, S. E. 1990. The effect of prenatal methadone exposure of development and nociception during the early postnatal period of the rat. *NIDA Res Monogr*, 105, 376-7.
- ESCHENROEDER, A. C., VESTAL-LABORDE, A. A., SANCHEZ, E. S., ROBINSON, S. E. & SATO-BIGBEE, C. 2012. Oligodendrocyte responses to buprenorphine uncover novel and opposing roles of μ -opioid- and nociceptin/orphanin FQ receptors in cell development: Implications for drug addiction treatment during pregnancy. *Glia*, 60, 125-136.
- FELDMAN, H. M., YEATMAN, J. D., LEE, E. S., BARDE, L. H. & GAMAN-BEAN, S. 2010. Diffusion tensor imaging: a review for pediatric researchers and clinicians. *J Dev Behav Pediatr*, 31, 346-56.
- FIELDS, R. D. 2008. Oligodendrocytes changing the rules: action potentials in glia and oligodendrocytes controlling action potentials. *The Neuroscientist : a review journal bringing neurobiology, neurology and psychiatry*, 14, 540.
- FINNEMORE, A., TOULMIN, H., MERCHANT, N., ARICHI, T., TUSOR, N., COX, D., EDERIES, A., NONGENA, P., KO, C., DIAS, R., EDWARDS, A. D. & GROVES, A. M. 2014. Chloral hydrate sedation for magnetic resonance imaging in newborn infants. *Paediatr Anaesth*, 24, 190-5.
- FRODL, T. & SKOKAUSKAS, N. 2012. Meta-analysis of structural MRI studies in children and adults with attention deficit hyperactivity disorder indicates treatment effects. *Acta Psychiatr Scand*, 125, 114-26.
- FUNDARO, C., FIGLIOLA BALDIERI, N., MICCINESI, N., GENOVESE, O., RENDELI, C. & SALVUCCI, S. 2002. Prenatal drug abuse exposure: Long-term developmental outcome of child. *Acta Medica Romana*, 40, 69-80.

- GAILLARD, M. C. & BORRUAT, F. X. 2002. [New finding: transitory horizontal pendular nystagmus secondary to neonatal abstinence syndrome]. *Klin Monbl Augenheilkd*, 219, 317-9.
- GARERI, J., LYNN, H., HANDLEY, M., RAO, C. & KOREN, G. 2008. Prevalence of fetal ethanol exposure in a regional population-based sample by meconium analysis of fatty acid ethyl esters. *Therapeutic Drug Monitoring*, 30, 239-245.
- GLANTZ, M. & CHAMBERS, J. 2006. Prenatal drug exposure effects on subsequent vulnerability to drug abuse. *Development and Psychopathology*, 18, 893-922.
- GLEESON, J., EOGAN, M. & CLEARY, B. 2017. Detoxification from opiates during pregnancy: additional risks.
- GRAY, T. R., CHOO, R. E., CONCHEIRO, M., WILLIAMS, E., ELKO, A., JANSSON, L. M., JONES, H. E. & HUESTIS, M. A. 2010. Prenatal methadone exposure, meconium biomarker concentrations and neonatal abstinence syndrome. *Addiction*, 105, 2151-9.
- GREVEN, C. U., BRALTEN, J., MENNES, M., O'DWYER, L., VAN HULZEN, K. J., ROMMELSE, N., SCHWEREN, L. J., HOEKSTRA, P. J., HARTMAN, C. A., HESLENFELD, D., OOSTERLAAN, J., FARAONE, S. V., FRANKE, B., ZWIERS, M. P., ARIAS-VASQUEZ, A. & BUITELAAR, J. K. 2015. Developmentally stable whole-brain volume reductions and developmentally sensitive caudate and putamen volume alterations in those with attention-deficit/hyperactivity disorder and their unaffected siblings. *JAMA Psychiatry*, 72, 490-9.
- GUO, H. Z., ENTERS, E. K., MCDOWELL, K. P. & ROBINSON, S. E. 1990. The effect of prenatal exposure to methadone on neurotransmitters in neonatal rats. *Brain Res Dev Brain Res*, 57, 296-8.
- GUPTA, M., MULVIHILL, A. O., LASCARATOS, G., FLECK, B. W. & GEORGE, N. D. 2012. Nystagmus and reduced visual acuity secondary to drug exposure in utero: long-term follow-up. *J Pediatr Ophthalmol Strabismus*, 49, 58-63.
- GUPTA, S. N., GUPTA, V. S. & WHITE, A. C. 2016. Spectrum of intracranial incidental findings on pediatric brain magnetic resonance imaging: What clinician should know? *World J Clin Pediatr*, 5, 262-72.
- GUYATT, G., OXMAN, A. D., AKL, E. A., KUNZ, R., VIST, G., BROZEK, J., NORRIS, S., FALCK-YTTER, Y., GLASZIOU, P., DEBEER, H., JAESCHKE, R., RIND, D., MEERPOHL, J., DAHM, P. & SCHUNEMANN, H. J. 2011a. GRADE guidelines: 1. Introduction-GRADE evidence profiles and summary of findings tables. *J Clin Epidemiol*, 64, 383-94.
- GUYATT, G. H., OXMAN, A. D., VIST, G., KUNZ, R., BROZEK, J., ALONSO-COELLO, P., MONTORI, V., AKL, E. A., DJULBEGOVIC, B., FALCK-YTTER, Y., NORRIS, S. L., WILLIAMS, J. W., JR., ATKINS, D., MEERPOHL, J. & SCHUNEMANN, H. J. 2011b. GRADE guidelines: 4. Rating the quality of evidence--study limitations (risk of bias). *J Clin Epidemiol*, 64, 407-15.
- GYARMATHY, V. A., GIRAUDON, I., HEDRICH, D., MONTANARI, L., GUARITA, B. & WIESSING, L. 2009. Drug use and pregnancy - challenges for public health. *Euro Surveill*, 14, 33-6.
- HAABREKKE, K. J., SLINNING, K., WALHOVD, K. B., WENTZEL-LARSEN, T. & MOE, V. 2014. The Perinatal Outcome of Children Born to Women With Substance

- Dependence Detoxified in Residential Treatment During Pregnancy. *Journal of Addictive Diseases*, 33, 114-123.
- HAMILTON, R., MCGLONE, L., MACKINNON, J. R., RUSSELL, H. C., BRADNAM, M. S. & MACTIER, H. 2010. Ophthalmic, clinical and visual electrophysiological findings in children born to mothers prescribed substitute methadone in pregnancy. *British Journal of Ophthalmology*, 94, 696-700.
- HANS, S. L. 1999. Demographic and psychosocial characteristics of substance-abusing pregnant women. *Clin Perinatol*, 26, 55-74.
- HANS, S. L. & JEREMY, R. J. 2001. Postneonatal mental and motor development of infants exposed in utero to opioid drugs. *Infant Mental Health Journal*, 22, 300-315.
- HARLAN, R. E. & SONG, D. D. 1994. Prenatal morphine treatment and the development of the striatum. *Regulatory Peptides*, 54, 117-118.
- HASAN, K. M., ALEXANDER, A. L. & NARAYANA, P. A. 2004. Does fractional anisotropy have better noise immunity characteristics than relative anisotropy in diffusion tensor MRI? An analytical approach. *Magn Reson Med*, 51, 413-7.
- HAUSER, K. F., GURWELL, J. A. & TURBEK, C. S. 1994. Morphine inhibits Purkinje cell survival and dendritic differentiation in organotypic cultures of the mouse cerebellum. *Exp Neurol*, 130, 95-105.
- HAUSER, K. F. & KNAPP, P. E. 2018. Opiate Drugs with Abuse Liability Hijack the Endogenous Opioid System to Disrupt Neuronal and Glial Maturation in the Central Nervous System. *Frontiers in Pediatrics*, 5.
- HENSLEY, L., SULO, S., KOZMIC, S. & PARILLA, B. V. 2018. Opioid Addiction in Pregnancy: Does Depression Negatively Impact Adherence With Prenatal Care? *J Addict Med*, 12, 61-64.
- HILLENBRAND, C. & REYKOWSKI, A. 2011. MR Imaging of the Newborn: A Technical Perspective. *Magnetic Resonance Imaging Clinics of North America*.
- HIMES, S. K., DUKES, K. A., TRIPP, T., PETERSEN, J. M., RAFFO, C., BURD, L., ODENDAAL, H., ELLIOTT, A. J., HERELD, D., SIGNORE, C., WILLINGER, M. & HUESTIS, M. A. 2015. Clinical sensitivity and specificity of meconium fatty acid ethyl ester, ethyl glucuronide, and ethyl sulfate for detecting maternal drinking during pregnancy. *Clin Chem*, 61, 523-32.
- HSHIEH, T. T., FOX, M. L., KOSAR, C. M., CAVALLARI, M., GUTTMANN, C. R., ALSOP, D., MARCANTONIO, E. R., SCHMITT, E. M., JONES, R. N. & INOUE, S. K. 2016. Head circumference as a useful surrogate for intracranial volume in older adults. *Int Psychogeriatr*, 28, 157-62.
- HU, S., SHENG, W. S., LOKENSGARD, J. R. & PETERSON, P. K. 2002. Morphine induces apoptosis of human microglia and neurons. *Neuropharmacology*, 42, 829-836.
- HULSE, G. K., MILNE, E., ENGLISH, D. R. & HOLMAN, C. D. 1997. The relationship between maternal use of heroin and methadone and infant birth weight. *Addiction*, 92, 1571-9.
- HUNT, R. W., TZIOUMI, D., COLLINS, E. & JEFFERY, H. E. 2008. Adverse neurodevelopmental outcome of infants exposed to opiate in-utero. *Early Human Development*, 84, 29-35.

- HUPPI, P. S. & DUBOIS, J. 2006. Diffusion tensor imaging of brain development. *Semin Fetal Neonatal Med*, 11, 489-97.
- HUTCHINSON, M. R., SHAVIT, Y., GRACE, P. M., RICE, K. C., MAIER, S. F. & WATKINS, L. R. 2011. Exploring the neuroimmunopharmacology of opioids: an integrative review of mechanisms of central immune signaling and their implications for opioid analgesia. *Pharmacol Rev*, 63, 772-810.
- IBRAHIM, T., FEW, K., GREENWOOD, R., SMITH, C., MALCOLM, P., JOHNSON, G., LALLY, P., THAYYIL, S. & CLARKE, P. 2015. 'Feed and wrap' or sedate and immobilise for neonatal brain MRI? *Arch Dis Child Fetal Neonatal Ed*, 100, F465-6.
- ISD SCOTLAND, N. 2014. Estimating the national and local prevalence of problem drug use in Scotland 2012/2013. *In: DIVISION, I. S. (ed.)*.
- JANE-LLOPIS, E. & MATYTSINA, I. 2006. Mental health and alcohol, drugs and tobacco: a review of the comorbidity between mental disorders and the use of alcohol, tobacco and illicit drugs. *Drug Alcohol Rev*, 25, 515-36.
- JANSEN, P. R., DREMMEN, M., VAN DEN BERG, A., DEKKERS, I. A., BLANKEN, L. M. E., MUETZEL, R. L., BOLHUIS, K., MULDER, R. M., KOCEVSKA, D., JANSEN, T. A., DE WIT, M. Y., NEUTEBOOM, R. F., POLDERMAN, T. J. C., POSTHUMA, D., JADDOE, V. W. V., VERHULST, F. C., TIEMEIER, H., VAN DER LUGT, A. & WHITE, T. J. H. 2017. Incidental Findings on Brain Imaging in the General Pediatric Population. *N Engl J Med*, 377, 1593-1595.
- JANSSON, L. M., DI PIETRO, J. A., ELKO, A., WILLIAMS, E. L., MILIO, L. & VELEZ, M. 2012. Pregnancies exposed to methadone, methadone and other illicit substances, and poly-drugs without methadone: a comparison of fetal neurobehaviors and infant outcomes. *Drug Alcohol Depend*, 122, 213-9.
- JANSSON, L. M., DIPIETRO, J. & ELKO, A. 2005. Fetal response to maternal methadone administration. *Am J Obstet Gynecol*, 193, 611-7.
- JANSSON, L. M., DIPIETRO, J. A., VELEZ, M., ELKO, A., KNAUER, H. & KIVLIGHAN, K. T. 2009. Maternal methadone dosing schedule and fetal neurobehaviour. *J Matern Fetal Neonatal Med*, 22, 29-35.
- JANSSON, L. M., DIPIETRO, J. A., VELEZ, M., ELKO, A., WILLIAMS, E., MILIO, L., O'GRADY, K. & JONES, H. E. 2011. Fetal neurobehavioral effects of exposure to methadone or buprenorphine. *Neurotoxicol Teratol*, 33, 240-3.
- JEFFERIES, A. L. 2011. Selective serotonin reuptake inhibitors in pregnancy and infant outcomes. *Paediatr Child Health*, 16, 562-3.
- JOHNSON, J. L. & LEFF, M. 1999. Children of substance abusers: overview of research findings. *Pediatrics*, 103, 1085-99.
- JONES, H. E., KALTENBACH, K., HEIL, S. H., STINE, S. M., COYLE, M. G., ARRIA, A. M., O'GRADY, K. E., SELBY, P., MARTIN, P. R. & FISCHER, G. 2010. Neonatal abstinence syndrome after methadone or buprenorphine exposure. *N Engl J Med*, 363, 2320-31.
- JONES, H. E., O'GRADY, K. E., MALFI, D. & TUTEN, M. 2008. Methadone maintenance vs. methadone taper during pregnancy: maternal and neonatal outcomes. *Am J Addict*, 17, 372-86.

- JONES, H. E., TERPLAN, M. & MEYER, M. 2017. Medically Assisted Withdrawal (Detoxification): Considering the Mother-Infant Dyad. *J Addict Med*, 11, 90-92.
- JUNG, G., SCHRODER, R., LANFERMANN, H., JACOBS, A., SZELIES, B. & SCHRODER, R. 1997. Evidence of acute demyelination around a developmental venous anomaly: magnetic resonance imaging findings. *Invest Radiol*, 32, 575-7.
- KAISER, D., LEACH, J., VANNEST, J., SCHAPIRO, M. & HOLLAND, S. 2015. Unanticipated findings in pediatric neuroimaging research: prevalence of abnormalities and process for reporting and clinical follow-up. *Brain Imaging Behav*, 9, 32-42.
- KALLEN, B., BORG, N. & REIS, M. 2013. The use of central nervous system active drugs during pregnancy. *Pharmaceuticals (Basel)*, 6, 1221-86.
- KALTENBACH, K. & FINNEGAN, L. P. Neonatal abstinence syndrome, pharmacotherapy and developmental outcome. *Neurobehavioral Toxicology & Teratology*, 8, 353-5.
- KALTENBACH, K. & FINNEGAN, L. P. 1987. Perinatal and developmental outcome of infants exposed to methadone in-utero. *Neurotoxicology & Teratology*, 9, 311-3.
- KALTENBACH, K. & FINNEGAN, L. P. 1989. Children exposed to methadone in utero: Assessment of developmental and cognitive ability. *Annals of the New York Academy of Sciences*, 562, 360-362.
- KALTENBACH, K., GRAZIANI, L. J. & FINNEGAN, L. P. 1979. Methadone exposure in utero: developmental status at one and two years of age. *Pharmacology Biochemistry and Behavior*, 11, 15-17.
- KANDALL, S. R., ALBIN, S., GARTNER, L. M., LEE, K. S., EIDELMAN, A. & LOWINSON, J. 1977. The narcotic-dependent mother: fetal and neonatal consequences. *Early Hum Dev*, 1, 159-69.
- KANDALL, S. R., ALBIN, S., LOWINSON, J., BERLE, B., EIDELMAN, A. I. & GARTNER, L. M. 1976. Differential effects of maternal heroin and methadone use on birthweight. *Pediatrics*, 58, 681-5.
- KANDALL, S. R., GAINES, J., HABEL, L., DAVIDSON, G. & JESSOP, D. 1993. Relationship of maternal substance abuse to subsequent sudden infant death syndrome in offspring. *Journal of Pediatrics*, 123, 120-6.
- KAYE, A. D., JONES, M. R., KAYE, A. M., RIPOLL, J. G., GALAN, V., BEAKLEY, B. D., CALIXTO, F., BOLDEN, J. L., URMAN, R. D. & MANCHIKANTI, L. 2017. Prescription Opioid Abuse in Chronic Pain: An Updated Review of Opioid Abuse Predictors and Strategies to Curb Opioid Abuse: Part 1. *Pain Physician*, 20, S93-s109.
- KELLY, L., DOOLEY, J., CROMARTY, H., MINTY, B., MORGAN, A., MADDEN, S. & HOPMAN, W. 2011. Narcotic-exposed neonates in a First Nations population in northwestern Ontario: incidence and implications. *Can Fam Physician*, 57, e441-7.
- KELLY, R., ZATZICK, D. & ANDERS, T. 2001. The detection and treatment of psychiatric disorders and substance use among pregnant women cared for in obstetrics. *Am J Psychiatry*, 158, 213-9.

- KELTY, E. & HULSE, G. 2017. A Retrospective Cohort Study of Birth Outcomes in Neonates Exposed to Naltrexone in Utero: A Comparison with Methadone-, Buprenorphine- and Non-opioid-Exposed Neonates. *Drugs*, 77, 1211-1219.
- KEUNEN, K., ISGUM, I., VAN KOOIJ, B. J., ANBEEK, P., VAN HAASTERT, I. C., KOOPMAN-ESSEBOOM, C., FIERET-VAN STAM, P. C., NIEVELSTEIN, R. A., VIERGEVER, M. A., DE VRIES, L. S., GROENENDAAL, F. & BENDERS, M. J. 2016. Brain Volumes at Term-Equivalent Age in Preterm Infants: Imaging Biomarkers for Neurodevelopmental Outcome through Early School Age. *J Pediatr*, 172, 88-95.
- KINNEY, H. C., BRODY, B. A., FINKELSTEIN, D. M., VAWTER, G. F., MANDELL, F. & GILLES, F. H. 1991. Delayed central nervous system myelination in the sudden infant death syndrome. *J Neuropathol Exp Neurol*, 50, 29-48.
- KLINGER, G. & MERLOB, P. 2008. Selective serotonin reuptake inhibitor induced neonatal abstinence syndrome. *Isr J Psychiatry Relat Sci*, 45, 107-13.
- KNAPP, P. E. & HAUSER, K. F. 1996. mu-Opioid receptor activation enhances DNA synthesis in immature oligodendrocytes. *Brain Res*. Netherlands.
- KNAPP, P. E., MADERSPACH, K. & HAUSER, K. F. 1998. Endogenous opioid system in developing normal and jimpy oligodendrocytes: mu and kappa opioid receptors mediate differential mitogenic and growth responses. *Glia*, 22, 189-201.
- KOCHERLAKOTA, P. 2014. Neonatal abstinence syndrome. *Pediatrics*, 134, e547-61.
- KONIJNENBERG, C. 2015. Methodological issues in assessing the impact of prenatal drug exposure. *Substance Abuse: Research and Treatment*, 9, 39-44.
- KONIJNENBERG, C., LUND, I. O. & MELINDER, A. 2015. Behavioural outcomes of four-year-old children prenatally exposed to methadone or buprenorphine: A test of three risk models. *Early Child Development and Care*, 185, 1641-1657.
- KONIJNENBERG, C. & MELINDER, A. 2011. Prenatal exposure to methadone and buprenorphine: a review of the potential effects on cognitive development. *Child Neuropsychol*, 17, 495-519.
- KONIJNENBERG, C. & MELINDER, A. 2015. Executive function in preschool children prenatally exposed to methadone or buprenorphine. *Child Neuropsychology*, 21, 570-585.
- KREITINGER, C., GUTIERREZ, H., HAMIDOVIC, A., SARANGARM, P., WOHLERT, E., STEPHENS, E., LEEMAN, L. & BAKHIREVA, L. 2013. Effect of prenatal alcohol co-exposure and other factors on neonatal abstinence syndrome in infants born to opioid-dependent mothers. *Pharmacotherapy*, 33 (10), e294.
- KRISTINE, B. W., VIBEKE, M., KARI, S., TORILL, S., ANDERS, M. F., ASTRID, B. & LARS, S. 2009. Effects of prenatal opiate exposure on brain development – a call for attention. *Nature Reviews Neuroscience*, 10, 390.
- KUNZ, N., ZHANG, H., VASUNG, L., O'BRIEN, K. R., ASSAF, Y., LAZEYRAS, F., ALEXANDER, D. C. & HUPPI, P. S. 2014. Assessing white matter microstructure of the newborn with multi-shell diffusion MRI and biophysical compartment models. *Neuroimage*, 96, 288-99.
- LABORIE, C., DUTRIEZ-CASTELOOT, I., MONTEL, V., DICKES-COOPMAN, A., LESAGE, J. & VIEAU, D. 2005. Prenatal morphine exposure affects sympathoadrenal axis

- activity and serotonin metabolism in adult male rats both under basal conditions and after an ether inhalation stress. *Neurosci Lett*, 381, 211-6.
- LACROIX, I., BERREBI, A., GARIPUY, D., SCHMITT, L., HAMMOU, Y., CHAUMERLIAC, C., LAPEYRE-MESTRE, M., MONTASTRUC, J. L. & DAMASE-MICHEL, C. 2011. Buprenorphine versus methadone in pregnant opioid-dependent women: a prospective multicenter study. *Eur J Clin Pharmacol*, 67, 1053-9.
- LASLO, J., BRUNNER, J. M., BURNS, D., BUTLER, E., CUNNINGHAM, A., KILLPACK, R., PYERITZ, C., RINARD, K., CHILDERS, J. & HORZEMPA, J. 2017. An overview of available drugs for management of opioid abuse during pregnancy. *Matern Health Neonatol Perinatol*, 3, 4.
- LESTER, B. M. & LAGASSE, L. L. 2010. Children of addicted women. *J Addict Dis*. England.
- LI, W., LI, Q., WANG, Y., ZHU, J., YE, J., YAN, X., LI, Y., CHEN, J., LIU, J., LI, Z., WANG, W. & LIU, Y. 2016a. Methadone-induced Damage to White Matter Integrity in Methadone Maintenance Patients: A Longitudinal Self-control DTI Study. *Sci Rep*, 6, 19662.
- LI, W., LI, Q., ZHU, J., QIN, Y., ZHENG, Y., CHANG, H., ZHANG, D., WANG, H., WANG, L., WANG, Y. & WANG, W. 2013. White matter impairment in chronic heroin dependence: a quantitative DTI study. *Brain Res*, 1531, 58-64.
- LI, W., ZHU, J., LI, Q., YE, J., CHEN, J., LIU, J., LI, Z., LI, Y., YAN, X., WANG, Y. & WANG, W. 2016b. Brain white matter integrity in heroin addicts during methadone maintenance treatment is related to relapse propensity. *Brain Behav*, 6, e00436.
- LIBERATI, A., ALTMAN, D. G., TETZLAFF, J., MULROW, C., GOTZSCHE, P. C., IOANNIDIS, J. P., CLARKE, M., DEVEREAUX, P. J., KLEIJNEN, J. & MOHER, D. 2009. The PRISMA statement for reporting systematic reviews and meta-analyses of studies that evaluate healthcare interventions: explanation and elaboration. *Bmj*, 339, b2700.
- LIFSCHITZ, M. H., WILSON, G. S., O'BRIAN SMITH, E. & DESMOND, M. M. 1985. Factors affecting head growth and intellectual function in children of drug addicts. *Pediatrics*, 75, 269-274.
- LIND, J. N., INTERRANTE, J. D., AILES, E. C., GILBOA, S. M., KHAN, S., FREY, M. T., DAWSON, A. L., HONEIN, M. A., DOWLING, N. F., RAZZAGHI, H., CREANGA, A. A. & BROUSSARD, C. S. 2017. Maternal Use of Opioids During Pregnancy and Congenital Malformations: A Systematic Review. *Pediatrics*, 139.
- LINSCOTT, L. L., LEACH, J. L., ZHANG, B. & JONES, B. V. 2014. Brain parenchymal signal abnormalities associated with developmental venous anomalies in children and young adults. *AJNR Am J Neuroradiol*, 35, 1600-7.
- LIU, H., HAO, Y., KANEKO, Y., OUYANG, X., ZHANG, Y., XU, L., XUE, Z. & LIU, Z. 2009. Frontal and cingulate gray matter volume reduction in heroin dependence: optimized voxel-based morphometry. *Psychiatry Clin Neurosci*. Australia.
- LIU, H., LI, L., HAO, Y., CAO, D., XU, L., ROHRBAUGH, R., XUE, Z., HAO, W., SHAN, B. & LIU, Z. 2008. Disrupted white matter integrity in heroin dependence: a controlled study utilizing diffusion tensor imaging. *Am J Drug Alcohol Abuse*, 34, 562-75.

- LOBSIEN, D., ETRICH, B., SOTIRIOU, K., CLASSEN, J., THEN BERGH, F. & HOFFMANN, K. T. 2014. Whole-brain diffusion tensor imaging in correlation to visual-evoked potentials in multiple sclerosis: a tract-based spatial statistics analysis. *AJNR Am J Neuroradiol*, 35, 2076-81.
- LOH, W. Y., ANDERSON, P. J., CHEONG, J. L. Y., SPITTLE, A. J., CHEN, J., LEE, K. J., MOLESWORTH, C., INDER, T. E., CONNELLY, A., DOYLE, L. W. & THOMPSON, D. K. 2017. Neonatal basal ganglia and thalamic volumes: very preterm birth and 7-year neurodevelopmental outcomes. *Pediatr Res*, 82, 970-978.
- LOZANO, J., GARCIA-ALGAR, O., VALL, O., DE LA TORRE, R., SCARAVELLI, G. & PICHINI, S. 2007. Biological matrices for the evaluation of in utero exposure to drugs of abuse. *Therapeutic Drug Monitoring*, 29, 711-734.
- LUTY, J., NIKOLAOU, V. & BEARN, J. 2003. Is opiate detoxification unsafe in pregnancy? *Journal of Substance Abuse Treatment*, 24, 363-367.
- MA, M., CHEN, J. Y., PLOWEY, E. D., FISCHBEIN, N. & IV, M. 2017. Tumefactive demyelination associated with developmental venous anomaly: Report of two cases. *Clin Imaging*, 43, 194-198.
- MA, X., QIU, Y., TIAN, J., WANG, J., LI, S., ZHAN, W., WANG, T., ZENG, S., JIANG, G. & XU, Y. 2015. Aberrant Default-Mode Functional and Structural Connectivity in Heroin-Dependent Individuals.(Report). 10.
- MAAS, U., KATTNER, E., WEINGART-JESSE, B., SCHAFER, A. & OBLADEN, M. 1990. Infrequent neonatal opiate withdrawal following maternal methadone detoxification during pregnancy. *J Perinat Med*, 18, 111-8.
- MACTIER, H., SHIPTON, D., DRYDEN, C. & TAPPIN, D. M. 2014. Reduced fetal growth in methadone-maintained pregnancies is not fully explained by smoking or socio-economic deprivation. *Addiction*, 109, 482-8.
- MAKROPOULOS, A., GOUSIAS, I. S., LEDIG, C., ALJABAR, P., SERAG, A., HAJNAL, J. V., EDWARDS, A. D., COUNSELL, S. J. & RUECKERT, D. 2014. Automatic whole brain MRI segmentation of the developing neonatal brain. *IEEE Trans Med Imaging*, 33, 1818-31.
- MALOVA, M., ROSSI, A., SEVERINO, M., PARODI, A., MORANA, G., SANNIA, A., CAMA, A. & RAMENGI, L. A. 2017. Incidental findings on routine brain MRI scans in preterm infants. *Arch Dis Child Fetal Neonatal Ed*, 102, F73-f78.
- MARCUS, J., HANS, S. L. & JEREMY, R. J. 1982. Patterns of 1-day and 4-month motor functioning in infants of women on methadone. *Neurobehavioral Toxicology and Teratology*, 4, 473-476.
- MARTIN, T. J., KAHN, W. R., XIAO, R. & CHILDERS, S. R. 2007. Differential regional effects of methadone maintenance compared to heroin dependence on mu-opioid receptor desensitization in rat brain. *Synapse*, 61, 176-84.
- MATTICK, R. P., BREEN, C., KIMBER, J. & DAVOLI, M. 2009. Methadone maintenance therapy versus no opioid replacement therapy for opioid dependence. *Cochrane Database of Systematic Reviews*.
- MATTICK, R. P., BREEN, C., KIMBER, J. & DAVOLI, M. 2014. Buprenorphine maintenance versus placebo or methadone maintenance for opioid dependence. *Cochrane Database Syst Rev*, 2, Cd002207.

- MCCARTHY, J. J., LEAMON, M. H., FINNEGAN, L. P. & FASSBENDER, C. 2017. Opioid dependence and pregnancy: minimizing stress on the fetal brain. *Am J Obstet Gynecol*, 216, 226-231.
- MCCARTHY, J. J., LEAMON, M. H., WILLITS, N. H. & SALO, R. 2015. The effect of methadone dose regimen on neonatal abstinence syndrome. *J Addict Med*, 9, 105-10.
- MCCARTHY, M. 2015. US babies born in withdrawal from opioids quadruples in nine years, study finds. *BMJ*, 350, h2313.
- MCCARTHY, M. 2016. Doctors are urged to limit opioid prescribing. *BMJ*, 352, i1614.
- MCGLONE, L., HAMILTON, R., MCCULLOCH, D. L., BOULTON, R., BRADNAM, M. S., WEAVER, L. T. & MACTIER, H. 2013a. Neonatal visual evoked potentials in infants born to mothers prescribed methadone. *Pediatrics*, 131, e857-63.
- MCGLONE, L., HAMILTON, R., MCCULLOCH, D. L., MACKINNON, J. R., BRADNAM, M. & MACTIER, H. 2014. Visual outcome in infants born to drug-misusing mothers prescribed methadone in pregnancy. *British Journal of Ophthalmology*, 98, 238-45.
- MCGLONE, L. & MACTIER, H. 2015. Infants of opioid-dependent mothers: Neurodevelopment at six months. *Early Human Development*, 91, 19-21.
- MCGLONE, L., MACTIER, H., COOPER, G. & HASSAN, H. 2012. Alcohol and drug exposure in infants born to mothers prescribed methadone during pregnancy. *Archives of Disease in Childhood*, 97, A4-A5.
- MCGLONE, L., MACTIER, H., HAMILTON, R., BRADNAM, M. S., BOULTON, R., BORLAND, W., HEPBURN, M. & MCCULLOCH, D. L. 2008. Visual evoked potentials in infants exposed to methadone in utero. *Arch Dis Child*, 93, 784-6.
- MCGLONE, L., MACTIER, H., HASSAN, H. & COOPER, G. 2013b. In utero drug and alcohol exposure in infants born to mothers prescribed maintenance methadone. *Arch Dis Child Fetal Neonatal Ed*, 98, F542-4.
- MCQUIRE, C., PARANJOTHY, S., HURT, L., MANN, M., FAREWELL, D. & KEMP, A. 2016. Objective Measures of Prenatal Alcohol Exposure: A Systematic Review. *Pediatrics*, 138.
- METZ, V., JAGSCH, R., EBNER, N., WURZL, J., PRIBASNIG, A., ASCHAUER, C. & FISCHER, G. 2011. Impact of treatment approach on maternal and neonatal outcome in pregnant opioid-maintained women. *Hum Psychopharmacol*, 26, 412-21.
- MIGUEL-HIDALGO, J. J. 2009. The role of glial cells in drug abuse. *Curr Drug Abuse Rev*, 2, 72-82.
- MINOZZI, S., AMATO, L., BELLISARIO, C., FERRI, M. & DAVOLI, M. 2013. Maintenance agonist treatments for opiate-dependent pregnant women. *Cochrane Database Syst Rev*, CD006318.
- MITEW, S., HAY, C. M., PECKHAM, H., XIAO, J., KOENNING, M. & EMERY, B. 2014. Mechanisms regulating the development of oligodendrocytes and central nervous system myelin. *Neuroscience*, 276, 29.
- MOE, V. 2002. Foster-placed and adopted children exposed in utero to opiates and other substances: prediction and outcome at four and a half years. *J Dev Behav Pediatr*, 23, 330-9.

- MONNELLY, V. J., ANBLAGAN, D., QUIGLEY, A., CABEZ, M. B., COOPER, E. S., MACTIER, H., SEMPLE, S. I., BASTIN, M. E. & BOARDMAN, J. P. 2018. Prenatal methadone exposure is associated with altered neonatal brain development. *Neuroimage Clin*, 18, 9-14.
- MONSON, B. B., ANDERSON, P. J., MATTHEWS, L. G., NEIL, J. J., KAPUR, K., CHEONG, J. L., DOYLE, L. W., THOMPSON, D. K. & INDER, T. E. 2016. Examination of the Pattern of Growth of Cerebral Tissue Volumes From Hospital Discharge to Early Childhood in Very Preterm Infants. *JAMA Pediatr*, 170, 772-9.
- MOOG, N. K., ENTRINGER, S., RASMUSSEN, J. M., STYNER, M., GILMORE, J. H., KATHMANN, N., HEIM, C. M., WADHWA, P. D. & BUSS, C. 2018. Intergenerational Effect of Maternal Exposure to Childhood Maltreatment on Newborn Brain Anatomy. *Biol Psychiatry*, 83, 120-127.
- MORRIS, Z., WHITELEY, W. N., LONGSTRETH, W. T., JR., WEBER, F., LEE, Y. C., TSUSHIMA, Y., ALPHS, H., LADD, S. C., WARLOW, C., WARDLAW, J. M. & AL-SHAHI SALMAN, R. 2009. Incidental findings on brain magnetic resonance imaging: systematic review and meta-analysis. *Bmj*, 339, b3016.
- NARDUCCI, R., BARONCELLI, L., SANSEVERO, G., BEGENISIC, T., PRONTERA, C., SALE, A., CENNI, M. C., BERARDI, N. & MAFFEI, L. 2018. Early impoverished environment delays the maturation of cerebral cortex. *Sci Rep*, 8, 1187.
- NECHANSKA, B., MRAVCIK, V., SKURTVEIT, S., LUND, I. O., GABRHELIK, R., ENGELAND, A. & HANDAL, M. 2018. Neonatal Outcomes after Fetal Exposure to Methadone and Buprenorphine: National Registry Studies from the Czech Republic and Norway. *Addiction*.
- NELSON, L. B., EHRLICH, S. & CALHOUN, J. H. 1987. Occurrence of strabismus in infants born to drug-dependent women. *American Journal of Diseases of Children*, 141, 175-178.
- NIESINK, R. J. M., VAN BUREN-VAN DUINKERKEN, L. & VAN REE, J. M. 1999. Social behavior of juvenile rats after in utero exposure to morphine: dose–time–effect relationship. *Neuropharmacology*, 38, 1207-1223.
- NIJHUIS, J. G. 2003. Fetal behavior. *Neurobiology of Aging*, 24, S41-S46.
- NISHIOKA, C., LIANG, H. F., CHUNG, C. F. & SUN, S. W. 2017. Disease stage-dependent relationship between diffusion tensor imaging and electrophysiology of the visual system in a murine model of multiple sclerosis. *Neuroradiology*, 59, 1241-1250.
- NORGAARD, M., NIELSSON, M. S. & HEIDE-JORGENSEN, U. 2015. Birth and Neonatal Outcomes Following Opioid Use in Pregnancy: A Danish Population-Based Study. *Subst Abuse*, 9, 5-11.
- NULMAN, I., KOREN, G., ROVET, J., BARRERA, M., PULVER, A., STREINER, D. & FELDMAN, B. 2012. Neurodevelopment of children following prenatal exposure to venlafaxine, selective serotonin reuptake inhibitors, or untreated maternal depression. *Am J Psychiatry*, 169, 1165-74.
- NYGAARD, E., SLINNING, K., MOE, V. & WALHOVD, K. B. 2016. Behavior and Attention Problems in Eight-Year-Old Children with Prenatal Opiate and Poly-Substance Exposure: A Longitudinal Study. *PLoS One*, 11, e0158054.

- NYGAARD, E., SLINNING, K., MOE, V. & WALHOVD, K. B. 2017. Cognitive function of youths born to mothers with opioid and poly-substance abuse problems during pregnancy. *Child Neuropsychol*, 23, 159-187.
- O'GORMAN, R. L., BUCHER, H. U., HELD, U., KOLLER, B. M., HÜPPI, P. S. & HAGMANN, C. F. 2014. Tract-based spatial statistics to assess the neuroprotective effect of early erythropoietin on white matter development in preterm infants. *Brain: A Journal Of Neurology*.
- OLSEN, G. D. & LEES, M. H. 1980. Ventilatory response to carbon dioxide of infants following chronic prenatal methadone exposure. *Journal of Pediatrics*, 96, 983-9.
- ORNOY, A. 2003. The impact of intrauterine exposure versus postnatal environment in neurodevelopmental toxicity: long-term neurobehavioral studies in children at risk for developmental disorders. *Toxicol Lett*, 140-141, 171-81.
- ORNOY, A., DAKA, L., GOLDZWEIG, G., GIL, Y., MJEN, L., LEVIT, S., SHUFMAN, E., BAR-HAMBURGER, R. & GREENBAUM, C. W. 2010. Neurodevelopmental and psychological assessment of adolescents born to drug-addicted parents: effects of SES and adoption. *Child Abuse Negl*, 34, 354-68.
- ORNOY, A., MICHAILEVSKAYA, V., LUKASHOV, I., BAR-HAMBURGER, R. & HAREL, S. 1996. The developmental outcome of children born to heroin-dependent mothers, raised at home or adopted. *Child Abuse & Neglect*, 20, 385-396.
- ORNOY, A., SEGAL, J., BAR-HAMBURGER, R. & GREENBAUM, C. 2001. Developmental outcome of school-age children born to mothers with heroin dependency: importance of environmental factors. *Dev Med Child Neurol*, 43, 668-75.
- PASTO, M. E., GRAZIANI, L. J. & TUNIS, S. L. 1985. Ventricular configuration and cerebral growth in infants born to drug-dependent mothers. *Pediatric Radiology*, 15, 77-81.
- PATRICK, S. W., DAVIS, M. M., LEHMANN, C. U. & COOPER, W. O. 2015a. Increasing incidence and geographic distribution of neonatal abstinence syndrome: United States 2009 to 2012. *J Perinatol*, 35, 650-5.
- PATRICK, S. W., DUDLEY, J., MARTIN, P. R., HARRELL, F. E., WARREN, M. D., HARTMANN, K. E., ELY, E. W., GRIJALVA, C. G. & COOPER, W. O. 2015b. Prescription opioid epidemic and infant outcomes. *Pediatrics*, 135, 842.
- PATRICK, S. W., SCHUMACHER, R. E., BENNEYWORTH, B. D., KRANS, E. E., MCALLISTER, J. M. & DAVIS, M. M. 2012. Neonatal abstinence syndrome and associated health care expenditures: United States, 2000-2009. *Jama*, 307, 1934-40.
- PAUL, J. A., LOGAN, B. A., KRISHNAN, R., HELLER, N. A., MORRISON, D. G., PRITHAM, U. A., TISHER, P. W., TROESE, M., BROWN, M. S. & HAYES, M. J. 2014. Development of auditory event-related potentials in infants prenatally exposed to methadone. *Dev Psychobiol*, 56, 1119-28.
- PERTSCHUK, L. P. & SHER, J. H. 1975. Demonstration of methadone in the human brain by immunofluorescence. *Res Commun Chem Pathol Pharmacol*, 11, 319-22.
- PIERSON, P. S., HOWARD, P. & KLEBER, H. D. 1972. Sudden deaths in infants born to methadone-maintained addicts. *JAMA*, 220, 1733-1734.

- PORTER, E. J., COUNSELL, S. J., EDWARDS, A. D., ALLSOP, J. & AZZOPARDI, D. 2010. Tract-based spatial statistics of magnetic resonance images to assess disease and treatment effects in perinatal asphyxial encephalopathy. *Pediatr Res*, 68, 205-9.
- PRITHAM, U. A., PAUL, J. A. & HAYES, M. J. 2012. Opioid dependency in pregnancy and length of stay for neonatal abstinence syndrome. *J Obstet Gynecol Neonatal Nurs*, 41, 180-90.
- QIU, Y., JIANG, G., SU, H., LV, X., ZHANG, X., TIAN, J. & ZHUO, F. 2013. Progressive white matter microstructure damage in male chronic heroin dependent individuals: a DTI and TBSS study. *PLoS One*. United States.
- QIU, Y. W., SU, H. H., LV, X. F. & JIANG, G. H. 2015. Abnormal white matter integrity in chronic users of codeine-containing cough syrups: a tract-based spatial statistics study. *AJNR Am J Neuroradiol*, 36, 50-6.
- RAGNHILDUR, K., NICOLA, B. H., YAMINA, B. & DAVID, A. 2008. Spiking and nonspiking classes of oligodendrocyte precursor glia in CNS white matter. *Nature Neuroscience*, 11, 450.
- RAJEGOWDA, B. K., KANDALL, S. R. & FALCIGLIA, H. 1978. Sudden unexpected death in infants of narcotic-dependent mothers. *Early Hum Dev*, 2, 219-25.
- RAMER, C. & LODGE, A. 1975. Neonatal addiction: a two-year study. Part I. Clinical and developmental characteristics of infants of mothers on methadone maintenance. *Addictive diseases*, 2, 227-234.
- RAVNDAL, E. & AMUNDSEN, E. J. 2010. Mortality among drug users after discharge from inpatient treatment: an 8-year prospective study. *Drug Alcohol Depend*, 108, 65-9.
- REMENTERIA, J. L. & NUNAG, N. N. 1973. Narcotic withdrawal in pregnancy: stillbirth incidence with a case report. *Am J Obstet Gynecol*, 116, 1152-6.
- RICALDE, A. A. & HAMMER, R. P., JR. 1990. Perinatal opiate treatment delays growth of cortical dendrites. *Neurosci Lett*, 115, 137-43.
- ROBINSON, S. E., GUO, H., MAHER, J. R., MCDOWELL, K. P. & KUNKO, P. M. 1996. Postnatal methadone exposure does not prevent prenatal methadone-induced changes in striatal cholinergic neurons. *Developmental Brain Research*, 95, 118-121.
- ROBINSON, S. E., MAHER, J. R., WALLACE, M. J. & KUNKO, P. M. 1997. Perinatal methadone exposure affects dopamine, norepinephrine, and serotonin in the weanling rat. *Neurotoxicol Teratol*, 19, 295-303.
- ROGERS, D. M., PECKHAM, M. E., SHAH, L. M. & WIGGINS, R. H., 3RD 2018. Association of Developmental Venous Anomalies with Demyelinating Lesions in Patients with Multiple Sclerosis. *AJNR Am J Neuroradiol*, 39, 97-101.
- ROLLINS, C. K., ASARO, L. A., AKHONDI-ASL, A., KUSSMAN, B. D., RIVKIN, M. J., BELLINGER, D. C., WARFIELD, S. K., WYPIJ, D., NEWBURGER, J. W. & SOUL, J. S. 2017. White Matter Volume Predicts Language Development in Congenital Heart Disease. *J Pediatr*, 181, 42-48.e2.
- ROSEN, T. S. & JOHNSON, H. L. 1985. Long-term effects of prenatal methadone maintenance. *NIDA Research Monograph*, 59, 73-83.

- ROZISKY, J. R., MEDEIROS, L. F., ADACHI, L. S., ESPINOSA, J., DE SOUZA, A., NETO, A. S., BONAN, C. D., CAUMO, W. & TORRES, I. L. 2011. Morphine exposure in early life increases nociceptive behavior in a rat formalin tonic pain model in adult life. *Brain Res.* Netherlands: 2010 Elsevier B.V.
- RUTHERFORD, M., MALAMATENIOU, C., ZEKA, J. & COUNSELL, S. 2004. MR imaging of the neonatal brain at 3 Tesla. *Eur J Paediatr Neurol*, 8, 281-9.
- SADRAIE, S. H., KAKA, G. R., SAHRAEI, H., DASHTNAVAR, H., BAHADORAN, H., MOFID, M., NASAB, H. M. & JAFARI, F. 2008. Effects of maternal oral administration of morphine sulfate on developing rat fetal cerebrum: A morphometrical evaluation. *Brain Research*, 1245, 36-40.
- SAKELLARIDIS, N., MANGOURA, D. & VERNADAKIS, A. 1986. Effects of opiates on the growth of neuron-enriched cultures from chick embryonic brain. *Int J Dev Neurosci*, 4, 293-302.
- SALISBURY, A. L., COYLE, M. G., O'GRADY, K. E., HEIL, S. H., MARTIN, P. R., STINE, S. M., KALTENBACH, K., WENINGER, M. & JONES, H. E. 2012. Fetal assessment before and after dosing with buprenorphine or methadone. *Addiction*, 36-44.
- SALZWEDEL, A. P., GREWEN, K. M., VACHET, C., GERIG, G., LIN, W. & GAO, W. 2015. Prenatal Drug Exposure Affects Neonatal Brain Functional Connectivity. *The journal of neuroscience*, 35, 5860-5869.
- SANCHEZ, E. S., BIGBEE, J. W., FOBBS, W., ROBINSON, S. E. & SATO-BIGBEE, C. 2008. Opioid addiction and pregnancy: Perinatal exposure to buprenorphine affects myelination in the developing brain. *Glia*, 56, 1017-1027.
- SANDBERG, D. E., MEYER-BAHLBURG, H. F. L., ROSEN, T. S. & JOHNSON, H. L. 1990. Effects of prenatal methadone exposure on sex-dimorphic behavior in early school-age children. *Psychoneuroendocrinology*, 15, 77-82.
- SCHNEIDER, J. W. & HANS, S. L. 1996. Effects of prenatal exposure to opioids on focused attention in toddlers during free play. *Journal of Developmental and Behavioral Pediatrics*, 17, 240-247.
- SEATRIZ, J. V. & HAMMER, R. P. 1993. Effects of opiates on neuronal development in the rat cerebral cortex. *Brain Research Bulletin*, 30, 523-527.
- SERAG, A., BLESA, M., MOORE, E. J., PATAKY, R., SPARROW, S. A., WILKINSON, A. G., MACNAUGHT, G., SEMPLE, S. I. & BOARDMAN, J. P. 2016. Accurate Learning with Few Atlases (ALFA): an algorithm for MRI neonatal brain extraction and comparison with 11 publicly available methods. *Sci Rep*, 6, 23470.
- SERAG, A., WILKINSON, A. G., TELFORD, E. J., PATAKY, R., SPARROW, S. A., ANBLAGAN, D., MACNAUGHT, G., SEMPLE, S. I. & BOARDMAN, J. P. 2017. SEGMA: An Automatic SEGmentation Approach for Human Brain MRI Using Sliding Window and Random Forests. *Front Neuroinform*, 11, 2.
- SETANEN, S., LEHTONEN, L., PARKKOLA, R., AHO, K. & HAATAJA, L. 2016. Prediction of neuromotor outcome in infants born preterm at 11 years of age using volumetric neonatal magnetic resonance imaging and neurological examinations. *Dev Med Child Neurol*, 58, 721-7.
- SHARPE, C. & KUSCHEL, C. 2004. Outcomes of infants born to mothers receiving methadone for pain management in pregnancy. *Arch Dis Child Fetal Neonatal Ed*, 89, F33-6.

- SHEN, Y., WANG, E., WANG, X. & LOU, M. 2012. Disrupted integrity of white matter in heroin-addicted subjects at different abstinent time. *J Addict Med*, 6, 172-6.
- SIMMONS, M. L. & CHAVKIN, C. 1996. Endogenous opioid regulation of hippocampal function. *Int Rev Neurobiol*, 39, 145-96.
- SINGH, H. H., PUROHIT, V. & AHLUWALIA, B. S. 1980. Effect of methadone treatment during pregnancy on the fetal testes and hypothalamus in rats. *Biol Reprod*, 22, 480-5.
- SIRNES, E., GRIFFITHS, S. T., AUKLAND, S. M., EIDE, G. E., ELGEN, I. B. & GUNDERSEN, H. 2018. Functional MRI in prenatally opioid-exposed children during a working memory-selective attention task. *Neurotoxicol Teratol*.
- SIRNES, E., OLTEDAL, L., BARTSCH, H., EIDE, G. E., ELGEN, I. B. & AUKLAND, S. M. 2017. Brain morphology in school-aged children with prenatal opioid exposure: A structural MRI study. *Early Hum Dev*, 106-107, 33-39.
- SKINNER, M. L., HAGGERTY, K. P., FLEMING, C. B. & CATALANO, R. F. 2009. Predicting functional resilience among young-adult children of opiate-dependent parents. *J Adolesc Health*, 44, 283-90.
- SLINNING, K. 2004. Foster placed children prenatally exposed to poly-substances. *European Child & Adolescent Psychiatry*, 13, 19-27.
- SMITH, D. K., JOHNSON, A. B., PEARS, K. C., FISHER, P. A. & DEGARMO, D. S. 2007. Child maltreatment and foster care: unpacking the effects of prenatal and postnatal parental substance use. *Child Maltreat*, 12, 150-60.
- SMITH, K. & LIPARI, R. 2013. Women of Childbearing Age and Opioids. *The CBHSQ Report*. Rockville (MD): Substance Abuse and Mental Health Services Administration (US).
- SMITH, S. M., JENKINSON, M., JOHANSEN-BERG, H., RUECKERT, D., NICHOLS, T. E., MACKAY, C. E., WATKINS, K. E., CICCARELLI, O., CADER, M. Z., MATTHEWS, P. M. & BEHRENS, T. E. J. 2006. Tract-based spatial statistics: Voxelwise analysis of multi-subject diffusion data. *Neuroimage*, 31, 1487-1505.
- SMITH, S. M. & NICHOLS, T. E. 2009. Threshold-free cluster enhancement: addressing problems of smoothing, threshold dependence and localisation in cluster inference. *Neuroimage*, 44, 83-98.
- SOEPATMI, S. 1994. Developmental outcomes of children of mothers dependent on heroin or heroin/methadone during pregnancy. *Acta Paediatr Suppl*, 404, 36-9.
- SPITTLE, A., ORTON, J., ANDERSON, P. J., BOYD, R. & DOYLE, L. W. 2015. Early developmental intervention programmes provided post hospital discharge to prevent motor and cognitive impairment in preterm infants. *Cochrane Database Syst Rev*, Cd005495.
- STEINGART, R. A., ABU-ROUMI, M., NEWMAN, M. E., SILVERMAN, W. F., SLOTKIN, T. A. & YANAI, J. 2000. Neurobehavioral damage to cholinergic systems caused by prenatal exposure to heroin or phenobarbital: cellular mechanisms and the reversal of deficits by neural grafts. *Brain Res Dev Brain Res*, 122, 125-33.
- STEWART, R. D., NELSON, D. B., ADHIKARI, E. H., MCINTIRE, D. D., ROBERTS, S. W., DASHE, J. S. & SHEFFIELD, J. S. 2013. The obstetrical and neonatal impact of

- maternal opioid detoxification in pregnancy. *American Journal of Obstetrics and Gynecology*, 209, 267.e1-267.e5.
- STRAUSS, M. E., LESSEN-FIRESTONE, J. K., CHAVEZ, C. J. & STRYKER, J. C. 1979. Children of methadone-treated women at five years of age. *Pharmacol Biochem Behav*, 11 Suppl, 3-6.
- STRAUSS, M. E., STARR JR, R. H. & OSTREA JR, E. M. 1976. Behavioral concomitants of prenatal addiction to narcotics. *Journal of Pediatrics*, 89, 842-846.
- SUFFET, F. & BROTMAN, R. A comprehensive care program for pregnant addicts: obstetrical, neonatal, and child development outcomes. *International Journal of the Addictions*, 19, 199-219.
- TAN, J. W., DUAN, T. T., ZHOU, Q. X., DING, Z. Y., JING, L., CAO, J., WANG, L. P., MAO, R. R. & XU, L. 2015. Impaired contextual fear extinction and hippocampal synaptic plasticity in adult rats induced by prenatal morphine exposure. *Addiction Biology*, 20, 652-662.
- TELFORD, E. J., COX, S. R., FLETCHER-WATSON, S., ANBLAGAN, D., SPARROW, S., PATAKY, R., QUIGLEY, A., SEMPLE, S. I., BASTIN, M. E. & BOARDMAN, J. P. 2017. A latent measure explains substantial variance in white matter microstructure across the newborn human brain. *Brain Struct Funct*, 222, 4023-4033.
- THOMPSON, B. L., LEVITT, P. & STANWOOD, G. D. 2009. Prenatal exposure to drugs: effects on brain development and implications for policy and education. *Nat Rev Neurosci*, 10, 303-12.
- TINELLI, F., GAMUCCI, A., BATTINI, R. & CIONI, G. 2013. Congenital nystagmus in two infants born from mothers exposed to methadone during pregnancy. *Italian Journal of Pediatrics*, 39, 40.
- TSUNEISHI, S. & CASAER, P. 1997. Stepwise decrease in VEP latencies and the process of myelination in the human visual pathway. *Brain Dev*, 19, 547-51.
- TUSOR, N., WUSTHOFF, C., SMEE, N., MERCHANT, N., ARICHI, T., ALLSOP, J. M., COWAN, F. M., AZZOPARDI, D., EDWARDS, A. D. & COUNSELL, S. J. 2012. Prediction of neurodevelopmental outcome after hypoxic-ischemic encephalopathy treated with hypothermia by diffusion tensor imaging analyzed using tract-based spatial statistics. *Pediatr Res*, 72, 63-69.
- VAIANO, F., FAVRETTO, D., PALUMBO, D., COOPER, G., MACTIER, H., BUSARDO, F. P., MARI, F. & BERTOL, E. 2016. A novel, simultaneous extraction of FAEE and EtG from meconium and analysis by LC-MS/MS. *Anal Bioanal Chem*, 408, 2587-94.
- VAN BAAR, A. 1990. Development of infants of drug dependent mothers. *J Child Psychol Psychiatry*, 31, 911-20.
- VAN BAAR, A. & DE GRAAFF, B. M. T. 1994. Cognitive development at preschool-age of infants of drug-dependent mothers. *Developmental Medicine and Child Neurology*, 36, 1063-1075.
- VAN KOOIJ, B. J., DE VRIES, L. S., BALL, G., VAN HAASTERT, I. C., BENDERS, M. J., GROENENDAAL, F. & COUNSELL, S. J. 2012. Neonatal tract-based spatial statistics findings and outcome in preterm infants. *AJNR Am J Neuroradiol*, 33, 188-94.

- VASU, V., DURIGHEL, G., THOMAS, E. L., MALAMATENIOU, C., BELL, J. D., RUTHERFORD, M. A. & MODI, N. 2014. Preterm nutritional intake and MRI phenotype at term age: a prospective observational study. *BMJ Open*, 4, e005390.
- VESTAL-LABORDE, A. A., ESCHENROEDER, A. C., BIGBEE, J. W., ROBINSON, S. E. & SATO-BIGBEE, C. 2014. The Opioid System and Brain Development: Effects of Methadone on the Oligodendrocyte Lineage and the Early Stages of Myelination. *Developmental Neuroscience*, 36, 409-421.
- VILLAPIANO, N. L., WINKELMAN, T. N., KOZHIMANNIL, K. B., DAVIS, M. M. & PATRICK, S. W. 2017. Rural and Urban Differences in Neonatal Abstinence Syndrome and Maternal Opioid Use, 2004 to 2013. *JAMA Pediatr*, 171, 194-196.
- VOLKOW, N. D., FRIEDEN, T. R., HYDE, P. S. & CHA, S. S. 2014. Medication-assisted therapies--tackling the opioid-overdose epidemic. *N Engl J Med*, 370, 2063-6.
- WACHMAN, E. M., HAYES, M. J., LESTER, B. M., TERRIN, N., BROWN, M. S., NIELSEN, D. A. & DAVIS, J. M. 2014. Epigenetic variation in the mu-opioid receptor gene in infants with neonatal abstinence syndrome. *J Pediatr*, 165, 472-8.
- WACHMAN, E. M., HAYES, M. J., SHERVA, R., BROWN, M. S., SHRESTHA, H., LOGAN, B. A., HELLER, N. A., NIELSEN, D. A. & FARRER, L. A. 2017. Association of maternal and infant variants in PNOC and COMT genes with neonatal abstinence syndrome severity. *American Journal on Addictions*, 26, 42-49.
- WALHOVD, K. B., BJØRNEBEKK, A., HAABREKKE, K., SIQVELAND, T., SLINNING, K., NYGAARD, E., FJELL, A. M., DUE-TØNNESEN, P., BJØRNERUD, A. & MOE, V. 2015. Child Neuroanatomical, Neurocognitive, and Visual Acuity Outcomes With Maternal Opioid and Polysubstance Detoxification. *Pediatric Neurology*, 52, 326-332.e3.
- WALHOVD, K. B., MOE, V., SLINNING, K., DUE-TONNESSEN, P., BJORNERUD, A., DALE, A. M., VAN DER KOUWE, A., QUINN, B. T., KOSOFSKY, B., GREVE, D. & FISCHL, B. 2007. Volumetric cerebral characteristics of children exposed to opiates and other substances in utero. *Neuroimage*, 36, 1331-44.
- WALHOVD, K. B., WATTS, R., AMLIEN, I. & WOODWARD, L. J. 2012. Neural tract development of infants born to methadone-maintained mothers. *Pediatric Neurology*, 47, 1-6.
- WALHOVD, K. B., WESTLYE, L. T., MOE, V., SLINNING, K., DUE-TONNESSEN, P., BJORNERUD, A., VAN DER KOUWE, A., DALE, A. M. & FJELL, A. M. 2010. White Matter Characteristics and Cognition in Prenatally Opiate- and Polysubstance-Exposed Children: A Diffusion Tensor Imaging Study. *AMERICAN JOURNAL OF NEURORADIOLOGY*, 31, 894-900.
- WALSH, C., MACMILLAN, H. L. & JAMIESON, E. 2003. The relationship between parental substance abuse and child maltreatment: findings from the Ontario Health Supplement. *Child Abuse Negl*, 27, 1409-25.
- WALSH, S. L., PRESTON, K. L., BIGELOW, G. E. & STITZER, M. L. 1995. Acute administration of buprenorphine in humans: partial agonist and blockade effects. *J Pharmacol Exp Ther*, 274, 361-72.
- WANG, X., ZHOU, X., LIAO, Y., TANG, J., LIU, T. & HAO, W. 2011. Microstructural disruption of white matter in heroin addicts revealed by diffusion tensor

- imaging: a controlled study. *Zhong Nan Da Xue Xue Bao Yi Xue Ban*, 36, 728-32.
- WANG, Y. & HAN, T. Z. 2009. Prenatal exposure to heroin in mice elicits memory deficits that can be attributed to neuronal apoptosis. *Neuroscience*, 160, 330-8.
- WEHNER, F., WEHNER, H., SCHIEFFER, M. C. & SUBKE, J. 2000. Immunohistochemical detection of methadone in the human brain. *Forensic Sci Int*, 112, 11-6.
- WHITHAM, J. N., SPURRIER, N. J., BAGHURST, P. A., WESTON, P. & SAWYER, M. G. 2015. Visual evoked potential latencies of three-year-old children prenatally exposed to buprenorphine or methadone compared with non-opioid exposed children: The results of a longitudinal study. *Neurotoxicol Teratol*, 52, 17-24.
- WHITHAM, J. N., SPURRIER, N. J., SAWYER, M. G., BAGHURST, P. A., TAPLIN, J. E., WHITE, J. M. & GORDON, A. L. 2010. The effects of prenatal exposure to buprenorphine or methadone on infant visual evoked potentials. *Neurotoxicol Teratol*, 32, 280-8.
- WILLIAMS, A. 1985. When the client is pregnant: information for counselors. *J Subst Abuse Treat*, 2, 27-34.
- WILSON, G. S. 1989. Clinical studies of infants and children exposed prenatally to heroin. *Annals of the New York Academy of Sciences*, 562, 183-194.
- WILSON, G. S., DESMOND, M. M. & WAIT, R. B. 1981. Follow-up of methadone-treated and untreated narcotic-dependent women and their infants: Health, developmental, and social implications. *Journal of Pediatrics*, 98, 716-722.
- WILSON, G. S., MCCREARY, R., KEAN, J. & BAXTER, J. C. 1979. The development of preschool children of heroin-addicted mothers: a controlled study. *Pediatrics*, 63, 135-41.
- WITTMANN, B. K. & SEGAL, S. 1991. A comparison of the effects of single- and split-dose methadone administration on the fetus: ultrasound evaluation. *Int J Addict*, 26, 213-8.
- WONG, C.-S., LEE, Y.-J., CHIANG, Y.-C., FAN, L.-W., HO, I.-K. & TIEN, L.-T. 2014. Effect of prenatal methadone on reinstated behavioral sensitization induced by methamphetamine in adolescent rats. *Behavioural Brain Research*, 258, 160-165.
- WOULDES, T. A., ROBERTS, A. B., PRYOR, J. E., BAGNALL, C. & GUNN, T. R. 2004. The effect of methadone treatment on the quantity and quality of human fetal movement. *Neurotoxicol Teratol*, 26, 23-34.
- WURST, K. E., ZEDLER, B. K., JOYCE, A. R., SASINOWSKI, M. & MURRELLE, E. L. 2016. A Swedish Population-based Study of Adverse Birth Outcomes among Pregnant Women Treated with Buprenorphine or Methadone: Preliminary Findings. *Subst Abuse*, 10, 89-97.
- YAMAZAKI, Y., FUJIWARA, H., KANEKO, K., HOZUMI, Y., XU, M., IKENAKA, K., FUJII, S. & TANAKA, K. F. 2014. Short- and long-term functional plasticity of white matter induced by oligodendrocyte depolarization in the hippocampus. *Glia*, 62, 1299-1312.
- YANG, S. N., LIU, C. A., CHUNG, M. Y., HUANG, H. C., YEH, G. C., WONG, C. S., LIN, W. W., YANG, C. H. & TAO, P. L. 2006. Alterations of postsynaptic density proteins

- in the hippocampus of rat offspring from the morphine-addicted mother: Beneficial effect of dextromethorphan. *Hippocampus*, 16, 521-530.
- YAZDY, M. M., DESAI, R. J. & BROGLY, S. B. 2015. Prescription Opioids in Pregnancy and Birth Outcomes: A Review of the Literature. *J Pediatr Genet*, 4, 56-70.
- YIN, X., CRAWFORD, T. O., GRIFFIN, J. W., TU, P. H., LEE, V. M., LI, C., RODER, J. & TRAPP, B. D. 1998. Myelin-associated glycoprotein is a myelin signal that modulates the caliber of myelinated axons. *The Journal of neuroscience : the official journal of the Society for Neuroscience*, 18, 1953.
- YOO, S. H., JANSSON, L. M. & PARK, H. J. 2017. Sensorimotor outcomes in children with prenatal exposure to methadone. *J AAPOS*, 21, 316-321.
- YUAN, Q., RUBIC, M., SEAH, J., RAE, C., WRIGHT, I. M., KALTENBACH, K., FELLER, J. M., ABDEL-LATIF, M. E., CHU, C. & OEI, J. L. 2014. Do maternal opioids reduce neonatal regional brain volumes? A pilot study. *J Perinatol*, 34, 909-13.
- ZELSON, C., LEE, S. J. & CASALINO, M. 1973. Neonatal narcotic addiction. Comparative effects of maternal intake of heroin and methadone. *N Engl J Med*, 289, 1216-20.
- ZHANG, X. L., ZHANG, Y. & QIU, S. J. 2007. Magnetic resonance imaging findings in comparison with histopathology of heroin-associated encephalopathy. *Nan Fang Yi Ke Da Xue Xue Bao*, 27, 121-5.
- ZHANG, Y., LOH, H. H. & LAW, P. Y. 2016. Effect of Opioid on Adult Hippocampal Neurogenesis. *ScientificWorldJournal*, 2016, 2601264.
- ZUSPAN, F. P., GUMPEL, J. A., MEJIA-ZELAYA, A., MADDEN, J. & DAVIS, R. 1975. Fetal stress from methadone withdrawal. *Am J Obstet Gynecol*, 122, 43-6.
- ZWICKER, J. G., MILLER, S. P., GRUNAU, R. E., CHAU, V., BRANT, R., STUDHOLME, C., LIU, M., SYNNES, A., POSKITT, K. J., STIVER, M. L. & TAM, E. W. 2016. Smaller Cerebellar Growth and Poorer Neurodevelopmental Outcomes in Very Preterm Infants Exposed to Neonatal Morphine. *J Pediatr*, 172, 81-87 e2.

APPENDIX I

Table 14 : Appendix I: Quality rating of all studies reporting childhood neurodevelopmental outcomes after prenatal methadone exposure

First author and Year	Domain ^a	Design ^b	Comparison unexposed group ^c	Population ^d and sample size ^e	Objective assessment tool ^f	Blinded assessor ^g	Confounders ^h	Attrition ⁱ	Clear findings ^j	Total quality score ^k
Ramer 1975	ND	0	0	0	1	0	0	0	1	2
Zarin-Ackerman 1976	ND	0	0	0	1	0	0	0	0	1
Strauss 1976 *	ND	0	1	½	1	0	0	0	1	3 ½
Kaltenbach 1979 ‡	ND	0	1	0	1	1	½	0	1	4 ½
Strauss 1979	ND	0	1	½	1	1	0	0	1	4 ½
Wilson 1981	ND	0	1	½	1	1	½	1	1	6
Marcus 1982	ND	0	1	0	1	1	½	0	1	4 ½
Chasnoff 1984 *‡	ND	0	1	½	1	0	½	0	1	4
Suffet 1984	ND	0	0	½	1	0	½	0	0	2 ½
Lifschitz 1985	ND	0	1	½	1	0	0	0	1	3 ½

*indicates studies included in meta-analysis at 6 months, ‡indicates studies included in meta-analysis at 18–24 months.

First author and Year	Domain ^a	Design ^b	Comparison unexposed group ^c	Population ^d and sample size ^e	Objective assessment tool ^f	Blinded assessor ^g	Confounders ^h	Attrition ⁱ	Clear findings ^j	Total quality score ^k
Rosen 1985 *	ND	0	1	½	1	0	½	0	1	4
Kaltenbach 1986	ND	0	0	0	1	0	0	0	1	2
Kaltenbach 1987	ND	0	1	1	1	0	½	0	1	4 ½
Davis 1988	ND	0	1	0	1	0	0	0	1	3
Doberczak 1988	ND	0	0	0	1	0	0	0	1	2
Kaltenbach 1989 *‡	ND	0	1	0	1	0	½	1	1	4 ½
Wilson 1989 ‡	ND	0	1	0	1	0	½	0	1	3 ½
Sandberg 1990	ND	0	1	½	1	0	0	0	1	3 ½
Van Baar 1990 *‡	ND	0	1	½	1	0	½	0	1	4
De Cubas 1993	ND	0	1	½	1	0	0	0	1	3 ½
Van Baar 1994	ND	0	1	½	1	0	0	0	1	3 ½
Schneider 1996	ND	0	1	½	1	1	1	0	1	5 ½
Bunikowski 1998	ND	0	1	0	1	0	1	0	0	3

*indicates studies included in meta-analysis at 6 months, ‡indicates studies included in meta-analysis at 18–24 months.

First author and Year	Domain ^a	Design ^b	Comparison unexposed group ^c	Population ^d and sample size ^e	Objective assessment tool ^f	Blinded assessor ^g	Confounders ^h	Attrition ⁱ	Clear findings ^j	Total quality score ^k
Hans 2001 ‡	ND	0	1	½	1	1	½	0	1	5
Hunt 2008	ND	0	1	½	1	0	½	0	1	4
Paul 2013	ND	0	0	0	1	0	0	0	1	2
Konijnenberg 2015	ND	0	0	½	1	0	1	0	1	3 ½
Bier 2015	ND	0	1	½	1	0	0	0	1	3 ½
McGlone 2015	ND	0	0	1	1	0	1	1	1	5
Nelson 1987	V	0	0	0	1	0	0	0	0	1
Gaillard 2002	V	0	0	0	1	0	0	0	0	1
McGlone 2008	V	0	1	1	1	1	0	0	1	5
Hamilton 2010	V	0	0	0	1	0	0	0	½	1.5
Whitham 2010	V	0	1	½	1	1	½	0	1	5
Gupta 2012	V	0	0	½	1	0	0	0	1	2 ½
McGlone 2013	V	0	1	1	1	1	1	0	1	6

*indicates studies included in meta-analysis at 6 months, ‡indicates studies included in meta-analysis at 18–24 months.

First author and Year	Domain ^a	Design ^b	Comparison unexposed group ^c	Population and sample size ^d	Objective assessment tool ^f	Blinded assessor ^g	Confounders ^h	Attrition ⁱ	Clear findings ^j	Total quality score ^k
Tinelli 2013	V	0	0	0	1	0	0	0	0	1
McGlone 2014	V	0	1	1	1	1	1	1	1	7
Konijnenberg 2015	V	0	1	½	1	0	0	0	1	3 ½
Whitham 2015	V	0	1	0	1	0	½	0	1	3 ½
Yoo 2017	V	0	0	0	1	0	0	0	1	2
Pasto 1985	NI	0	1	½	1	0	½	0	1	4
Walhovd 2012	NI	0	1	0	1	0	1	0	1	4

*indicates studies included in meta-analysis at 6 months, ‡indicates studies included in meta-analysis at 18–24 months.

Notes relating to Table 14.

^a ND = neurodevelopmental, NI = neuroimaging, V = visual; ^b Randomised controlled trial = 1, observational study = 0; ^c Comparison unexposed group (not exposed to opioids) = 1, no comparison unexposed group = 0; ^d ≥36 w GA = ½, <36w GA = 0; ^e Sample size ≥20 = ½, sample size <20 = ½; ^f Objective assessment tool used = 1, no objective tool = 0; ^g Blinded = 1, not blinded = 0; ^h Confounder identified = ½, confounder adjusted for = ½, confounder not identified or not adjusted for = 0 (potential confounders included socio-economic status and/or polydrug use); ⁱ Attrition ≤20% of original cohort = 1, >20% of original cohort = 0, if not applicable due to study design, score 0; ^j Clear finding with statistical analysis = 1, findings not clear and no statistical analysis = 0; ^k Total scores 0–3 = poor, 3½–6 = intermediate, 6½–8 = good

APPENDIX II

Table 15 : Appendix II: All studies reporting childhood neurodevelopmental outcomes after prenatal methadone exposure

Author Year	QR ^a	Meth	Un- exposed	Age ^b	Drug info ^c	Assessment instrument	Main findings <i>Results appear as methadone vs unexposed</i>	Comments ^d
Ramer <i>et al</i> , 1975 Case series	C	10 7 5 7 4	0 0 0 0 0	2-3 m 4-6 m 7-9 m 10-15 m 16-24 m	No dosing information IUS (heroin metabolites in 6 urine)	BSID (MDI, PDI) at all ages	2-3 m: MDI 117.3 (16.56); PDI 136.4 (18.67) 4-6 m: MDI 116.43 (17.91), PDI 116.29 (19.2) 7-9 m: MDI 110.4 (31.41), PDI 110.4 (30.26) 10-15 m: MDI 113.57 (13.25), PDI 105.71 (14.65) 16 -24m: MDI 99.25 (16.76), PDI 107.25 (18.7)	Original cohort 35 methadone- exposed infants (26 females, 9 males), no unexposed group. One early neonatal death from meconium aspiration. Mean GA at birth not stated; one infant was 36 weeks, the remainder were >36 weeks GA. Pharmacological treatment for NAS in 14; Diazepam first line, paregoric (morphine) used as second agent in 2 cases, phenobarbital used for some cases of NAS. 10 infants received 'medication for comfort' and 4 'required regular sedative medications'. Medications used: diazepam, morphine and phenobarbital

Author Year	QR ^a	Meth	Un-exposed	Age ^b	Drug info ^c	Assessment instrument	Main findings <i>Results appear as methadone vs unexposed</i>	Comments ^d
Beschnell (Zarin-Acherman) Case series	C	16 14	0 0	3 m 6 m	Mean dose in 3 rd trimester 13.9; max 65; No screening or PD information	BSID (MDI, PDI) at all ages	3 m: MDI 101 (no sd); PDI 112 (no sd), <i>no statistics reported</i> 6 m: MDI 103 (no sd); PDI 107 (no sd), <i>no statistics reported</i>	Original cohort 42 methadone exposed infants, no unexposed group. 10 infants were preterm. No information about NAS or treatment.
Strauss <i>et al*</i> , Prospective cohort study	B	25 25 25	26 26 26	3 m 6 m * 1 yr	No information	BSID (MDI, PDI) at all ages	3 m: MDI 112.5 (11.5) vs 115.3 (13.5); PDI 119.4 (9.1) vs 117.1 (14.5) 6 m*: MDI 115.7 (16.8) vs 114.3 (20.9); PDI 109.4 (12.2) vs 111.7 (14.5) 1 yr: MDI 113.4 (10.2) vs 114.8 (11.3); PDI 102.8 (11.0) vs 110.4 (9.8). Decline in PDI between 3m and 12m in methadone exposed infants compared with unexposed: 16.6 points vs 6.7 points (p<0.01)	Original cohort 60 methadone-exposed infants and 53 unexposed infants; all infants were African-American. Data reported only for infants who underwent BSID at all 3 ages. One case of sudden infant death in the methadone-exposed group. Matching of unexposed group not stated. GA at birth not stated. Assessor blinding not stated. No information about NAS or treatment

Author Year	QR ^a	Meth	Un-exposed	Age ^b	Drug info ^c	Assessment instrument	Main findings <i>Results appear as methadone vs unexposed</i>	Comments ^d
Kaltenbach <i>et al</i> ‡, Prospective cohort study	B	26 17	27 24	1 yr 2 yr ‡	Mean dose for 1 yr cohort: 30; Mean dose for 2 yr cohort: 18 No screening or PD information	BSID (MDI) at all ages	1 yr: MDI 103.4 (9.26) vs 109.4 (9.35), p<0.05 2 yr ‡: MDI 90.88 (8.26) vs 94.62 (11.93), ns	Original cohort 43 methadone vs 51 un-exposed (matched for maternal SES, ethnicity and medical conditions). Assessors blinded to group. 1 year cohort had a mean GA of 40 weeks vs 39 weeks for unexposed infants. No range stated. 62% treated for NAS. 2 year cohort mean GA of 39 weeks vs 39 weeks, no range stated. 67% 2 year old cohort had been treated for NAS in the neonatal period. No pharmacological agent stated. Unclear whether overlap of participants assessed at 1 year and 2 years.
Strauss <i>et al</i> , Prospective cohort study	B	31	27	5 yr	No information	MSCA; Modified IBR (15 of 27 scales)	GCI 86.8 (13.3) vs 86.2 (16.2), ns. Modified IBR: Gross bodily movement: 5.5 (1.4) vs 4.7 (1.4), p<0.05. Levels of energy: 3.4 (0.9) vs 2.8 (1.1), p<0.05. Fine motor coordination: 3.0 (0.8) vs 2.5 (1.0), ns. Irrelevant motor movement: 5.3 (1.7) vs 4.1 (1.7), p<0.01. Immaturity in interaction: 33% vs 7%, p<0.05	Original cohort 60 methadone-vs 53 un-exposed infants (matching not stated). Same cohort as Strauss <i>et al</i> 1976. 1 case of SIDS in the methadone-exposed group. All infants African American. Mean GA at birth not stated, range GA not stated. Assessor blinding not consistent. No information about NAS or treatment.

Author Year	QR ^a	Meth	Un-exposed	Age ^b	Drug info ^c	Assessment instrument	Main findings <i>Results appear as methadone vs unexposed</i>	Comments ^d
Wilson <i>et al</i> , Prospective cohort study	B	33	54	9 m	20–60 (32 cases) <20 (6 cases) 90 (1 case) Maternal urine screening; PD >50%, alcohol 10% Only 3/33 used solely methadone	BSID (MDI, PDI) IBR	MDI 99.3 (15.5) vs 105.5 (15.6) ns; PDI 89.9 (12.6) vs 99.0 (14.5), p<0.01 IBR: Poor fine motor co-ordination: 27/33 vs 27/55 unexposed (χ^2 =8.80, p<0.01) Less attentive: 8/33 vs 4/55 (χ^2 =4.88, p<0.05)	Original cohort 39 methadone-exposed and 59 unexposed, one SIDS death in methadone-exposed group before assessment at 12 months. Unexposed matched for maternal age, SES, marital status and ethnicity. Mean GA not stated; 7/39 (18%) methadone-exposed group were preterm, compared with 6/57 (10%) of unexposed group. Assessor blinded to group. 34/39 severe NAS, treatment not stated
Marcus <i>et al</i> , Prospective cohort study	B	15	23	4 m	No information	IBR	IBR sum score: 22.2 (2.57) vs 18.13 (2.72), p<0.001 Tension: 5.33 (0.9) vs 4.61 (1.47), ns. Activity: 4.93 (1.49) vs 3.87 (1.18), p<0.05 Interest in body motion: 5.4 (1.24) vs 3.96 (0.98), p<0.001 Co-ordination (gross motor): 3.13 (0.64) vs 2.68 (0.57), p<0.05. Co-ordination (fine motor): 3.4 (0.63) vs 2.96 (0.56), p<0.001	No information about original cohort of infants. All African-American infants. One case in methadone-group of SIDS and one stroke. Unexposed infants matched for maternal age and SES. Mean GA not stated, range GA not stated. Assessors blinded to group. No methadone-exposed infants treated for NAS

Author Year	QR ^a	Meth	Un- exposed	Age ^b	Drug info ^c	Assessment instrument	Main findings <i>Results appear as methadone vs unexposed</i>	Comments ^d
Chasnoff <i>et al</i> *‡, Prospective cohort study	B	31 13 11 6	34 29 27 14	3 m 6 m * 1 yr 2 yr ‡	Mean dose 14.6 ± 10.2 (range 5– 40); MUS; 4/31 used drugs in addition to heroin during pregnancy	BSID (MDI, PDI) at all ages	3 m: MDI 105.0 (12.5) vs 99.2 (9.0); PDI 105.0 (18.6) vs 102.8 (7.0) 6 m*: MDI 105.9 (12.4) vs 111.0 (12.3); PDI 103.9 (9.0) vs 107.6 (15.1) 1 yr: MDI 104.2 (7.1) vs 105.8 (8.1); PDI 106.0 (14.4) vs 103.8 (12.5) 2 yr ‡: MDI 97.5 (16.1) vs 96.2 (15.9); PDI 99.3 (16.9) vs 98.2 (8.9) <i>No p values stated</i>	Original cohort 39 methadone exposed and 34 unexposed. Unexposed group matched for maternal age, education, gravidity and smoking. All infants were term (Ballard criteria), no range stated. Assessor blinding not stated. No information about NAS or treatment.

Author Year	QR ^a	Meth	Un-exposed	Age ^b	Drug info ^c	Assessment instrument	Main findings <i>Results appear as methadone vs unexposed</i>	Comments ^d
Suffet <i>et al</i> , Case series	C	131 106 99 35	0 0 0 0	3 m 6 m 1 yr 2 yr	Median 25–40 No screening information No PD information stated, although a condition of joining the programme was detoxification from non-narcotics.	BSID (MDI, PDI) at all ages	3 m: MDI 106.3 (13.5) PDI 112.1 (15.5). 6 m: MDI 108.1 (19.4) PDI 105.0 (14.5). 1 yr: MDI 97.7 (17.3) PDI 101.3 (16.1). 2 yr: MDI 89.3 (17.0) PDI 106.5 (19.1) Compared by gender at all ages, girls vs boys: 1 yr: MDI 108.8 (11.2) vs 102.7 (12.8), p<0.05; PDI 102.3 (14.2) vs 95.7 (14.5), p<0.05. At 2 yr: MDI 99.2 (14.8) vs 82.0 (14.9), p<0.01 Significant associations between both BW and gender and MDI and PDI: A. Positive correlation between birthweight and MDI at 6 months (p<0.001) PDI and 6 months (p<0.01), which is still present at 24 months: MDI p<0.01, PDI p<0.05). B. Male vs female. 1 year: MDI 108.8 (11.2) vs 102.7 (12.8), p<0.05, PDI 102.3 (14.2) vs 95.7 (14.5), p<0.05	Original cohort 220 infants, all methadone exposed; 3.2% congenital anomalies; 5 deaths, 3 neonatal and 2 sudden infant deaths. 20% original cohort were <37 weeks GA at birth. 18.2% original cohort treated for NAS with paregoric (morphine).

Author Year	QR ^a	Meth	Un- exposed	Age ^b	Drug info ^c	Assessment instrument	Main findings <i>Results appear as methadone vs unexposed</i>	Comments ^d
Lifschitz <i>et al</i> , Prospective cohort study	B	26	41	Mean 3 yr 5 m (range 3 yr – 5 yr 11 m)	No dosing or screening information; 95% PD use (heroin or psychoactive drugs)	MSCA (GCI)	GCI: 90.4 (13) vs 89.4 (10.8), ns 9 children (35%) in methadone-exposed group scored >1 sd below mean compared to 56% heroin exposed children and 22% unexposed children, p<0.01.	Original cohort 33 methadone- exposed and 57 unexposed infants (matched for maternal age, parity, SES and marital status). Mean GA 38.8 weeks vs 39.2 weeks in unexposed group, range GA not stated. Assessors blinded to group. 88% methadone- exposed were treated for NAS, pharmacological agent not stated.
Rosen <i>et al</i> * ‡, Prospective cohort study	B	41 41 38 34 39	23 22 23 22 21	6 m * 1 yr 18 m 2 yr ‡ 3 yr	42 (mean of original cohort) MUS; PD use 56% of original cohort (BDZ, opiates, cocaine, barbiturates, TCA)15% reported “mod- severe” alcohol intake	BSID (MDI, PDI) at 6m,12m,18m and 2yr; M-P at 3yr	6 m *: MDI 95 (2.5) vs 100.7 (4.2), ns; PDI 101 (2.8) vs 105.1 (2.9), ns 1 yr: MDI 98.4 (2.7) vs 107 (2.8) p=0.05; PDI 94.9 (2.5) vs 102.8 (2.3) p=0.05 18 m: MDI 96 (2.3) vs 106.4 (3.6), p =0.05; PDI 92.6 (2.4) vs 105.3 (2.2) p=0.05 2 yr ‡: MDI 90.4 (2.6) vs 96.9 (3.1) ns; PDI 99.1 (2.7) vs 108 (2.7), p=0.05 3 yr: M-P 44.6 (2.1) vs 46.3 (2.3) ns. <i>All scores are mean (SE)</i>	Original cohort 61 methadone- exposed infants and 32 unexposed infants (matched for maternal ethnicity, SES, infant gender, BW and GA). 15.4% preterm vs 11%, no range GA stated. Assessor blinding not stated. 75% methadone-exposed had NAS; number treated pharmacologically not stated.

Author Year	QR ^a	Meth	Un-exposed	Age ^b	Drug info ^c	Assessment instrument	Main findings <i>Results appear as methadone vs unexposed</i>	Comments ^d
Kaltenbach <i>et al</i> , Case series	C	85 21 17 31 16	0 n/a n/a n/a n/a	6 months Group 1 Group 2 Group 3 Group 4	No information	BSID (MDI)	<i>MDI at 6 months reported by groups depending on the treatment for NAS:</i> Group 1: paregoric (morphine) MDI 103 (no sd stated) Group 2: phenobarbital MDI 104 (no sd stated) Group 3: >1 agent MDI 103 (no sd stated) Group 4: no treatment MDI 101 (no sd stated) No difference between the 4 groups, p>0.1	All infants were term. No mean GA stated. 69/85 treated for NAS with either paregoric (morphine), phenobarbital, or diazepam, or a combination.
Kaltenbach <i>et al</i> , Prospective cohort study	B	105	63	6 months	39 (5–85) No screening or PD information	BSID (MDI)	6 m: MDI 103.53 vs 104.39 (no sd stated) t=0.45, ns	Original cohort was 141 methadone exposed at birth. Unexposed group matched for maternal ethnicity, SES and medical background. All infants >36 weeks GA, mean GA at birth 38.7 vs 39.4 weeks, no GA range stated. Blinding of assessors not stated. 70% methadone-exposed were treated for NAS with paregoric (morphine) and/or phenobarbital.

Author Year	QR ^a	Meth	Un-exposed	Age ^b	Drug info ^c	Assessment instrument	Main findings <i>Results appear as methadone vs unexposed</i>	Comments ^d
Davis <i>et al</i> , Cross-sectional study	C	12	28	Mean 8.5yr vs 11.2 yr (range 6 – 15 yr)	No information	WISC-R	<i>Results stated are methadone subgroup vs unexposed at mean age of 8.5 yr vs 11.2 yr</i> Verbal IQ: 89 (11.33) vs 94.29 (9.13) no p-value Performance IQ: 92.75 (10.16) vs 100.0 (9.82) no p-value Full scale IQ: 89.58 (10.32) vs 96.32 (8.72) no p-value	Original cohort 28 opiate-exposed, of which there was a subgroup of 12 methadone-exposed and 9 heroin exposed and 28 unexposed (no prenatal exposure to methadone but living in a 'narcotic environment' with an addicted parent/partner). Mean GA not stated, range GA not stated. Assessor not blinded. No information about NAS or treatment
Doberczak <i>et al</i> , Cohort study	C	8 vs 32 7 vs 16	n/a n/a	5 – 7 m 8 – 16 m	Mean dose seizure: 56 Mean dose no seizure: 55. MUS, IUS; Both groups PD use with heroin, cocaine, barbiturates, AMP and BDZ.	BSID (MDI, PDI) at both ages	<i>Results stated are seizures vs no seizures</i> 5 - 7 m: MDI 103.1 (19.9) vs 111.5 (19.6), ns; PDI 114.7 (14.9) vs 103.4 (16.6), ns 8 - 16 m: MDI 114.0 (8.2) vs 109.7 (10.9), ns; PDI 109.7 (10.9) vs 99.5 (13.5), ns	Original cohort was 14 infants with methadone-related NAS-associated seizures and a comparison group of methadone-exposed infants with no seizures. No unexposed comparison group. Mean GA of seizure group 39 weeks (range 33 – 43 weeks). Mean GA of no seizure group 39 weeks (range 33 – 42 weeks). Assessor blinded to infant history. Treatment for NAS with morphine and/or phenobarbital.

Author Year	QR ^a	Meth	Un- exposed	Age ^b	Drug info ^c	Assessment instrument	Main findings <i>Results appear as methadone vs unexposed</i>	Comments ^d
Kaltenbach <i>et al</i> , *‡, Prospective cohort study	B	27 27 27 27	17 17 17 17	6 m * 1 yr 2 yr ‡ 3.5 – 4.5 yr	Mean dose 38.42; No screening or PD information	BSID (MDI) at 6m, 1 yr and 2 yr. MSCA (GCI) at 3.5-4.5 yr	6 m*: MDI 107.9 (12.23) vs 105.6 (7.31) no p value 1 yr: MDI 102.5 (11.38) vs 106 .53 (6.41), no p value 2 yr ‡: MDI 100.9 (18.04) vs 103.9 (11.49) no p value 3.5 - 4.5 yr: GCI 106.5 (12.96) vs 106.05 (13.10), <i>t</i> =0.11	Limited information about original cohort. Unexposed group were matched for maternal ethnicity and SES. GA infants not stated, no GA range stated. Blinding of assessors not stated. 92% were treated for NAS, pharmacological agent not stated.
Wilson <i>et</i> <i>al</i> , ‡, Prospective cohort study	B	33 29 42 26 12	54 42 48 41 12	9 m 18 m 2 yr ‡ 3 – 5 yr 6 – 11 yr	No dosing information MUS; 93% used psychoactive drugs	BSID (MDI) at 9m, 18m, 2yr. MSCA (GCI) at 3-5yr; School performance 6- 11 yr (survey, school reports and IQ testing)	9 m: published in Wilson <i>et al</i> ¹² 1981 18 m: MDI 92 (14.5) vs 97.4 (14.4) ns 2 yr ‡: MDI 88.8 (15.5) vs 90.2 (14.6) ns 3 – 5 yr: GCI 90.4 (13.0) vs 89.4 (10.8) ns 6 – 11 yrs: IQ 1- 2 sd below norm 8% vs 5%; Language disability 8% vs 5%; Special education needs 16% vs 19%; Behavioural problems 75% vs 48%; Psychiatric referral 16% vs 5%.	Original cohort 39 methadone- exposed, 57 unexposed infants (matched for maternal age, ethnicity, SES and marital status). Mean GA not stated for either group, range GA not stated. Assessor blinding not stated. 87% original cohort treated for NAS, pharmacological agent not stated.

Author Year	QR ^a	Meth	Un- exposed	Age ^b	Drug info ^c	Assessment instrument	Main findings <i>Results appear as methadone vs unexposed</i>	Comments ^d
Sandberg <i>et al</i> , Prospective cohort study	B	30	16	5 – 8 years	39.5 (boys), 38.7 (girls) MUS; Original cohort, 68% PD use; 15% moderate to heavy alcohol intake.	CGPQ ^f CBAQ ^f (boys only)	Methadone-exposed boys showed more feminine game play than unexposed boys ($p < 0.04$). No significant differences in girls. Overall CBAQ scores were not significantly different between methadone-exposed and unexposed boys. Group split by gender and by PD use creating: boys methadone only (n=5) and boys methadone + PD (n=9) In the methadone only boys group (n=5), ANCOVA with age as covariate of individual elements of the feminine behaviour sub-scale showed higher (more feminine behaviour) scores for 2 items: “he is good at imitating females”, $p < 0.001$, “he dresses in female clothing”, $p < 0.05$ In the PD boys group (n=9) there were higher feminine scores on “he is good at imitating females”, $p < 0.05$, and “he does things with female relatives’, $p < 0.05$	Original cohort 61 methadone- exposed infants and 32 unexposed infants (matched for maternal ethnicity, SES, infant gender, BW (± 250 g), GA (± 2 w) and APGAR score). 2 methadone-exposed infants from original cohort died of sudden infant death. GA at birth and range GA not stated. NAS treatment not stated. Mother or primary care-giver completed questionnaire.

Author Year	QR ^a	Meth	Un- exposed	Age ^b	Drug info ^c	Assessment instrument	Main findings <i>Results appear as methadone vs unexposed</i>	Comments ^d
Van Baar <i>et al</i> * ‡, Prospective cohort study	B	21 21 18 21 19	37 34 34 34 34	6 m * 1 yr 18 m 2 yr ‡ 2.5 yr	No dosing or screening information; Original cohort; 6 IV drug users; 94% used multiple drugs; 60% use cocaine	BSID (MDI, PDI, NDI) at all ages WWPA ^f at 18 m (n=14), 2 yr (n=16) and 2.5 yr (n=15)	*6 m MDI 103 (12) vs 107 (13); PDI 116 (18) vs 114 (21); NDI 105 (13) vs 109 (14). At 1 yr: MDI 108(13) vs 114 (17); PDI 112 (21) vs 119 (20); NDI 109 (12) vs 112 (18). At 18 m: MDI 94 (14) vs 99 (19); PDI 108 (20) vs 112 (19); NDI 97 (15) vs 99 (17). At 2 yr ‡: MDI 86 (15) vs 98 (16) p<0.05; PDI 102 (16) vs 100 (18) ns; NDI 93 (16) vs 102 (22). At 2.5 yr: MDI 87 (15) vs 101 (20) p<0.05; PDI 96 (19) vs 101(24) ns; NDI 100 (19) vs 108 (19) ns. <i>WWPA^d, medians (range)</i> 18m: 1.78 (1.40 – 2.62) vs 1.73 (1.23 – 2.52) ns 2y: 1.66 (1.33 – 2.660 vs 1.76 (1.19 – 2.52) ns. At 2.5 yr: 1.62 (1.03 – 2.66) vs 1.64 (1.28 – 2.52) ns; Methadone had lower MDI at 2 and 2 ½ years due to delayed early language. No differences in motor t, non- verbal or hyperactivity scores.	Original cohort 35 methadone- exposed and 37 unexposed infants (not matched). In methadone-exposed group 9/35 were preterm but results presented are subgroup of term only methadone-exposed infants. Assessor blinding not stated. 28/35 (80%) were treated for NAS, pharmacological agent not stated

Author Year	QR ^a	Meth	Un-exposed	Age ^b	Drug info ^c	Assessment instrument	Main findings <i>Results appear as methadone vs unexposed</i>	Comments ^d
De Cubas <i>et al</i> , Cross-sectional study	B	20	20	Mean age 8.5 vs 7.8 yr	No drug information; “moderate alcohol use”	SBIS; KABC-A; RATC; CBCL ^f	SBIS: 97.6 vs 98.1 ns. Within methadone-exposed group: NAS (n=5) vs No NAS (n=15) 89.9 vs 100.2, $t=3.65$, $p<0.002$ KABC-A: 98.8 vs 102.4, <i>no p value</i> ; Faces and places subtest 95 vs 103, $p<0.02$; RATC: Methadone-exposed scored higher on anxiety, aggression, rejection and maladaptive outcome, $p<0.01$ for all; CBCL: More behaviour problems [depressed, social withdrawal, somatic complaints, hyperactive, aggressive, delinquent, internalising and externalising behaviour] reported by parents in methadone group, $p<0.05$ all categories.	Unexposed group matched for demographics (not stated) age/grade level, sex, ethnicity, SES, family structure, maternal education, maternal alcohol/tobacco, perinatal complications. Mean GA not stated; In methadone-exposed one child was preterm; In the unexposed group 10/20 had “perinatal complications such as prematurity or SGA”. Assessor blinding not stated. 3/20 treated for NAS with phenobarbital

Author Year	QR ^a	Meth	Un-exposed	Age ^b	Drug info ^c	Assessment instrument	Main findings <i>Results appear as methadone vs unexposed</i>	Comments ^d
Van Baar <i>et al</i> , Prospective cohort study	B	23 26 23 22	32 32 31 30	3.5 yr 4 yr 4.5 yr 5.5 yr	No dosing information; Maternal interview and MUS, IUS; PD use: 16/35 heroin and cocaine, 2/35 solely methadone	SON IQ at 3.5 yr; RC and RE at 4 yr; RAKIT at 4.5 and 5.5 yr; IBR at 3.5 yr (n=22), 4.5 yr (n=23) 5.5 yr (n=22)	SON IQ 99 (9) vs 109 (11), p<0.01. RC 46 (6) vs 52 (6), p<0.01. RE 46 (9) vs 50 (6), p<0.05. RAKIT 4.5 yr: 85 (11) vs 103 (15), p<0.01; 14 / 23 methadone-exposed children had developmental delay (defined as scores >1 sd), p<0.01. RAKIT 5.5yr: 90 (12) vs 102 (17), p<0.05 <i>IBR: Results median(range)</i> 3.5 yr: Free of fear: 9 (4-9) vs 6.5 (2-9), p<0.05; Activity level: 6 (3-9) vs 5 (2-9), p<0.05; Attention: 5 (1-7) vs 5.5 (1-9) p<0.05; Fine motor: 3 (1-5) vs 3 (1-5), p<0.05 4.5 yr: Co-operation: 6 (2-9) vs 7 (3-9), p<0.01; Endurance: 4 (2-9) vs 6 (1-9), p<0.01; Attention: 8 (2-9) vs 5 (2-8), ns 5.5 yr: Co-operation: 6 (1-9) vs 8 (4-9), p<0.01; Free of fear: 8 (2-9) vs 9 (5-9), ns; Attention: 5 (2-8) vs 5 (3-9), ns.	Original cohort 35 methadone-exposed vs 35 unexposed (unmatched). Mean GA for original cohort 38 weeks vs 39.7 weeks, no range stated; 7/23 methadone exposed infants were preterm, range of GA not stated. Blinding of assessors not stated. 28/35 were treated for NAS, all treated with phenobarbital. After correcting for behaviour that differed between groups, significant differences between methadone-exposed and unexposed children still existed at 3.5 years for SON IQ, p<0.05, and at 4.5 years for RAKIT, p<0.01, but not at 5.5 years for RAKIT, p=0.13

Author Year	QR ^a	Meth	Un-exposed	Age ^b	Drug info ^c	Assessment instrument	Main findings <i>Results appear as methadone vs unexposed</i>	Comments ^d
Schneider <i>et al</i> , Prospective cohort Study	B	30	44	2 yr	Mean <20 (range 3 – 40) “most women occasionally used cannabis, alcohol and BDZ”	Focus Ratio	Focus ratio at 2 yr: 0.35 (0.15) vs 0.31 (0.13) ns ANCOVA to unexposed for cannabis (F(1,69) = 0.16), nicotine (F(1,69) = 0.16) and alcohol (F(1,69) = 0.04) ns	Unexposed comparable for low income. 2 methadone-exposed were preterm (30w and 33w). Attrition: 4 children died before 2 years of age in the methadone-exposed group, and one had massive cerebral haemorrhage. 1 in unexposed group withdrew following diagnosis of cerebral palsy. Assessors scoring videotaped encounter were blinded to group. No methadone-exposed child was treated for NAS.
Bunikowski <i>et al</i> , Prospective cohort study	C	18	42	1 yr	No information	Griffiths subscales:	<i>Methadone sub group data extracted:</i> Hearing and speech: 99.7 (8.1) vs 98.8 (9.1) Intellectual performance at 1 yr: 104.3 (11.6) vs 108.5 (11.1) <i>No p values as these results taken out of text as subgroup analysis</i>	Original cohort 46 opiate exposed versus 47 unexposed infants (matched for maternal smoking). 27 opiate exposed underwent Griffiths assessment at 1 year; opiate group split into methadone (n=18) and heroin (n=9), sub-group analysis reported. Mean GA not stated; 13/34 opiate exposed were preterm, range not stated Assessor blinding not stated. 28/46 treated for NAS with phenobarbital

Author Year	QR ^a	Meth	Un-exposed	Age ^b	Drug info ^c	Assessment instrument	Main findings <i>Results appear as methadone vs unexposed</i>	Comments ^d
Hans <i>et al</i> , ‡, Prospective cohort study	B	33 33 33 33 33	45 45 45 45 45	4 m 8 m 1 yr 18 m 2 yr ‡	Mean <20 (Range 3 - 40mg); Maternal interview and MUS; PD reported: 13/33 cocaine, 18/33 cannabis, 9/33 mild-mod alcohol use, 2/33 heavy alcohol use	BSID (MDI, PDI) at all ages	4 m: MDI 111 (12.3) vs 114 (15.1); PDI 116 (12.5) vs 121 (12.3) 8 m: MDI 116 (19.5) vs 120 (20.2); PDI 111 (12.4) vs 111 (12.4) 1 yr: MDI 107 (14.3) vs 109 (13.7); PDI 106 (18) vs 110 (17.7) 18 m: MDI 95 (16.3) vs 103 (13.1); PDI 105 (14.2) vs 109 (14.9) 2 yr ‡: MDI 92 (12.7) vs 96 (12.3); PDI 100 (14.2) vs 108 (14.9) Mean across all ages: MDI 104 (7.8) vs 108 (8.3), p<0.05; PDI 108 (9.2) vs 112 (10.4), ns	Original cohort 47 methadone-exposed infants and 45 unexposed infants (matched for maternal age, SES and IQ). 4 SIDS in the methadone-exposed group. All infants included were African-American. 3 subjects not tested at 2 years, not stated which group these subjects were in. Scores were estimated, based on their median 18 month scores and the average scores for the entire sample. Mean GA not stated, no GA range stated. Assessors blinded to group. No information about NAS or treatment

Author Year	QR ^a	Meth	Un-exposed	Age ^b	Drug info ^c	Assessment instrument	Main findings <i>Results appear as methadone vs unexposed</i>	Comments ^d
Hunt <i>et al</i> , Australia, 2008 ¹¹ Prospective cohort study	B	79 67	61 44	18 m 3 y	No information	BSID III (MDI, PDI) at 18 m; VL at 18m and 3 yr; SBIS and MSCA (GCI) at 3 yr; RC and RE at 3 yr	18 m: MDI 88.2 (16.4) vs 105.02 (23), p<0.001; PDI 107.5 (16.8) vs 110.13 (14.7) SBIS: 99.9 (15.1) vs 107.5 (13.4), p<0.01 GCI: 49.5 (8.7) vs 53.9 (8.3), p<0.05 VL 18 m: 113.2 (15.6) vs 119.15 (17.5), p<0.05 VL 3 yr: 38.4 (8.1) vs 46.1 (7.7), p<0.05 RC 42.4 (11.6) vs 49.2 (11.4), p<0.05; RE 35.5 (7.9) vs 42.8 (12.8), p<0.05	Original cohort 133 methadone exposed and 103 unexposed infants (matched for maternal age, height and ethnicity). In families lost to follow up, 10/133 methadone taking mothers had died before their child was 3 years old. Original cohort mean GA at birth 37.7 vs 40.2 weeks, 32/133 were preterm, all singleton pregnancies. Assessor blinding not stated. 74 /133 infants were treated for NAS with morphine. No NAS related seizures.
Paul <i>et al</i> , Maine, 2013 ²⁸ Case series	C	19 17 24	0 0 0	4-15 d 16 – 32 d 33 – 120 d	No drug information; Maternal questionnaire asked about alcohol use	Auditory ERP: P2 amplitude; P2 latency; Mismatch negativity	P2 amplitude greater in 16 – 32 d and 22 – 120 d in the frontal region, p<0.001 both Latency to oddball stimulus longer only in in 4 – 15 day group, p 0.04, but not in the Mismatch negativity amplitude became less negative as P2 amplitude to oddball stimulus increased at Fz, r ² 0.38, p<0.001	Three separate cohorts of infants, no unexposed group. Mean GA at birth for all groups was 38 weeks, no range stated. All infants in the 4 – 15 day group were receiving pharmacological treatment for NAS at the time of ERP testing. Pharmacological agent used for NAS treatment not stated.

Author Year	QR ^a	Meth	Un-exposed	Age ^b	Drug info ^c	Assessment instrument	Main findings <i>Results appear as methadone vs unexposed</i>	Comments ^d
Konijnenberg <i>et al</i> , Oslo, 2015 ⁵⁶ Prospective cohort study	B	24	0	Mean 52 m	Mean 85.96 ^g Maternal interview and medical records, IUS; PD reported in 40% and 25% alcohol	CBCL ^f	Methadone-exposed children aged 4 scored >55 on aggressive behaviour and withdrawn behaviour.	Part of a larger cohort study comparing buprenorphine with methadone. No unexposed group. Mean GA at birth 38.7 weeks. Inclusion of preterm infants not stated. No assessor blinding. 13/24 treated for NAS, pharmacological agent not stated
Bier <i>et al</i> , Boston, 2015 ⁴⁷ Retrospective cohort	B	High 81 Low 84	n/a n/a	4 m	Mean dose not stated. IUS; Co-treatment with psychiatric medication in 43% (high dose) and 10% (low dose)	BSID III (MDI); AIMS	<i>Subgroup methadone data extracted from paper. Scores presented are low dose vs high dose. MDI 96.6 (7) vs 94.3 (9), ns. AIMS percentile (sd): 44.8 (24) vs 38.1 (24) ns</i> High dose methadone associated with decreased HC z-score compared with low dose methadone, p<0.025. <i>No statistics reported on BSID or AIMS scores comparing low vs high, as subgroup data extracted from paper</i> Regression analysis confirmed an association between high dose methadone and lower HC z-score, p <0.025	Part of a study comparing low doses of methadone, high doses of methadone and buprenorphine. No unexposed comparator group. Subgroup results presented. Mean GA low group 37 weeks, 18/84 preterm. Mean GA high group 38 weeks, 13/81 preterm. Assessor blinded to infant background NAS treated in 72/84 in low group and 73/81 in the high group, treated with morphine and /or phenobarbital

Author Year	QR ^a	Meth	Un-exposed	Age ^b	Drug info ^c	Assessment instrument	Main findings <i>Results appear as methadone vs unexposed</i>	Comments ^d
McGlone <i>et al</i> , UK, 2015 ⁴⁹ Prospective cohort study	B	81	26	6 m	No dosing information MUS, IUS; Infant meconium; 75% opiates 67% BDZ 64% cannabis 26% stimulants	Griffiths Scales of Mental Development 1996 revision.	<i>All data median (IQR) and adjusted p values (correcting for maternal smoking and alcohol) GQ: 97 (93 -100) vs 105 (101-108) p<0.001; Locomotor: 102 (97 – 107) vs 111 (101-111) p=0.006; Personal-social: 94 (88-96) vs 99 (94 – 103) p=0.001; Language – hearing: 105 (105 – 109) vs 109 (105 – 109) p=0.007; Eye – Hand: 94 (86 – 99) vs 104 (99 – 104) p=0.001; Performance: 96 (86-100) vs 101 (101 – 111) p=0.002. 8/81 methadone-exposed had GQ <85. All infants in unexposed group scored ≥95. Infants treated for NAS vs those not treated for NAS had different scores: Median GQ 95 vs 99, (p=0.008). Infants exposed to multiple drugs had lower scores for locomotor and hand-eye skills (p=0.002)</i>	Original cohort 100 methadone-exposed infants and 50 unexposed infants (matched for gestation, birthweight and maternal postcode). Infants excluded if GA <36 weeks. Single assessor, not blinded to allocation. 39 infants treated for NAS with morphine, 15/39 treated with morphine and phenobarbital. Adjusted p values correct for maternal smoking and excess alcohol . Visual impairment independently associated with adverse neurodevelopmental outcome defined as GQ <85 (P<0.001)..

Notes relating to Table 15.

*indicates studies included in meta-analysis at 6 months, ‡ indicates studies included in meta-analysis at 2 years.

^a QR = quality rating: A = good, B = intermediate, C = poor, based on modified GRADE criteria; ^b Age expressed in days (d), months (m) or years (yr);

^c Drug information includes mean daily methadone dose (in milligrams), maternal urine screening (MUS) and/or infant urine screening (IUS) for drug exposure and information on maternal polydrug (PD) use, where these are reported. Unless otherwise stated, all information in this column refers to methadone-exposed group only;

^d Scores are presented as mean values (standard deviation) unless otherwise stated; ^e Comments include information on attrition, matching, gestation, blinding, proportion of infants treated for NAS and pharmacological treatment for NAS, where this is provided in the original publication; ^f Questionnaire completed by parent or care-giver; ^g Mean methadone dose excludes outlier daily dose of 660mg methadone

AIMS = Alberta Infant Motor Scales, ANCOVA = analysis of co-variance, BDZ = benzodiazepine, BSID = Bayley Scales of Infant Development, 1969, BSID III = Bayley Scales of Infant Development, 3rd edition, BW = birth weight, CBAQ = Child Behaviour Attitude Questionnaire CBCL = Child Behaviour Checklist, GCI = Cognitive General Index (used in the MSCA), CGPQ = Child Game Participation Questionnaire, ERP = event-related-potentials, Focus ratio = focussed attention : total play time (as observed by an assessor during 3 minutes of free play), GA = gestational age, GQ = Griffiths Quotient, KABC-A = Kaufman Assessment Battery for Children, achievement component (tests the acquired knowledge of fact), NAS = neonatal abstinence syndrome, NDI = Non-verbal Developmental Index, PD = polydrug (defined as methadone plus any other drug use during pregnancy, excluding tobacco), RATC = Robert's Apperception Test for Children (tests the child's perception of common interpersonal situations), RAKIT = Revision of the Amsterdam Children's Intelligence Test, RC = Reynell Developmental Language Scales, comprehension, RE = Reynell Developmental Language Scales, expression, SBIS = Stanford-Binet Intellectual Scale, sd = standard deviation, SES = socio-economic status, SIDS = sudden infant death syndrome, SGA = small for gestational age, SON-IQ = Snijders-Oomen Nonverbal Intelligence Test, Vineland SM = Vineland social maturity Scale, WICS-R = Weschler Intelligence Scale for Children – Revised, WWPA = Werry-Weiss Peters Activity Scale

APPENDIX III


The original format of the following first author paper is shown in this appendix.

Monnelly et al (2018) is reproduced from John Willey and Sons with permission:

Monnelly VJ, Hamilton R, Chappell FM, Mactier H, Boardman JP: Childhood neurodevelopment after prescription of maintenance methadone for opioid-dependency in pregnancy: a systematic review and meta-analysis.

Dev Med Child Neurol. 2018 Dec 3. doi: 10.1111/14117. [Epub ahead of print]

Childhood neurodevelopment after prescription of maintenance methadone for opioid dependency in pregnancy: a systematic review and meta-analysis

VICTORIA J MONNELLY¹ | RUTH HAMILTON² | FRANCESCA M CHAPPELL³ | HELEN MACTIER⁴ | JAMES P BOARDMAN^{1,3} 

¹ MRC Centre for Reproductive Health, University of Edinburgh, Edinburgh; ² Department of Clinical Physics and Bioengineering, Royal Hospital for Children, Glasgow; ³ Centre for Clinical Brain Sciences, University of Edinburgh, Edinburgh; ⁴ Princess Royal Maternity, Glasgow, UK.

Correspondence to James P Boardman, MRC Centre for Reproductive Health, Room W1.26, Queen's Medical Research Institute, 47 Little France Crescent, Edinburgh EH16 4TJ, UK. E-mail: james.boardman@ed.ac.uk

This article is commented on by Skranes.

PUBLICATION DATA

Accepted for publication 12th October 2018

Published online

ABBREVIATIONS

MAT	Medically assisted treatment
MDI	Mental Development Index
NAS	Neonatal abstinence syndrome
PDI	Psychomotor Development Index
VEP	Visual evoked potential
WMD	Weighted mean difference

AIM To systematically review and meta-analyse studies of neurodevelopmental outcome of children born to mothers prescribed methadone in pregnancy.

METHOD MEDLINE, Embase, and PsycINFO were searched for studies published from 1975 to 2017 reporting neurodevelopmental outcomes in children with prenatal methadone exposure.

RESULTS Forty-one studies were identified (2283 participants). Eight studies were amenable to meta-analysis: at 2 years the Mental Development Index weighted mean difference of children with prenatal methadone exposure compared with unexposed infants was -4.3 (95% confidence interval [CI] -7.24 to -1.63), and the Psychomotor Development Index weighted mean difference was -5.42 (95% CI -10.55 to -0.28). Seven studies reported behavioural scores and six found scores to be lower among methadone-exposed children. Twelve studies reported visual outcomes: nystagmus and strabismus were common; five studies reported visual evoked potentials of which four described abnormalities. Factors that limited the quality of some studies, and introduced risk of bias, included absence of blinding, small sample size, high attrition, uncertainty about polydrug exposure, and lack of comparison group validity.

INTERPRETATION Children born to mothers prescribed methadone in pregnancy are at risk of neurodevelopmental problems but risk of bias limits inference about harm. Research into management of opioid use disorder in pregnancy should include evaluation of childhood neurodevelopmental outcome.

Opioid use, both prescribed and illicit, has been increasing globally since 2007. Past-year prevalence of heroin use has almost doubled since 2007, and the rate of increase is higher among women compared with men. In the USA, the average rate of past-year heroin use between 2013 and 2015 was 2.0 per 1000 women,¹ and since 2000 there has been an almost fivefold increase in the prevalence of neonatal abstinence syndrome (NAS), a drug withdrawal syndrome commonly used as a proxy for opioid exposure during pregnancy.² It is estimated that in the current opioid crisis up to 14.4% of pregnant women have opioid prescriptions dispensed during pregnancy.³

Pregnant women who use heroin are recommended medically assisted treatment (MAT) with an opioid substitute such as methadone as part of a comprehensive antenatal care plan because it is associated with improved use of

antenatal services, reduced use of heroin during pregnancy, and reduced risk of preterm delivery, when compared with no treatment.⁴⁻⁹ Fetal benefits of MAT include improved growth^{7,10} and less risk of intrauterine death.⁸

Methadone is a synthetic long acting μ -opioid agonist which freely crosses the placenta; despite the potential for methadone to affect the developing fetal brain, this treatment was introduced into practice without a randomized controlled study of childhood neurodevelopmental outcome. Preclinical studies suggest that exogenous opioids may exert pleiotropic harmful effects on the central nervous system,¹¹⁻¹³ and diffusion magnetic resonance imaging studies show that the tract tissue microstructure of white matter (fractional anisotropy) is altered in neonates exposed prenatally to methadone.^{14,15} Improved understanding of the neurodevelopmental outcome of children

born to opioid-dependent mothers and exposed prenatally to methadone is essential to inform management of their mothers during pregnancy.^{16–18} The issue is prescient because the optimal methadone dose regimen is uncertain,¹⁹ and alternative opioids such as buprenorphine may have a different risk profile for neonatal outcome,^{16,17,20} leading to equipoise about the optimal MAT strategy.

The aims of this study were to perform a systematic review of published literature on childhood neurodevelopmental outcomes after prescription of maintenance methadone in pregnancy, and to undertake a meta-analysis of studies that used a common assessment tool.

METHOD

The study protocol was registered with the international prospective register of systematic reviews (PROSPERO), registration number CRD42017063987 (<https://www.crd.york.ac.uk/prospéro/>). Methodology is reported according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) statement.²¹

We included all studies that reported neurodevelopmental outcome, including visual development, of children whose opioid-dependent mothers were prescribed methadone during pregnancy. There was no language restriction. Exclusion criteria were prescription of alternative opioid substitutes during pregnancy and studies reporting only neonatal neurodevelopment.

Two reviewers (VJM, RH) independently searched MEDLINE, Embase, and PsycINFO for studies published between 1975 and 2017. Medical Subject Headings terms used were ‘methadone’ and ‘prenatal’ or ‘prenatal exposure’ or ‘prenatal drug exposure’ or ‘prenatal exposure delayed effects’ or ‘in utero’. Bibliographies of primary studies and review articles meeting the inclusion criteria were searched manually to identify further eligible studies.

Three reviewers (VJM, RH, HM) independently screened titles and abstracts to identify potentially eligible studies. Where necessary to determine eligibility, full text was retrieved and reviewed. Duplication was avoided if it was clear that the same cohort was reported in two publications; where more than one publication for a study was retrieved, only the report that contained the maximum data points was included.

Four reviewers (VJM, RH, HM, JPB) independently extracted data from included studies using a standardized template. Extracted information included study setting, design, population and participant demographics, details of methadone exposure if available, control conditions, recruitment and completion rates, age at outcome measurement, assessment tool, and outcome of assessment. Data were extracted from each study by two reviewers independently, and templates were combined to ensure complete data collection. Disagreements were resolved through discussion.

A quality assessment instrument was developed using the Grading of Recommendations Assessment Development and Evaluation Guidelines^{22–24} to provide a structured

What this Study Adds

- Children born to opioid-dependent mothers prescribed methadone are at risk of neurodevelopmental impairment.
- Exposed infants have lower Mental Development Index and Psychomotor Development Index scores than unexposed children.
- Atypical visual evoked potentials, strabismus, and nystagmus have increased prevalence.
- Estimates of impairment may be biased by intermediate to poor quality evidence.

scoring system that aimed to describe quality and sources of bias in studies of neurodevelopment after prenatal drug exposure. It incorporated objective criteria about study design, sample size and characteristics, use of validated outcome measures, risk of bias (blinding, confounding, attrition), and data analysis. Each study was assessed independently by two reviewers (VJM, JPB) and scored as good (A, 6.5–8), intermediate (B, 3.5–6), or poor (C, 1–3) quality (Table S1, online supporting information).

Statistical analysis

Where studies used the same assessment tool for any outcome domain, quantitative data were pooled in random effects meta-analysis using R software, version 3.2.2 (K Hornik; R Foundation for Statistical Computing, Vienna, Austria; <https://cran.r-project.org/>) with mean difference weighted by the inverse of the variance.²⁵ Effect sizes were expressed as weighted mean differences (WMD) and their 95% confidence intervals (CI). For longitudinal studies, data for assessments at 6 months and at 2 years were analysed. Heterogeneity was assessed using the standard I^2 and τ statistics and graphically using forest plots. Where statistical pooling was not possible, data were collated in tables for outcomes across two domains (neurodevelopmental and visual development), and statements generated to represent the body of literature reviewed.

RESULTS

Characteristics of included studies

Forty-one eligible studies were identified, including a total of 1441 methadone-exposed children and 842 unexposed children (Fig. S1, online supporting information). Twenty-nine studies reported neurodevelopmental outcome (1247 methadone-exposed vs 740 unexposed children), eight of which were amenable to meta-analysis; 12 reported visual outcome (275 methadone-exposed vs 128 unexposed). There were no randomized trials.

Only one study fulfilled criteria for good quality;²⁶ 26 (63%) studies were intermediate and 14 (34%) were poor quality (Table S1). Study deficiencies included lack of blinding, small sample size, high attrition rates, and lack of comparison group validity. Twenty-four of 41 studies reported information about polydrug use during pregnancy, and 20 studies provided information about methadone dose exposure.

Thirty-three of 41 studies reported infants receiving pharmacological treatment of NAS; in 19 of these the treatment regimen was described, with the most frequently

used drugs being morphine, phenobarbital, benzodiazepines, or a combination. Sixteen studies stated that infants born preterm were included, while only seven explicitly excluded infants born before 36 weeks' gestation. In 18 studies, it could not be determined whether infants born preterm were included.

Neurodevelopmental outcome

Of 29 studies reporting neurodevelopmental outcome, 15 used the original Bayley Scales of Infant Development.²⁷ Five of these 15 studies had no comparison individuals,^{28–32} one did not report a measure of variance,³³ and one assessed children at 9 months only;³⁴ leaving eight studies that were eligible for meta-analysis of neurodevelopmental outcome based on the Bayley Scales of Infant Development (Table I).

Five studies reported Mental Development Index (MDI) at 6 months of age,^{35–39} and four of these reported Psychomotor Development Index (PDI)^{35–37,39} (Fig. 1). Studies were all of intermediate quality with attrition rates ranging from 31% to 70%; three studies described maternal methadone doses, and gestational age was variably reported. For both MDI and PDI at 6 months, the difference in exposed versus non-exposed infants was marginal and 95% CIs included the possibility of no difference: MDI, WMD of -1.56 (95% CI -4.98 to 1.87 ; Fig. 1a); PDI, WMD of -2.46 (95% CI -6.75 to 1.82 ; Fig. 1b).

Seven studies reported MDI at 2 years of age,^{36–42} four of which reported PDI^{36,37,39,42} (Table I). All seven studies were rated intermediate quality, with attrition rates ranging from 18% to 84%; maternal methadone dose was variably reported; and where polydrug use was reported (five of seven studies), this ranged from 56% to more than 90% of mothers. The gestational age of participants was not stated in four studies.^{38,40–42} Five studies reported rates of NAS between 67% and 92%; and no study described treatment for NAS. Compared with non-exposed children, methadone exposure was associated with lower MDI, WMD of -4.43 (95% CI -7.24 to -1.63 ; Fig. 2a), and lower PDI, WMD of -5.42 (95% CI -10.55 to -0.28 ; Fig. 2b).

Of the remaining 21 neurodevelopmental studies, 13 were rated as having intermediate quality and eight as poor quality. Infant Behavior Record was reported in two studies: Marcus et al. reported poorer motor performance at 4 months in 15 methadone-exposed infants compared with 23 unexposed infants;⁴³ and Wilson et al.³⁴ matched 33 methadone-exposed infants with 55 unexposed infants for maternal age, ethnicity, socio-economic status, and marital status, and reported poorer fine motor coordination, less attentiveness, and lower motor scores on the Bayley Scales of Infant Development at 9 months of age, but no difference in cognitive scores. Schneider and Hans reported no difference in focused attention during free play at 24 months between 30 exposed and 44 unexposed toddlers.⁴⁴

Suffet et al.³⁰ reported MDI and PDI in the normal range at 1 year, with females performing better than males (MDI mean 108.8 vs 102.7, $p < 0.05$; PDI mean 102.3 vs

95.7, $p < 0.05$). This association persisted for cognition up to 2 years of age (MDI 99.2 vs 82.0, $p < 0.01$). Bier et al.⁴⁵ reported MDI scores in the normal range at 4 months of age, in a cohort of 165 methadone-exposed infants, with no difference between those infants exposed prenatally to either low dose (< 100 mg/d) or high dose (≥ 100 mg/d) methadone.

At 6 months of age, using the Griffiths Scales of Mental Development, McGlone et al.⁴⁶ noted reduced median scores across all domains which persisted after adjustment for prenatal alcohol exposure and maternal smoking. This study included 81 methadone-exposed infants and 26 non-drug-exposed infants, matched for gestation and socio-economic status. Scores were also lower for infants who had been treated for NAS (median general quotient 95 vs 99, $p < 0.008$). Bunikowski et al.⁴⁷ reported reductions in quotients for two subscales (hearing and speech; intellectual performance) at 1 year of age in a case series of 18 prenatally exposed infants compared with 42 unexposed children.

Twelve studies evaluated children older than 2 years using a range of assessment tools (Table II). Participants included 323 methadone-exposed children and 321 unexposed children.

Six out of 10 studies measuring cognitive outcomes reported no difference between methadone-exposed and unexposed children,^{37,38,41,48–50} and four studies reported lower cognitive performance in methadone-exposed children at 2 years 6 months,³⁹ 3 years,⁵¹ 3 years 6 months,⁵² 4 years 6 months,⁵² 5 years 6 months,⁵² and 8 years 6 months⁵³ respectively. The Reynell Developmental Language Scales were used in two studies (93 methadone-exposed vs 76 unexposed children) at 3 years⁵¹ and at 4 years;⁵² both reported reduced performance in expressive and comprehensive language. Of the seven studies assessing behaviour, six reported more behavioural problems in methadone-exposed children than in unexposed children.^{41,50–52,54,55} Details of all studies reporting childhood neurodevelopmental outcome after prenatal methadone exposure are summarized in Table SII (online supporting information).

Visual development and function

Twelve studies reported visual outcomes, five of which measured visual evoked potentials (VEPs; Table SIII, online supporting information). The VEP studies consisted of a total of 143 methadone-exposed and 103 unexposed children, with one rated poor quality, three rated intermediate, and one rated good quality.

In two different cohorts, flash VEPs at 1 day to 4 days after birth were more frequently absent or immature and were smaller on average in methadone-exposed infants compared with non-exposed newborns.^{56,57} At 4 months⁵⁸ and at 6 months of age,²⁶ pattern-reversal and pattern-onset VEP abnormalities persisted in methadone-exposed infants. One follow-up study of 10 3-year-old children previously tested at 4 months found no group difference in pattern-reversal VEP peak times.⁵⁹

Table 1: Summary of the eight studies included in the meta-analysis

Study	Quality rating ^a	Methodone-exposed	Unexposed	Age ^b	Drug information ^c	Assessment tool	Main findings ^d (results appear as methadone vs unexposed)	Comments ^e
Strauss et al. ^{35,f}	B	25	26	3mo 6mo ^f 1y	No dosing information No drug screening or polydrug information	BSID (MDI, PDI) at all ages	6mo ^f : MDI 115.7 (16.8) vs 114.3 (20.9); PDI 109.4 (12.2) vs 111.7 (14.5)	Original cohort 60 methadone-exposed infants vs 53 unexposed infants (no information on matching); data reported only for infants who underwent BSID at all three time points. Attrition rate at 6mo was 58.4% vs 51%. One case of SIDS in the methadone-exposed group. Gestational age at birth not stated. Assessor blinding not stated.
Kaltenbach et al. ^{40,g}	B	26	27	1y 2y ^g	Mean dose for 1y cohort: 30; mean dose for 2y cohort: 18 No drug screening or polydrug information	BSID (MDI) at all ages	2y ^g : MDI 90.88 (8.26) vs 94.62 (11.93) ns	No information about NAS or treatment. Original cohort 43 methadone-exposed infants vs 51 unexposed (matched for maternal SES, ethnicity and medical conditions). Attrition rate at 2y 60.5% vs 53%. Assessors blinded to group. 62% 1y-olds and 67% 2y-olds had been treated for NAS in the neonatal period. No pharmacological agent stated
Chasnoff et al. ^{36,f,g}	B	31	34	3mo 6mo ^f 1y 2y ^g	Mean dose 14.6±10.2 (5–40) Maternal interview and urine screening; 4 out of 31 used drugs in addition to heroin during pregnancy	BSID (MDI, PDI) at all ages	6mo ^f : MDI 105.9 (12.4) vs 111.0 (12.3); PDI 103.9 (9.0) vs 107.6 (15.1) 2y ^g : MDI 97.5 (16.1) vs 96.2 (15.9); PDI 99.3 (16.9) vs 98.2 (8.9)	Original cohort 39 methadone exposed vs 34 unexposed (matched for maternal age, education, gravidity, and smoking). Attrition rate at 6mo was 66.7% vs 14.7%; at 2y attrition rate was 84.6% vs 58.8%. All infants were born at term. Assessor blinding not stated. No information about NAS or treatment.
Rosen and Johnson ^{37,f,g}	B	41	23	6mo ^{h,i} 1y 18mo 2y ^a 3y	42 (mean of original cohort) Maternal urine screening; 56% of original cohort polydrug use (BDZ, opiates, cocaine, barbiturates, TCA) 15% reported 'mod-severe' alcohol intake	BSID (MDI, PDI) at 6mo, 12mo, 18mo, and 2y; M-P at 3y	No <i>p</i> -values stated 6mo ^f : MDI 95 (2.5) vs 100.7 (4.2), ns; PDI 101 (2.8) vs 105.1 (2.9), ns 2y ^a : MDI 90.4 (2.6) vs 96.9 (3.1) ns; PDI 99.1 (2.7) vs 108 (2.7), <i>p</i> = 0.05	Original cohort 61 methadone-exposed infants vs 32 unexposed infants (matched for maternal ethnicity, SES, infant sex, birthweight, and gestational age). Attrition rate at 6mo was 32.8% vs 28.1%; at 2y was 44.3% vs 31.3%. Both groups included infants born preterm. Assessor blinding not stated. 75% methadone-exposed had NAS; number treated pharmacologically not stated
Kaltenbach and Finnegan ^{38,f,g}	B	27	17	6mo ^f 1y 2y ^g 3y 6mo 4y 6mo	Mean dose 38.42 No drug screening or polydrug information	BSID (MDI) at 6mo, 1y, and 2y; MSCA (GCI) at 3y 6mo–4y 6mo	6mo ^f : MDI 107.9 (12.23) vs 105.6 (7.31), no <i>p</i> -value 2y ^g : MDI 100.9 (18.04) vs 103.9 (11.49) no <i>p</i> -value	No information about original cohort, therefore attrition rates unknown. Unexposed group were matched for maternal ethnicity and SES. Mean gestational age infants not stated. Blinding of assessors not stated. 92% were treated for NAS; pharmacological agent not stated.

Table 1: Continued

Study	Quality rating ^a	Methadone-exposed	Unexposed	Age ^b	Drug information ^c	Assessment tool	Main findings ^d (results appear as methadone vs unexposed)	Comments ^e
Wilson ^{41,g}	B	33 29 32 26 12	54 42 48 41 12	9mo 18mo 2y ^d 3-5y 6-11y	No dosing information Maternal urine screening; 93% used psychoactive drugs	BSID (MDI) at 9mo, 18mo, and 2y ^c	2y ^d : MDI 88.8 (15.5) vs 90.2 (14.6) ns	Original cohort 39 methadone-exposed vs 57 unexposed infants (matched for maternal age, ethnicity, SES, and marital status). Attrition rate at 2y was 18% vs 16%. Mean gestational age not stated for either group. Assessor blinding not stated. 87% original cohort treated for NAS, pharmacological agent not stated.
van Baar ^{39,f,g}	B	21 21 18 21 19	37 34 34 34 34	6mo ^f 1y 18mo 2y ^d 2y 6mo	No dosing or screening information; Original cohort; six IV drug users; 94% used multiple drugs; 60% use cocaine	BSID (MDI, PDI, NDI) at all ages WWPA ^f at 18mo (n=14), 2y (n=16), and 2y 6mo (n=15)	6mo ^f : MDI 103 (12) vs 107 (13), PDI 116 (18) vs 114 (21), NDI 105 (13) vs 109 (14) 2y ^d : MDI 86 (15) vs 98 (16) p<0.05 PDI 102 (16) vs 100 (18) ns, NDI 93 (16) vs 102 (22)	Original cohort 35 methadone-exposed vs 37 unexposed infants (not matched). Attrition rate at 6mo was 19.2% vs 0% and at 2y was 19.3% vs 8.1%. Assessor blinding not stated. 28 out of 35 (80%) were treated for NAS, pharmacological agent not stated.
Hans and Jeremy ^{42,g}	B	33 33 33 33 33	45 45 45 45 45	4mo 8mo 1y 18mo 2y ^d	Mean <20 (range 3-40) Maternal interview and MUS; 13 out of 33 cocaine, 18 out of 33 cannabis, 11 out of 33 alcohol use	BSID (MDI, PDI) at all ages	2y ^d : MDI 92 (12.7) vs 96 (12.3); PDI 100 (14.2) vs 108 (14.9)	Original cohort 47 methadone-exposed vs 45 unexposed infants (matched for maternal age, SES, and IQ). Attrition rate 29.8% vs 0%. Assessors blinded to group. No information about NAS or treatment.

^aQuality rating: A, good; B, intermediate; C, poor; based on modified Grading of Recommendations Assessment Development and Evaluation (GRADE) criteria (Table S1, online supporting information). ^bAge expressed in months (mo) or years (y). ^cDrug information includes mean daily methadone dose (in milligrams), maternal urine screening, and/or infant urine screening for drug exposure and information on maternal polydrug use (defined as methadone plus any other drug use during pregnancy, excluding tobacco), where these are reported. Unless otherwise stated, all information in this column refers to methadone-exposed group only. ^dFull details from these studies is available in Table S11 (online supporting information). Scores are presented as mean values (standard deviation) unless otherwise stated. ^eComments include information on attrition, matching, gestation, blinding, proportion of infants treated for NAS, and pharmacological treatment for NAS, where provided in the original study. ^fStudies included in meta-analysis at 6mo. ^gStandard errors were converted to standard deviations for the meta-analysis. BSID, Bayley Scales of Infant Development; MDI, Mental Developmental Index; PDI, Psychomotor Developmental Index; SIDS, sudden infant death syndrome; NAS, neonatal abstinence syndrome; SES, socio-economic status; BDZ, benzodiazepine; TCA, tricyclic antidepressant; M-P, Merrill-Palmer Scale; MSCA, McCarthy Scales of Childhood Abilities; GCI, General Cognitive Index; ns, not significant; IV, intravenous; NDI, non-verbal developmental index; WWPA, Werry-Weiss Peters Activity Scale; MUS, maternal urine screening.

A further six case series have described abnormal visual outcomes in a total of 108 methadone-exposed children^{60–65} (Table SIV, online supporting information). All six studies were rated as having poor quality evidence because they did not have comparison groups and did not correct for confounders owing to their observational design. However, collectively they describe common visual abnormalities, nystagmus (50 out of 108) and strabismus (51 out of 108), which may occur together (22 out of 108 cases). Nystagmus in all described cases was horizontal and either jerk or pendular in waveform.

In a case-control study of 100 methadone-exposed infants, 81 of whom were followed up at 6 months of age, abnormal visual outcomes were present in 40% of methadone-exposed children (nystagmus nine out of 81 cases, strabismus 20 out of 81 cases; both five out of 81) compared with two out of 26 non-drug exposed infants matched for gestation and socio-economic status.²⁶ One intermediate quality study of methadone-exposed 4-year-old children reported reduced visual selective attention in methadone-exposed children compared with unexposed children.⁶⁶

DISCUSSION

This systematic review of neurodevelopmental and visual outcomes of children born to opioid-dependent mothers prescribed methadone in pregnancy has synthesized data from 41 studies (1441 children whose mothers were prescribed methadone and 842 children whose mothers were not prescribed methadone during pregnancy). In the meta-analysis, we found that point estimates of MDI and PDI in children exposed to prenatal methadone compared with children whose mothers were not prescribed methadone are reduced at 6 months of age, and by 2 years the 95% CIs of these estimates make the possibility of no group difference in MDI and PDI unlikely. The emergence of difficulties as children grow older is well-recognized after complications during the perinatal period and is likely to reflect the ontogeny of higher-order functions through childhood. The finding of behavioural problems in six out of seven studies that measured this domain, and lower cognitive performance in four out of 10 studies that reported outcome after 2 years, suggests that children of opioid-dependent mothers prescribed methadone may be at increased risk of longer-term problems.

An association between prenatal methadone exposure and atypical visual development has been described, with significant differences in VEPs in infancy and childhood, reflecting altered visual pathways.^{56,58} McGlone et al.,²⁶ in their cohort of 81 methadone-exposed and 29 unexposed infants at 6 months of age, describe a methadone-attributable risk of abnormal visual assessment of 80%, after correcting for excess prenatal alcohol exposure. The prevalence of childhood strabismus and nystagmus in the methadone-exposed population is higher than expected, which suggests that disorders of childhood visual function,

as well as altered electrophysiological measures, are associated with prenatal methadone exposure.

Our findings are consistent with a recent meta-analysis of five studies of infants and preschool children exposed to chronic intrauterine illicit heroin and/or prescribed methadone, which reported neurobehavioural impairment in the opioid-exposed group.^{67,68} Our data provide additional information by focusing on studies of women prescribed methadone, analysis of studies that reported a wide range of outcomes including visual development, and inclusion criteria designed to achieve maximum representation of the target population. Specifically, because use of prescribed and non-prescribed drugs and tobacco is common among pregnant women prescribed methadone (but ascertainment and reporting of polydrug exposure in studies is variable),⁶⁹ and because our purpose was to determine outcomes of methadone-exposed children rather than to investigate causation, we took a pragmatic approach and did not attempt to exclude on the basis of polydrug use.

The data are also consistent with the observation that fractional anisotropy is reduced throughout the white matter skeleton of neonates born to mothers who were prescribed methadone,¹⁵ because neonatal fractional anisotropy is associated with later neurodevelopmental impairment.^{70,71} More broadly, these results contribute to an emerging literature suggesting that exposure of the brain to psychoactive drugs during the perinatal period may modify its development.^{72,73}

A strength of this work is its pragmatic and systematic approach to summarizing childhood neurodevelopmental outcome after prescribed prenatal methadone exposure. We excluded studies of neonatal neurodevelopment to prevent confounding by NAS, and we excluded studies of alternative opioid substitutes to derive maximum inference about methadone.

However, limitations of included studies mean that the risk of impairment in children whose mothers were prescribed methadone may be biased. In particular, comparison groups were often poorly described beyond the definition of 'non-opioid exposed', with inadequate control for socio-economic status, or environmental factors, making it difficult to know who the methadone-exposed children were being compared with. Reporting of maternal methadone dosing and polydrug or alcohol use was variable and therefore it was difficult to obtain an accurate exposure profile of included children; only one study examined prenatal exposure in all infants in detail using extensive toxicology.⁵⁷ Finally, only 15 of the 41 studies were published in the past decade, which might affect application of results to contemporary populations because patterns of drug misuse change over time and strategies for MAT of opioid use disorder in pregnancy have evolved. For example, the dose of methadone prescribed for MAT in current practice is typically higher than that reported in historical studies. Further study of contemporary populations is required to determine the neurodevelopmental and

Table II: Summary of 12 studies reporting neurodevelopmental outcomes in children beyond age 2y

Study	Quality rating ^a	Methadone-exposed	Unexposed	Age ^b	Drug information ^c	Assessment tool	Main findings ^d (results appear as methadone vs unexposed)
Strauss et al. ⁴⁸	B	31	27	5y	No information	MSCA	86.8 (13.3) vs 86.2 (16.2), ns
Lifschitz et al. ⁴⁹	B	26	41	3y 5mo	95% taking heroin or psychoactive drugs	MSCA	90.4 (13) vs 89.4 (10.8), ns
Rosen and Johnson ⁷⁷	B	39	21	3y	42 (mean of original cohort)	M-P	44.6 (2.1) vs 46.3 (2.3) ns
Davis and Templer ⁵³	C	12	28	8y 6mo	No information	WISC-R	89.58 (10.32) vs 96.32 (8.72) no <i>p</i> -value
Wilson ⁴¹	B	26 12	41 12	3-5y 6-11y	No information	MSCA (GCI) Survey and school reports IQ testing	GCI: 90.4 (13.0) vs 89.4 (10.8) ns IQ 1-2 SD below norm 8% vs 5%, language disability 8% vs 5%, special education needs 16% vs 19%, behavioural problems 75% vs 48%, psychiatric referral 16% vs 5%
Kaltenbach and Finnegan ³⁸	B	27	17	3y 6mo-4y 6mo	Mean dose 38.42	MSCA (GCI)	GCI: 106.5 (12.96) vs 106.05 (13.10), <i>t</i> =0.11
Sandberg et al. ⁵⁴	B	30	16	5-8y	39.5 (males), 38.7 (females) Original cohort, 68% polydrug use; 15% moderate to heavy alcohol intake	CGPQ [*] CBAQ [*] (males only)	Methadone-exposed males showed more feminine game play than comparison males (<i>p</i> <0.04)
van Baar ³⁹	B	19	34	2y 6mo	No information	BSID (MDI, PDI, NDI) WWPA [*]	MDI 86 (15) vs 98 (16) <i>p</i> <0.05 PDI 102 (16) vs 100 (18) ns NDI 93 (16) vs 102 (22) WWPA 1.62 (1.03-2.66) vs 1.64 (1.28-2.52) ns
de Cubas and Field ⁵⁰	B	20	20	8y 6mo	No drug information. 'Moderate alcohol use'	SBIS KABC-A RATC CBCL [*]	SBIS: 97.6 vs 98.1, ns KABC-A: 98.8 vs 102.4, no <i>p</i> -value RATC: methadone-exposed scored higher on anxiety, aggression, rejection, maladaptive outcome, <i>p</i> <0.01 for all CBCL: more behaviour problems <i>p</i> <0.05
van Baar and de Graaf ⁶²	B	23 26 23 22	32 32 31 30	3y 6mo 4y 6mo 5y 6mo	Polydrug use; 16 out of 35 heroin and cocaine, only 2 out of 35 solely methadone	SON-IQ at 3y 6mo; RDLSC and RDLSE at 4y; RAKIT at 4y 6mo and 5y 6mo; IBR at 3y 6mo (<i>n</i> =22), 4y 6mo (<i>n</i> =23), 5y 6mo (<i>n</i> =22)	SON-IQ: 99 (9) vs 109 (11), <i>p</i> <0.01 RDLSC: 46 (6) vs 52 (6), <i>p</i> <0.01 RDLSE: 46 (9) vs 50 (6), <i>p</i> <0.05 RAKIT 4y 6mo: 85 (11) vs 103 (15), <i>p</i> <0.01 RAKIT 5y 6mo: 90 (12) vs 102 (17), <i>p</i> <0.05 IBR: results median (range): 3y 6mo: free of fear: 9 (4-9) vs 6.5 (2-9), <i>p</i> <0.05; activity level: 6 (3-9) vs 5 (2-9), <i>p</i> <0.05; attention: 5 (1-7) vs 5.5 (1-9) <i>p</i> <0.05; fine motor: 3 (1-5) vs 3 (1-5), <i>p</i> <0.05 4y 6mo: cooperation: 6 (2-9) vs 7 (3-9), <i>p</i> <0.01; endurance: 4 (2-9) vs 6 (1-9), <i>p</i> <0.01; attention: 8 (2-9) vs 5 (2-8), ns 5y 6mo: cooperation: 6 (1-9) vs 8 (4-9), <i>p</i> <0.01; free of fear: 8 (2-9) vs 9 (5-9), ns; attention: 5 (2-8) vs 5 (3-9), ns
Hunt et al. ⁵¹	B	67	44	3y	No information	SBIS; VSMS; MSCA; RDLSC and RDLSE	SBIS: 99.9 (15.1) vs 107.5 (13.4), <i>p</i> <0.01 VSMSL: 38.4 (8.1) vs 46.1 (7.7), <i>p</i> <0.05 MSCA: 49.5 (8.7) vs 53.9 (8.3), <i>p</i> <0.05 RDLSC: 42.4 (11.6) vs 49.2 (11.4), <i>p</i> <0.05 RDLSE: 35.5 (7.9) vs 42.8 (12.8), <i>p</i> <0.05

Table II: Continued

Study	Quality rating ^a	Methodone-exposed	Unexposed	Age ^b	Drug information ^c	Assessment tool	Main findings ^d (results appear as methadone vs unexposed)
Konijnenberg et al. ⁵⁵	B	24	0	4y	Mean 85.96 ^f ; polydrug use in 40% (illegal drug use), 25% alcohol	CBCL ^e	Scores >55 on aggressive behaviour and withdrawn behaviour

^aQuality rating: A, good; B, intermediate; C, poor; based on modified Grading of Recommendations Assessment Development and Evaluation criteria (Table S1, online supporting information). ^bAge expressed in months (mo) or years (y). ^cDrug information includes mean daily methadone dose (in milligrams) and information on maternal polydrug use (defined as methadone plus any other drug use during pregnancy, excluding tobacco), where these are reported. Unless otherwise stated, all information in this column refers to methadone-exposed group only. ^dScores are presented as mean values (standard deviation) unless otherwise stated. ^eQuestionnaire completed by parent or caregiver. ^fMean methadone dose excludes outlier daily dose of 660mg methadone. MSCA, McCarthy Scales of Childhood Abilities; ns, not significant; M-P, Merrill-Palmer Scale; WISC-R, Wechsler Intelligence Scale for Children-Revised; GCI, general cognitive index (used in the MSCA); SD, standard deviation; CGPQ, Child Game Participation Questionnaire; CBAQ, Child Behavior Attitude Questionnaire; BSID, Bayley Scales of Infant Development (original version 1969); MDI, mean developmental index (cognitive score); PDI, psychomotor developmental index (motor score); NDI, non-verbal developmental index; WWPA, Werry-Weiss Peters Activity Scale; SBIS, Stanford-Binet Intellectual Scale; KABC-A, Kaufman Assessment Battery for Children, achievement component (tests the acquired knowledge of fact); RATC, Robert's Apperception Test for Children (tests the child's perception of common interpersonal situations); CBCL, Child Behavior Checklist; SON-IQ, Snijders-Oomen Nonverbal Intelligence Test; RDLSC, Reynell Developmental Language Scales (Comprehensive); RDLSE, Reynell Developmental Language Scales (Expressive); RAKIT, Revision of the Amsterdam Children's Intelligence Test; IBR, Infant behaviour record; VSMS, Vineland Social Maturity Scale.

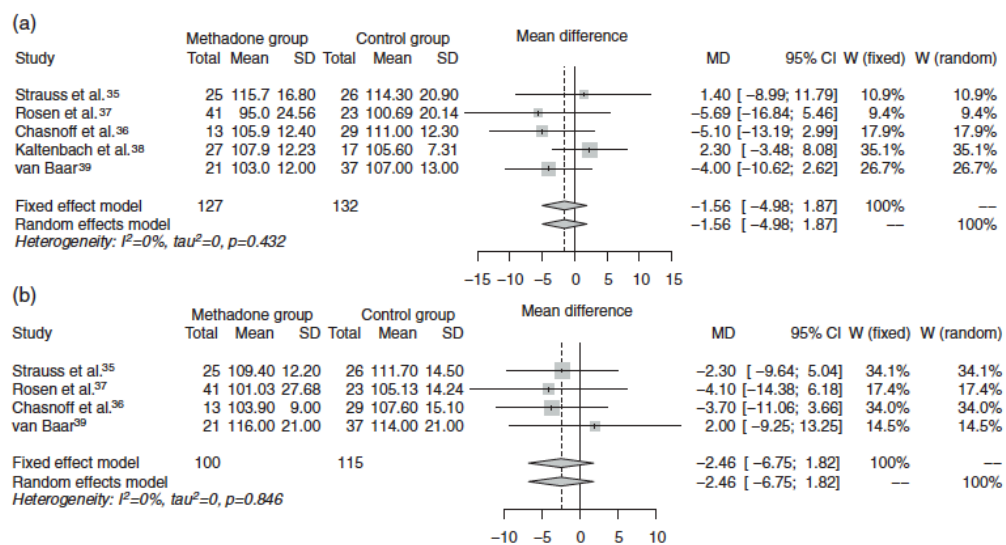


Figure 1: (a) Weighted mean difference in Mental Developmental Index and (b) Psychomotor Developmental Index of the Bayley Scales of Infant Development at age 6 months between methadone-exposed and unexposed infants.

visual outcomes of children born to opioid-dependent mothers.

Improved understanding of the effects of prenatal opioid use disorder and its treatment, including the use of alternative substitutes, has been identified as a research priority.⁷⁴ Buprenorphine has been evaluated as an opioid substitute in pregnancy. Although less severe NAS, improved growth, shorter hospital stay, and longer gestation are all reported in buprenorphine-exposed compared with methadone-exposed

infants,^{16,75-77} a recent Cochrane review concluded that there are insufficient data to establish whether buprenorphine is equivalent for all maternal outcomes, including adherence to treatment.¹⁸ Furthermore, confounding by indication could explain improved neonatal outcomes in buprenorphine groups.¹⁷ Therefore, there remains clinical equipoise about the safest opioid substitute for mother and child.

The data presented highlight that being born to an opioid-dependent mother who has been prescribed

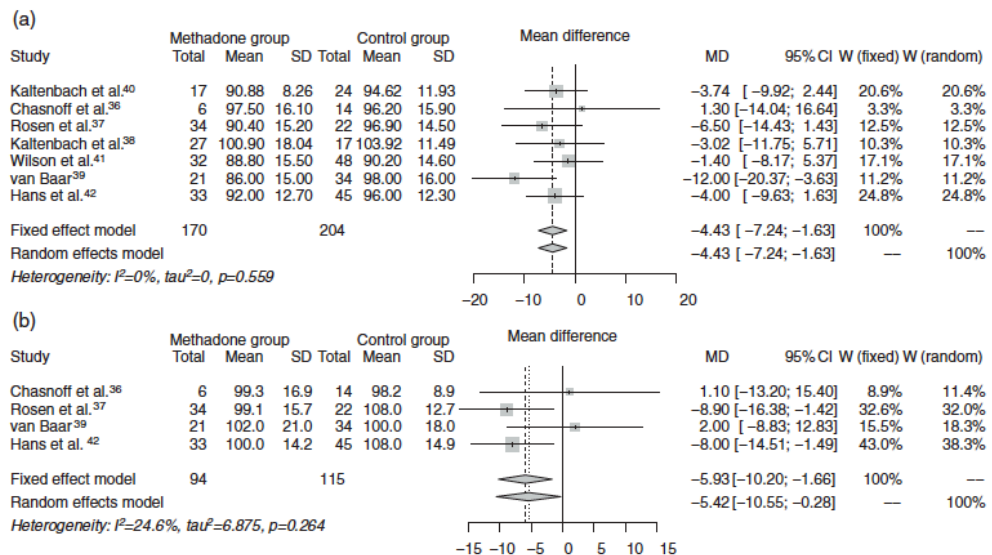


Figure 2: (a) Weighted mean difference in Mental Developmental Index and (b) Psychomotor Developmental Index of the Bayley Scales of Infant Development at age 18 to 24 months between methadone-exposed and unexposed infants.

maintenance methadone in pregnancy is associated with adverse visual and neurodevelopmental outcomes in infancy and early childhood, but deficiencies in the existing literature limit causal inference about harm and factors other than methadone per se could account for these observations. Further research into optimal management of opioid-dependent pregnant women is required; future studies should consider fetal brain development and long term neurodevelopmental and visual outcomes of the child.

ACKNOWLEDGEMENTS

We acknowledge Marshal Dozier, librarian at the University of Edinburgh, for her help and guidance with the original literature search and Oliver Koch for his help translating two articles written in German. This work was undertaken in the Medical Research Council Centre for Reproductive Health, which is funded by a Medical Research Council Centre grant (Medical

Research Council G1002033). The authors have stated that they had no interests that might be perceived as posing a conflict or bias.

SUPPORTING INFORMATION

The following additional material may be found online:

Table SI: Quality assessment of 41 studies assessing childhood outcomes after prenatal methadone exposure

Table SII: Twenty-nine studies reporting childhood neurodevelopmental outcomes after prenatal methadone exposure

Table SIII: Studies reporting childhood visual evoked potentials (VEPs) after prenatal methadone exposure

Table SIV: Studies reporting childhood visual outcomes after prenatal methadone exposure

Figure S1: Identification and selection. Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) flowchart showing process of inclusion and exclusion of studies.

REFERENCES

- United Nations Office on Drugs and Crime. World Drug Report 2017. United Nations publication E.17.XI.6: United Nations, 2017.
- Patrick SW, Davis MM, Lehmann CU, Cooper WO. Increasing incidence and geographic distribution of neonatal abstinence syndrome: United States 2009 to 2012. *J Perinatol* 2015; **35**: 650-5.
- Bateman BT, Hernandez-Diaz S, Rathmell JP, et al. Patterns of opioid utilization in pregnancy in a large cohort of commercial insurance beneficiaries in the United States. *Anesthesiology* 2014; **120**: 1216-24.
- Burns L, Mattick RP, Lim K, Wallace C. Methadone in pregnancy: treatment retention and neonatal outcomes. *Addiction* 2007; **102**: 264-70.
- Mattick RP, Breen C, Kimber J, Davoli M. Methadone maintenance therapy versus no opioid replacement therapy for opioid dependence. *Cochrane Database Syst Rev* 2009; **3**: CD00220.
- Zelson C, Lee SJ, Casalino M. Neonatal narcotic addiction. Comparative effects of maternal intake of heroin and methadone. *N Engl J Med* 1973; **289**: 1216-20.
- Kandall SR, Albin S, Lowinson J, Berle B, Eidelman AI, Gartner LM. Differential effects of maternal heroin and methadone use on birthweight. *Pediatrics* 1976; **58**: 681-5.
- Kandall SR, Albin S, Gartner LM, Lee KS, Eidelman A, Lowinson J. The narcotic-dependent mother: fetal and neonatal consequences. *Early Hum Dev* 1977; **1**: 159-69.

9. Doberczak TM, Thornton JC, Bernstein J, Kandall SR. Impact of maternal drug dependency on birth weight and head circumference of offspring. *Am J Dis Child* 1987; **141**: 1163–7.
10. Hulse GK, Milne E, English DR, Holman CD. The relationship between maternal use of heroin and methadone and infant birth weight. *Addiction* 1997; **92**: 1571–9.
11. Vestal-Laborde AA, Eschenroeder AC, Bigbee JW, Robinson SE, Sato-Bigbee C. The opioid system and brain development: effects of methadone on the oligodendrocyte lineage and the early stages of myelination. *Dev Neurosci* 2014; **36**: 409–21.
12. Thompson BL, Levitt P, Stanwood GD. Prenatal exposure to drugs: effects on brain development and implications for policy and education. *Nat Rev Neurosci* 2009; **10**: 303–12.
13. Hutchinson MR, Shavit Y, Grace PM, Rice KC, Maier SF, Watkins LR. Exploring the neuroimmunopharmacology of opioids: an integrative review of mechanisms of central immune signaling and their implications for opioid analgesia. *Pharmacol Rev* 2011; **63**: 772–810.
14. Walhovd KB, Watts R, Anlien I, Woodward LJ. Neural tract development of infants born to methadone-maintained mothers. *Pediatric Neurol* 2012; **47**: 1–6.
15. Monnelly VJ, Anblagan D, Quigley A, et al. Prenatal methadone exposure is associated with altered neonatal brain development. *Neuroimage Clin* 2017; **18**: 9–14.
16. Jones HE, Kaltenbach K, Heil SH, et al. Neonatal abstinence syndrome after methadone or buprenorphine exposure. *N Engl J Med* 2010; **363**: 2320–31.
17. Brogly SB, Saia KA, Walley AY, Du HM, Sebastiani P. Prenatal buprenorphine versus methadone exposure and neonatal outcomes: systematic review and meta-analysis. *Am J Epidemiol* 2014; **180**: 673–86.
18. Minozzi S, Amato L, Bellisario C, Ferri M, Davoli M. Maintenance agonist treatments for opiate-dependent pregnant women. *Cochrane Database Syst Rev* 2013; **12**: CD006318.
19. McCarthy JJ, Leamon MH, Finnegan LP, Fassbender C. Opioid dependence and pregnancy: minimizing stress on the fetal brain. *Am J Obstet Gynecol* 2017; **216**: 226–31.
20. Zedler BK, Mann AL, Kim MM, et al. Buprenorphine compared with methadone to treat pregnant women with opioid use disorder: a systematic review and meta-analysis of safety in the mother, fetus and child. *Addiction* 2016; **111**: 2115–28.
21. Liberati A, Altman DG, Tetzlaff J, et al. The PRISMA statement for reporting systematic reviews and meta-analyses of studies that evaluate healthcare interventions: explanation and elaboration. *BMJ* 2009; **339**: b2700.
22. Guyatt G, Oxman AD, Akl EA, et al. GRADE guidelines: 1. Introduction-GRADE evidence profiles and summary of findings tables. *J Clin Epidemiol* 2011; **64**: 383–94.
23. Guyatt GH, Oxman AD, Vist G, et al. GRADE guidelines: 4. Rating the quality of evidence—study limitations (risk of bias). *J Clin Epidemiol* 2011; **64**: 407–15.
24. Balshem H, Helfand M, Schünemann HJ, et al. GRADE guidelines: 3. Rating the quality of evidence. *J Clin Epidemiol* 2011; **64**: 401–6.
25. DerSimonian R, Laird N. Meta-analysis in clinical trials. *Control Clin Trials* 1986; **7**: 177–88.
26. McGlone L, Hamilton R, McCulloch DL, MacKinnon JR, Bradnam M, Mactier H. Visual outcome in infants born to drug-misusing mothers prescribed methadone in pregnancy. *Br J Ophthalmol* 2014; **98**: 238–45.
27. Bayley N. Bayley Scales of Infant Development: Birth to Two Years. New York, NY: Psychological Corporation, 1969.
28. Ramer C, Lodge A. Neonatal addiction: a two-year study. Part I. Clinical and developmental characteristics of infants of mothers on methadone maintenance. *Addict Dis* 1975; **2**: 227–34.
29. Zarin-Ackerman J. In: Beschner GM, Brotman R, Abuse Nlo D editors. Symposium on Comprehensive Health Care for Addicted Families and Their Children, 20th and 21st May 1976, New York, NY. National Institute on Drug Abuse, 1977.
30. Suflet F, Brotman R. A comprehensive care program for pregnant addicts: obstetrical, neonatal, and child development outcomes. *Int J Addict* 1984; **19**: 199–219.
31. Kaltenbach K, Finnegan LP. Neonatal abstinence syndrome, pharmacotherapy and developmental outcome. *Neurobehav Toxicol Teratol* 1986; **8**: 353–5.
32. Doberczak TM, Shanzer S, Cutler R, Senie RT, Loucopoulos JA, Kandall SR. One-year follow-up of infants with abstinence-associated seizures. *Arch Neurol* 1988; **45**: 649–53.
33. Kaltenbach K, Finnegan LP. Perinatal and developmental outcome of infants exposed to methadone in-utero. *Neurotoxicol Teratol* 1987; **9**: 311–3.
34. Wilson GS, Desmond MM, Wait RB. Follow-up of methadone-treated and untreated narcotic-dependent women and their infants: health, developmental, and social implications. *J Pediatr* 1981; **98**: 716–22.
35. Strauss ME, Starr RH, Ostrea EM, Chavez CJ, Stryker JC. Behavioral concomitants of prenatal addiction to narcotics. *J Pediatrics* 1976; **89**: 842–6.
36. Chasnoff IJ, Schnoll SH, Burns WJ, Burns K. Maternal nonnarcotic substance abuse during pregnancy: effects on infant development. *Neurobehav Toxicol Teratol* 1984; **6**: 277–80.
37. Rosen TS, Johnson HL. Long-term effects of prenatal methadone maintenance. *NIDA Res Monogr* 1985; **59**: 73–83.
38. Kaltenbach K, Finnegan LP. Children exposed to methadone in utero: assessment of developmental and cognitive ability. *Ann N Y Acad Sci* 1989; **562**: 360–2.
39. van Baar A. Development of infants of drug dependent mothers. *J Child Psychol Psychiatry* 1990; **31**: 911–20.
40. Kaltenbach K, Graziani LJ, Finnegan LP. Methadone exposure in utero: developmental status at one and two years of age. *Pharmacol Biochem Behav* 1979; **11**: 15–7.
41. Wilson GS. Clinical studies of infants and children exposed prenatally to heroin. *Ann N Y Acad Sci* 1989; **562**: 183–94.
42. Hans SL, Jeremy RJ. Postneonatal mental and motor development of infants exposed in utero to opioid drugs. *Infant Ment Health J* 2001; **22**: 300–15.
43. Marcus J, Hans SL, Jeremy RJ. Patterns of 1-day and 4-month motor functioning in infants of women on methadone. *Neurobehav Toxicol Teratol* 1982; **4**: 473–6.
44. Schneider JW, Hans SL. Effects of prenatal exposure to opioids on focused attention in toddlers during free play. *J Dev Behav Pediatr* 1996; **17**: 240–7.
45. Bier JB, Finger AS, Bier BA, Johnson TA, Coyle MG. Growth and developmental outcome of infants with in-utero exposure to methadone vs buprenorphine. *J Perinatol* 2015; **35**: 656–9.
46. McGlone L, Mactier H. Infants of opioid-dependent mothers: neurodevelopment at six months. *Early Hum Dev* 2015; **91**: 19–21.
47. Bunikowski R, Grimmer I, Heiser A, Metzke B, Schäfer A, Obladen M. Neurodevelopmental outcome after prenatal exposure to opiates. *Eur J Pediatr* 1998; **157**: 724–30.
48. Strauss ME, Lessen-Firestone JK, Chavez CJ, Stryker JC. Children of methadone-treated women at five years of age. *Pharmacol Biochem Behav* 1979; **11**: 3–6.
49. Lifschitz MH, Wilson GS, O'Brian Smith E, Desmond MM. Factors affecting head growth and intellectual function in children of drug addicts. *Pediatrics* 1985; **75**: 269–74.
50. de Cubas MM, Field T. Children of methadone-dependent women: developmental outcomes. *Am J Orthopsychiatry* 1993; **63**: 266–76.
51. Hunt RW, Tzioumi D, Collins E, Jeffery H. Adverse neurodevelopmental outcome of infants exposed to opiate in-utero. *Early Hum Dev* 2008; **84**: 29–35.
52. van Baar A, de Graaff BM. Cognitive development at preschool-age of infants of drug-dependent mothers. *Dev Med Child Neurol* 1994; **36**: 1063–75.
53. Davis DD, Templar DI. Neurobehavioral functioning in children exposed to narcotics in utero. *Addict Behav* 1988; **13**: 275–83.
54. Sandberg DE, Meyer-Bahlburg HF, Rosen TS, Johnson HL. Effects of prenatal methadone exposure on sex-dimorphic behavior in early school-age children. *Psychoneuroendocrinology* 1990; **15**: 77–82.
55. Konijnenberg C, Lund IO, Melinder A. Behavioural outcomes of four-year-old children prenatally exposed to methadone or buprenorphine: a test of three risk models. *Early Child Dev Care* 2015; **185**: 1641–57.
56. McGlone L, Mactier H, Hamilton R, et al. Visual evoked potentials in infants exposed to methadone in utero. *Arch Dis Child* 2008; **93**: 784–6.
57. McGlone L, Hamilton R, McCulloch DL, et al. Neonatal visual evoked potentials in infants born to mothers prescribed methadone. *Pediatrics* 2013; **131**: e857–63.
58. Whitham JN, Spurrier NJ, Sawyer MG, et al. The effects of prenatal exposure to buprenorphine or methadone on infant visual evoked potentials. *Neurotoxicol Teratol* 2010; **32**: 280–8.
59. Whitham JN, Spurrier NJ, Baghurst PA, Weston P, Sawyer MG. Visual evoked potential latencies of three-year-old children prenatally exposed to buprenorphine or methadone compared with non-opioid exposed children: the results of a longitudinal study. *Neurotoxicol Teratol* 2015; **52**: 17–24.
60. Nelson LB, Ehrlich S, Calhoun JH. Occurrence of strabismus in infants born to drug-dependent women. *Am J Dis Child* 1987; **141**: 175–8.
61. Gaillard MC, Borrut FX. New finding: transitory horizontal pendular nystagmus secondary to neonatal

- abstinence syndrome (In French). *Klin Monats Augenhilf* 2002; **219**: 317–9.
62. Hamilton R, McGlone L, MacKinnon JR, Russell HC, Bradrum MS, MacTier H. Ophthalmic, clinical and visual electrophysiological findings in children born to mothers prescribed substitute methadone in pregnancy. *Br J Ophthalmol* 2010; **94**: 696–700.
63. Gupta M, Mulvihill AO, Lascantos G, Fleck BW, George ND. Nystagmus and reduced visual acuity secondary to drug exposure in utero: long-term follow-up. *J Pediatr Ophthalmol Strabismus* 2012; **49**: 58–63.
64. Tinelli F, Ganucci A, Butti R, Cioni G. Congenital nystagmus in two infants born from mothers exposed to methadone during pregnancy. *Ital J Pediatr* 2013; **39**: 40.
65. Yoo SH, Jansson LM, Park HJ. Sensorimotor outcomes in children with prenatal exposure to methadone. *J AAPOS* 2017; **21**: 316–21.
66. Konijnenberg C, Melinder A. Visual selective attention is impaired in children prenatally exposed to opioid agonist medication. *Eur Addict Rev* 2015; **21**: 63–70.
67. Baldacchino A, Arbuckle K, Petrie DJ, McCowan C. Erratum: neurobehavioral consequences of chronic intrauterine opioid exposure in infants and preschool children: a systematic review and meta-analysis. *BMC Psychiatry* 2015; **15**: 134.
68. Baldacchino A, Arbuckle K, Petrie DJ, McCowan C. Neurobehavioral consequences of chronic intrauterine opioid exposure in infants and preschool children: a systematic review and meta-analysis. *BMC Psychiatry* 2014; **14**: 104.
69. McGlone L, MacTier H, Hassan H, Cooper G. In utero drug and alcohol exposure in infants born to mothers prescribed maintenance methadone. *Arch Dis Child Fet Neonat Ed* 2013; **98**: F542–4.
70. van Kooij BJ, de Vries LS, Ball G, et al. Neonatal tract-based spatial statistics findings and outcome in preterm infants. *AJNR Am J Neuroradiol* 2012; **33**: 188–94.
71. Bassi L, Ricci D, Volzone A, et al. Probabilistic diffusion tractography of the optic radiations and visual function in preterm infants at term equivalent age. *Brain* 2008; **131**: 573–82.
72. Duerden EG, Guo T, Dodbiba L, et al. Midazolam dose correlates with abnormal hippocampal growth and neurodevelopmental outcome in preterm infants. *Ann Neurol* 2016; **79**: 548–59.
73. Salzwald AP, Grewen KM, Vachet C, Geisig G, Lin W, Gao W. Prenatal drug exposure affects neonatal brain functional connectivity. *J Neurosci* 2015; **35**: 5860–9.
74. World Health Organization. Guidelines for the Identification and Management of Substance Use and Substance Use Disorders in Pregnancy. Geneva: World Health Organization, 2014.
75. Metz V, Jagsch R, Ehmer N, et al. Impact of treatment approach on maternal and neonatal outcome in pregnant opioid-maintained women. *Hum Psychopharmacol* 2011; **26**: 412–21.
76. Pritham UA, Paul JA, Hayes MJ. Opioid dependency in pregnancy and length of stay for neonatal abstinence syndrome. *J Obstet Gynecol Neonat Nurs* 2012; **41**: 180–90.
77. Lacroix I, Berrebi A, Garipuy D, et al. Buprenorphine versus methadone in pregnant opioid-dependent women: a prospective multicenter study. *Eur J Clin Pharmacol* 2011; **67**: 1053–9.

APPENDIX IV

The original format of the following first author paper is shown in this appendix.

Monnelly et al (2018) is reproduced from Elsevier with permission:

Monnelly VJ, Anblagan D, Quigley A, Cabez MB, Cooper ES, Mactier H, Semple SI, Bastin ME, Boardman JP: Prenatal methadone exposure is associated with altered neonatal brain development. *Neuroimage Clin* 2018;18:9-14.



Prenatal methadone exposure is associated with altered neonatal brain development

Victoria J. Monnelly^a, Devasuda Anblagan^b, Alan Quigley^c, Manuel Blesa Cabeza^a,
E. Sarah Cooper^d, Helen Mactier^c, Scott I. Semple^e, Mark E. Bastin^b, James P. Boardman^{a,b,*}

^a MRC Centre for Reproductive Health, University of Edinburgh, UK

^b Centre for Clinical Brain Sciences, University of Edinburgh, UK

^c Department of Paediatric Radiology, Royal Hospital for Sick Children, NHS Lothian, Edinburgh, UK

^d Department of Obstetrics, Royal Infirmary of Edinburgh, NHS Lothian, UK

^e Princess Royal Maternity and the University of Glasgow, UK

^f British Heart Foundation Centre for Cardiovascular Science, University of Edinburgh, UK



ARTICLE INFO

Keywords:
Prenatal
Methadone
Brain
Neonate
MRI
Opioid

ABSTRACT

Methadone is used for medication-assisted treatment of heroin addiction during pregnancy. The neurodevelopmental outcome of children with prenatal methadone exposure can be sub-optimal. We tested the hypothesis that brain development is altered among newborn infants whose mothers were prescribed methadone.

20 methadone-exposed neonates born after 37 weeks' postmenstrual age (PMA) and 20 non-exposed controls underwent diffusion MRI at mean PMA of 39⁺² and 41⁺¹ weeks, respectively. An age-optimized Tract-based Spatial Statistics (TBSS) pipeline was used to perform voxel-wise statistical comparison of fractional anisotropy (FA) data between exposed and non-exposed neonates.

Methadone-exposed neonates had decreased FA within the centrum semiovale, inferior longitudinal fasciculus (ILF) and the internal and external capsules after adjustment for GA at MRI ($p < 0.05$, TFCE corrected). Median FA across the white matter skeleton was 12% lower among methadone-exposed infants. Mean head circumference (HC) z-scores were lower in the methadone-exposed group (-0.52 (0.99) vs 1.15 (0.84), $p < 0.001$); after adjustment for HC z-scores, differences in FA remained in the anterior and posterior limbs of the internal capsule and the ILF. Polydrug use among cases was common.

Prenatal methadone exposure is associated with microstructural alteration in major white matter tracts, which is present at birth and is independent of head growth. Although the findings cannot be attributed to methadone *per se*, the data indicate that further research to determine optimal management of opioid use disorder during pregnancy is required. Future studies should evaluate childhood outcomes including infant brain development and long-term neurocognitive function.

1. Introduction

Globally, in 2015 there were estimated to be 17.7 million past-year users of heroin or opium, and increased heroin use is a major driver of the current opioid epidemic (World Drug Report, 2017). Pregnant women with opioid use disorder (OUD) due to heroin are recommended medication-assisted treatment (MAT) with an alternative opioid (usually methadone or buprenorphine) because treatment is associated with improved use of antenatal services, reduced use of heroin during pregnancy and reduced preterm delivery. Fetal benefits of MAT include improved growth and lower risk of intrauterine death (American College of Obstetricians and Gynecologists, 2017).

Methadone is a synthetic long acting μ -opioid agonist, which crosses the placenta freely, thereby exposing the developing fetus to exogenous opioid at a critical period of brain development. Pre-clinical studies suggest that prenatal methadone exposure may modify developing dopaminergic, cholinergic and serotonergic systems, and alter myelination. Antenatal exposure to the drug has behavioral consequences including depression, anxiety, and impaired learning, memory and social function (Chen et al., 2015; Robinson et al., 1996; Vestal-Laborde et al., 2014; Wong et al., 2014). In humans, prenatal methadone exposure is associated with increased incidence and severity of neonatal abstinence syndrome (NAS) (Wilson et al., 1981; Zelson et al., 1973) compared with heroin exposure, and with altered visual maturation in

* Corresponding author at: W1.26 MRC Centre for Reproductive Health, Queen's Medical Research Institute, 49 Little France Crescent, Edinburgh EH16 4TJ, UK.

E-mail address: james.boardman@ed.ac.uk (J.P. Boardman).

<https://doi.org/10.1016/j.nicl.2017.12.033>

Received 16 November 2017; Received in revised form 15 December 2017; Accepted 22 December 2017

Available online 24 December 2017

2213-1582/ © 2017 The Authors. Published by Elsevier Inc. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

childhood (McGlone et al., 2013a; McGlone et al., 2008; Whitham et al., 2010). These observations raise the possibility that prenatal methadone exposure may modify early brain development; however, the possible role of confounding by postnatal events, including pharmacotherapy with opioid for NAS and environmental factors, leaves uncertainty about the impact of prenatal methadone exposure on the developing brain.

Diffusion MRI (dMRI) is an established technique for studying brain development in early life. It provides objective measures of white matter microstructure that are sensitive to atypical developmental and injurious processes in the perinatal period, and which correlate with neurodevelopmental outcome in childhood (Counsell et al., 2014). Specifically, fractional anisotropy (FA) is a voxel-wise measure of the directional dependence of water molecule diffusion in tissue which is influenced by fiber density, axonal diameter and myelination, thereby enabling inference about underlying tissue microstructure. Tract-based Spatial Statistics (TBSS) enables unbiased group-wise analysis of FA volumes derived from dMRI data (Ball et al., 2010; Smith et al., 2006). It has been applied to neonatal dMRI to map microstructural change in white matter tracts of preterm infants at term equivalent age (Anjari et al., 2007), to identify clinical risk factors for altered brain development (Anblagan et al., 2016; Ball et al., 2010; Boardman et al., 2014), and to investigate neuroprotective treatment strategies in randomized clinical trials (Azzopardi et al., 2016; O’Gorman et al., 2015; Porter et al., 2010).

Based on the harmful effects of prenatal methadone exposure on neural systems and abnormal behavioral outcomes in pre-clinical models; and on human studies which suggest a modifying effect of prenatal methadone on postnatal behavior and development, we hypothesized that white matter development of neonates would be altered in neonates exposed to methadone *in utero*. We used TBSS to examine risks associated with prenatal methadone exposure, while minimizing the role of confounding by postnatal events and drug exposures.

2. Methods and materials

2.1. Participants

The study was conducted according to the principles of the Declaration of Helsinki and ethical approval was obtained from the UK National Ethics Service (South East Scotland Research Ethics Committee 02, 14/SS/1106). Written informed parental consent was obtained for all participants. The study group consisted of infants > 37 weeks’ postmenstrual age (PMA) whose mothers had been prescribed methadone during pregnancy for the treatment of OUD (cases) and a comparator group of healthy infants born at > 37 weeks’ PMA whose mothers did not use opioids (controls).

Mothers of cases were identified through a specialist antenatal clinic for pregnant women with substance misuse. All cases were born at the Royal Infirmary of Edinburgh between February 2015 and April 2017 and underwent MRI brain scanning at the Clinical Research Imaging Centre, University of Edinburgh. The controls were selected, based on age matching, from a previously described group of healthy term neonates recruited as part of a study of typical brain development (Blesa et al., 2016) (South East Scotland Research Ethics Committee 02, 13/SS/0143). For cases and controls, exclusion criteria were congenital infection or chromosomal abnormalities, or any implanted medical device.

Clinical and demographic information was extracted from the mother and infant clinical records. Birth weight and head circumference (HC) were described in terms of z-score for week of gestational age, calculated using INTERGROWTH-21st reference standards (Villar et al., 2014). The Scottish Index of Multiple Deprivation (SIMD) was used to characterize deprivation. The SIMD is the official Government tool used to identify areas of deprivation: it divides Scotland into around 6505 areas each containing around 350 households and assigns an index to

each area based on multiple measures of deprivation. The data are ranked from most to least deprived and are presented as deciles.

2.2. Ascertainment of maternal drug use

Details of methadone use, tobacco smoking, alcohol intake, and use of non-prescribed drugs were ascertained from medical records (including prescription charts), biological screening samples when these were performed as part of clinical care, and maternal interview at the time of delivery (V.M.)

2.3. MRI acquisition

MRI was performed on a Siemens Magnetom Verio 3T system (Siemens Healthcare GmbH, Erlangen, Germany) using a 12-channel matrix phased array head coil. All infants were scanned axially to acquire: 3D T1-weighted MPRAGE volume (1 mm³ resolution), T2-weighted STIR (0.9 mm³ resolution), T2-weighted FLAIR (1 mm³ resolution), and diffusion MRI (dMRI) (11 T2- and 64 diffusion encoding direction ($b = 750 \text{ s/mm}^2$) single-shot spin-echo echo planar imaging (EPI) volumes with 2 mm isotropic voxels, TE = 106 ms and TR = 7300 ms. Images were reported by a pediatric radiologist with experience in neonatal MRI (AQ), according to the system described by Woodward et al. (Woodward et al., 2006), with the modification for grey matter scores proposed by Leuchter et al. (Leuchter et al., 2014).

MRI was performed in the neonatal period during natural sleep, without sedation. A neonatologist was present for the duration of each MRI scan, and the infant had continuous oxygen saturation and heart rate monitoring. For acoustic protection, flexible earplugs and neonatal earmuffs (MiniMuffs, Nat’s Medical Inc., CA) were used.

2.4. Tract-based spatial statistics

dMRI data were preprocessed using FSL tools (FMRIB, Oxford, UK; <http://www.ndcn.ox.ac.uk/divisions/fmrib>). This included brain extraction, and removal of bulk infant motion and eddy current induced artefacts by registering the diffusion-weighted volumes to the first T2-weighted EPI volume for each subject. Using DTIFIT, FA volumes were generated for every subject. Diffusion volumes were assessed visually and were excluded if there was motion corruption.

TBSS analysis was performed using a pipeline optimized for neonatal dMRI data (Ball et al., 2010). An average FA volume and mean FA skeleton (thresholded at FA > 0.15) were created from the aligned data. Statistical comparison between groups with and without exposure to methadone during pregnancy was performed with FSL’s Randomise using a general linear univariate model, with GA at image acquisition and HC z-score at image acquisition listed as covariates. All FA data were subject to family-wise error correction for multiple comparisons following threshold-free cluster enhancement (TFCE) and are shown at $p < 0.05$ (Smith and Nichols, 2009).

2.5. Statistics

Student’s *t*-test or the Mann-Whitney test was used to investigate differences in clinical and demographic variables between infants exposed to methadone ($n = 20$) and those not exposed ($n = 20$) and chi-squared or Fisher’s exact test was used to compare proportions. Statistical analysis was performed using SPSS v22.0 (SPSS Inc., Chicago, IL).

3. Results

3.1. Participants

Conventional structural and dMRI data amenable to TBSS analysis were acquired from 40 neonates: 20 cases (10 female), who were

Table 1
Maternal and infant characteristics of participants. BMI, body mass index; SIMD, Scottish Index of Multiple Deprivation; PMA, postmenstrual age; HC, head circumference.

	Methodone	Control	p value
	n = 20	n = 20	
Maternal characteristics			
Mean age (range)/years	30 (23–41)	30.9 (19–39)	0.56
Mean BMI (range)	25.8 (21–41)	23.2 (19–39)	0.08
Median SIMD decile (Interquartile range)	3 (2–5)	8 (6–10)	0.012
Infant characteristics			
Mean PMA at birth (range)/weeks	38 ⁺⁵ (37 ⁺⁵ –41 ⁺⁹)	39 ⁺¹ (37 ⁺² –41 ⁺³)	0.32
Gender (M:F)	10:10	13:7	0.52
Mean birth weight (range)/g	2721 (2150–3440)	3349 (2346–4550)	< 0.01
Mean birth weight z-score (sd)	−1.062 (0.68)	0.443 (0.86)	< 0.01
Median (range) postnatal age at scan/days	3 (1 to 21)	13 (5 to 29)	0.005
Mean PMA at scan (range)/weeks	39 ⁺² (37 ⁺² –41 ⁺⁶)	41 ⁺¹ (39 ⁺⁰ –42 ⁺²)	0.004
Mean HC at scan (range)/cm	33.1 (31.2–35.0)	35.9 (32.6–37.4)	< 0.01
Mean HC z-score (sd)	−0.523 (0.986)	1.146 (0.837)	< 0.01

exposed to prenatal methadone, and 20 unexposed controls (7 female). Table 1 summarizes maternal and infant characteristics.

The mean methadone dose prescribed at pregnancy booking was 55 mg/day (range 0–160) and the mean dose at delivery was 70 mg/day (range 8–160). Nineteen (95%) of the women prescribed methadone smoked tobacco, one reported drinking excessive alcohol (4 units/day at booking), and nineteen women had illicit or prescribed polydrug use (Fig. 1). Additional prescribed medications included paroxetine (n = 1), mirtazapine (n = 1), gabapentin (n = 2), and pregabalin (n = 1).

None of the cases had neonatal encephalopathy, seizures or hypoglycemia. The mean arterial cord pH of the group was 7.26 (range 7.16–7.36). Three cases had minor congenital anomalies (1 hypospadias, 1 cleft lip, 1 fixed bilateral talipes). One methadone exposed infant required admission to the Neonatal Unit for treatment of transient tachypnoea of the newborn.

No infant had received pharmacological treatment for NAS at the time of image acquisition and none of the control group was exposed prenatally to opioid drugs.

3.2. Magnetic Resonance Imaging

None of the cases or controls had features consistent with injury to



Fig. 1. Euler diagram indicating prenatal drug exposures. Stimulant includes cocaine and amphetamine; *codeine phosphate and tramadol.

white matter or grey matter on conventional structural T1- or T2-weighted structural MRI. Four cases had mild enlargement of the lateral ventricular system; 1 case had asymmetric myelination of the posterior limb of the internal capsule (but had developed symmetric myelination on repeat MRI four weeks later); and no case had abnormalities in brainstem, cerebellum, deep or cortical grey matter, or extracerebral space. Two (10%) methadone exposed infants had developmental venous anomalies: one consisted of an area of low T2-weighted signal in the left peritrigonal white matter (most likely haemosiderin), with a curvilinear vessel extending peripherally and draining into the superior anastomotic vein of Troland, which continues up to the superior sagittal sinus; and the second was characterized by an area of low T2-weighted signal in the right peritrigonal white matter with a low T2-weighted signal vessel that drains toward the choroid plexus.

3.3. White matter correlates of prenatal methadone exposure

Methadone-exposed neonates had decreased FA within the centrum semiovale, inferior longitudinal fasciculi (ILF), and the internal and external capsules after adjustment for PMA at MRI ($p < 0.05$, TCFE corrected) (Fig. 2A). Mean HC z-scores were lower in the methadone exposed group (-0.52 (0.99) vs 1.15 (0.84), $p < 0.001$). After adjustment for HC z-scores, differences in FA remained in the anterior and posterior limbs of the internal capsule and the ILF (Fig. 2B). Radial diffusivity was increased in internal capsule and inferior longitudinal fasciculus in neonates with prenatal methadone exposure (Fig. 3). There were no differences in mean or axial diffusivities between groups.

Median FA across the white matter skeleton was 12% lower among methadone-exposed infants (Fig. 4).

4. Discussion

These data show that prenatal exposure to methadone is associated with altered microstructure in major white matter tracts of the newborn brain, independent of head growth. Children whose mothers take methadone during pregnancy are at increased risk of neurodevelopmental impairment, behavioral difficulties, and visual problems, but study designs have left uncertainty about the role of confounding by prematurity, postnatal opioid exposure for treatment of NAS, and environmental factors, in mediating adverse outcomes (Hans and Jeremy, 2001; Hunt et al., 2008; Konijnenberg and Melinder, 2015; McGlone et al., 2014; McGlone and Mactier, 2015; Rosen and Johnson, 1985; van Baar, 1990; Wilson, 1989). An association between prenatal methadone exposure and reduced somatic and head growth is documented (Mactier et al., 2014) but to our knowledge, this is first study to demonstrate brain tissue effects present around the time of birth after methadone exposure *in utero*.

We used TBSS to investigate brain development because of its sensitivity to group-wise differences in FA when used to survey the entire white matter skeleton (Ball et al., 2013). FA is a robust marker of tract microstructure that reflects fiber density, axonal diameter, wrapping by pre-myelinating oligodendrocytes and myelination. Therefore, these data suggest that neonates exposed to methadone *in utero* have less coherently organized and more immature fiber tracts compared to controls. Furthermore, correspondent increases in radial diffusivity without changes in axial diffusivity imply that abnormal myelination may contribute to altered FA among the cases. Since neonatal FA values in major white matter tracts correlate with later neurodevelopment, the findings may explain the prevalence of neurobehavioral problems seen in children with prenatal methadone exposure.

Quantitative MRI techniques have identified specific vulnerabilities of the developing brain to psychoactive drugs. Midazolam exposure during neonatal intensive care of preterm infants is associated with attenuated hippocampal growth (Duerden et al., 2016), and functional connectivity of the amygdala–frontal and thalamic networks is altered in neonates with prenatal cocaine exposure (Salzwedel et al., 2016;

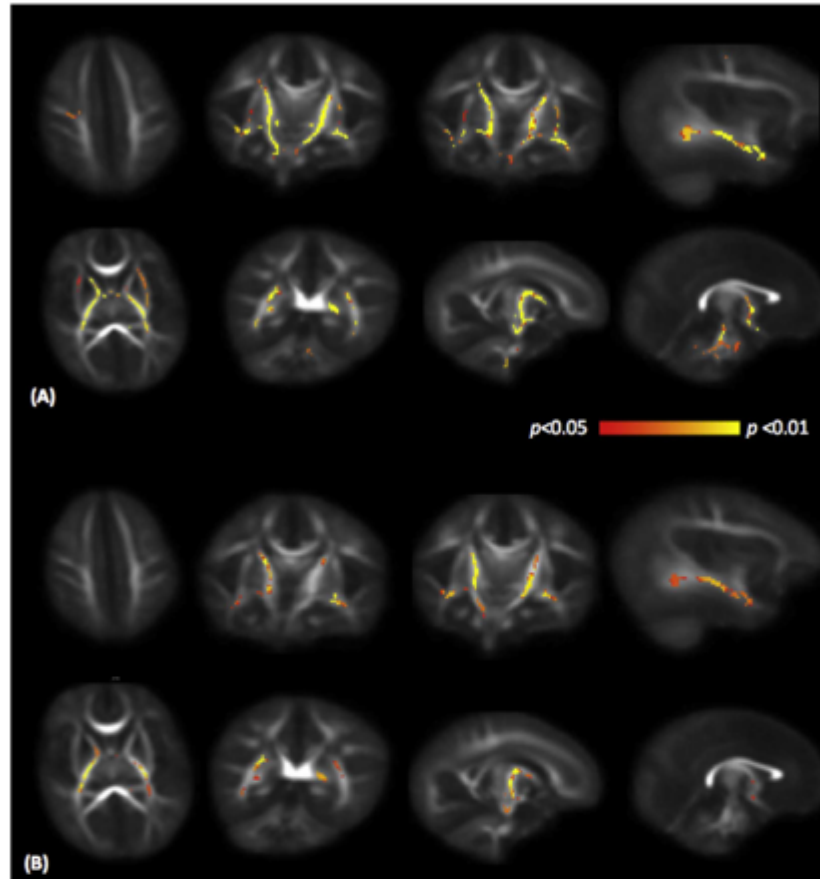


Fig. 2. Mean FA map of the subjects in transverse, coronal and sagittal planes. Voxels with significantly lower FA in neonates with prenatal methadone exposure are shown in yellow-red color scale. Fig. 2A shows results adjusted for PMA at scan, and Fig. 2B shows results adjusted for PMA at scan and head circumference z-score. FA, fractional anisotropy.

Salzweidel et al., 2015). In a preliminary study, Walhovd and colleagues reported higher mean diffusivity in the superior longitudinal fasciculus of 13 methadone-exposed cases compared to 7 controls, but infants were scanned at mean age of 3 weeks after birth and 85% of the cases had been treated with morphine for NAS, which limits inference about the effects of prenatal opioid exposure (Walhovd et al., 2012). Two (10%) cases had developmental venous anomalies (DVA), which was higher than expected based on estimated prevalence of 1.5% in neonates (Brinjikji et al., 2017). These did not occur in the cases exposed to cocaine, which is known to be associated with central nervous system vascular anomalies (Frank et al., 1999); therefore the possibility that prenatal methadone exposure is associated with CNS vascular malformation warrants further study.

All of our cases had been exposed to once daily dosing with methadone. Although the benefits of MAT with an opioid substitute during pregnancy are unequivocal (ACOG, 2017), there is no consensus regarding the optimal dosing regimen for methadone, or the role of buprenorphine as an alternative substitute. A single daily dose of methadone is commonly prescribed, but some authors suggest that accelerated metabolism of methadone by physiological induction of CYP450 enzymes during pregnancy might predispose women and

fetuses to daily withdrawal stress and risk of relapse to illicit drugs (Bogen et al., 2013; McCarthy et al., 2017; McCarthy et al., 2015). Studies that support the use of divided dosing to minimize fluctuations in serum concentration report favorable effects on maternal symptoms of withdrawal, and on fetal neurobehavior and NAS prevalence (Jansson et al., 2009; McCarthy et al., 2015; Wittmann and Segal, 1991), but no study has evaluated the impact of dosing regimen on fetal/neonatal brain development or long-term outcome. Monitoring of maternal plasma methadone concentration during the peripartum period with dose titration to keep levels within the maternal therapeutic range has been suggested, but this is not practiced widely (McCarthy et al., 2017). Buprenorphine is a partial mu-opioid agonist and kappa-opioid antagonist that is an acceptable substitute to pregnant women and is associated with lower risk of preterm birth, improved growth parameters at birth and less NAS, without apparent harm, when compared with methadone (Jones et al., 2010; Zedler et al., 2016). These neonatal outcomes suggest that buprenorphine may have a more favorable safety profile for the child, although long-term outcome studies are required.

Polydrug use, both illicit and prescribed, was very common among women prescribed methadone in our study. This is consistent with

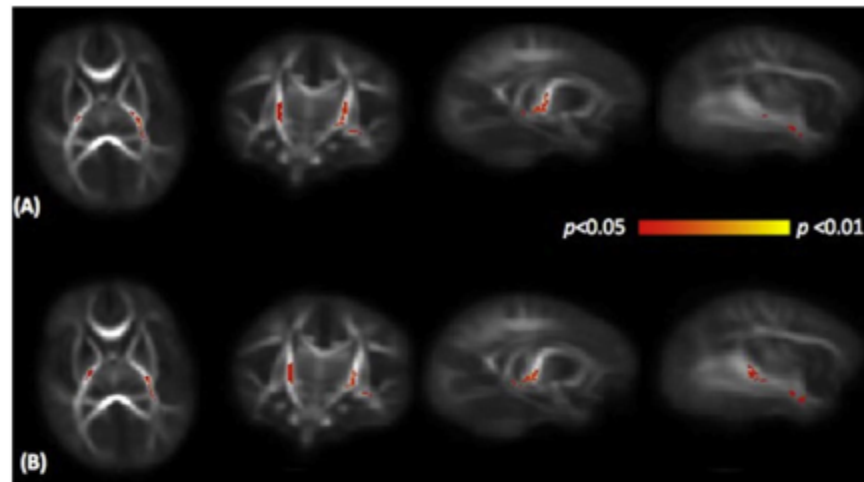


Fig. 3. Mean RD map of the subjects in transverse, coronal and sagittal planes. Voxels with significantly higher RD in neonates with prenatal methadone exposure are shown in yellow-red colour scale. Fig. 3A shows results adjusted for PMA at scan, and Fig. 3B shows results adjusted for PMA at scan and head circumference z-score. RD, radial diffusivity.

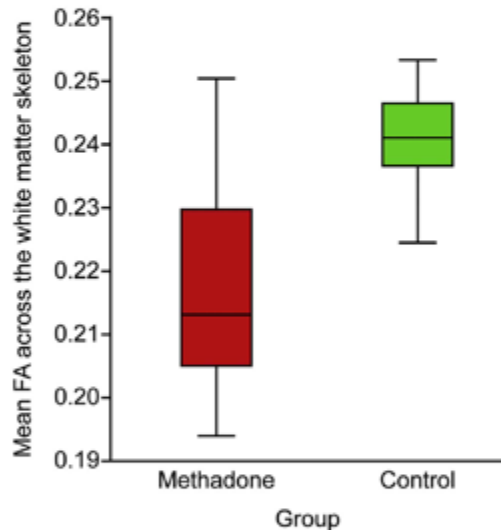


Fig. 4. Mean FA across the white matter skeleton of neonates with prenatal methadone exposure compared with unexposed controls. FA, fractional anisotropy.

observations from other cohorts of methadone using pregnant women (McGlone et al., 2013b; Rosen and Johnson, 1985; van Baar, 1990). In our study population, heroin and oral benzodiazepines were used by 11 (55%) and 12 (60%) of cases respectively, so it is possible that either drug could have confounded the observed association. No other drug class (anti-depressant, anti-epileptic, stimulant) was taken by > 15% of the cases so it is unlikely that exposure to these classes of drug explained the findings. The research implication of highly prevalent polydrug use in the target population is that future studies into optimal management of OUD in pregnancy are likely to require pragmatic designs that define case definition based on prescribed substitute, with *post hoc* adjustment for other drug exposures if they are shown to differ between groups.

The strengths of the study are that: dMRI acquisition took place

soon after birth before exposure to postnatal opioids or other pharmacological treatment for NAS; preterm birth, which is an important source of confounding for neurodevelopmental outcome was excluded; and detailed information about methadone dose and exposure to other drugs was available. Our study was limited by reliance on maternal report for drug exposures among control women; however, none of the control participants were prescribed opioids during pregnancy, and the likelihood of undisclosed heroin use or non-prescription opioids was low. A limitation of this study is that we could not evaluate causation, and therefore cannot exclude potential mediating or interacting factors such timing and dose effects of methadone and the role of other prenatal drug exposures. However, our data support pre-clinical studies, objective studies of visuo-cortical function in the newborn period and later infancy, and neurodevelopmental and behavioral studies, which strongly suggest an adverse effect of prenatal methadone upon the developing fetal brain and upon long-term childhood outcomes.

In conclusion, prenatal methadone exposure is associated with altered white matter microstructure that is apparent soon after birth. The data focus research attention on determining optimal management of pregnant women with OUD, including a pressing need to evaluate methadone dose regimens and alternative substitutes; future study designs should evaluate fetal or neonatal brain development and long-term neurocognitive outcome.

Acknowledgements

We thank the families that participated in this research; the specialist substance misuse midwives (LC and SC); and the radiographers at the Clinical Research Imaging Centre, Edinburgh, UK. We thank Thorsten Feiweier at Siemens Healthcare for collaborating with dMRI acquisitions (Works-in-Progress Package for Advanced EPI Diffusion Imaging). This work was supported by Theirworld (www.theirworld.org) and was undertaken in the MRC Centre for Reproductive Health, which is funded by MRC Centre Grant (MRC G1002033). The study was sponsored by the University of Edinburgh.

Financial disclosures

None of the authors report biomedical financial interests or potential conflicts of interest.

References

- American College of Obstetricians and Gynecologists, 2017. Committee opinion no. 711: opioid use and opioid use disorder in pregnancy. *Obstet. Gynecol.* 130, e91–e94.
- Anblagan, D., Putaky, R., Evans, M.J., Telford, E.J., Serag, A., Sparrow, S., Piyasena, C., Sempke, S.I., Wilkinson, A.G., Bastin, M.E., Boardman, J.P., 2016. Association between preterm brain injury and exposure to chorioamnionitis during fetal life. *Sci. Rep.* 6, 37932.
- Anjari, M., Srinivasan, L., Allsop, J.M., Hajnal, J.V., Rutherford, M.A., Edwards, A.D., Counsell, S.J., 2007. Diffusion tensor imaging with tract-based spatial statistics reveals local white matter abnormalities in preterm infants. *NeuroImage* 35, 1021–1027.
- Azopardi, D., Robertson, N.J., Bainbridge, A., Cady, E., Charles-Edwards, G., Deieri, A., Fajolis, G., Franks, R.P., Griffiths, J., Hajnal, J., Juszczak, E., Kapetanakis, B., Linsell, L., Maize, M., Omar, O., Stechin, B., Tisor, N., Edwards, A.D., 2016. Moderate hypothermia within 6 h of birth plus inhaled xenon versus moderate hypothermia alone after birth asphyxia (TOBY-Xe): a proof-of-concept, open-label, randomised controlled trial. *Lancet Neurol.* 15, 145–152.
- van Baar, A., 1990. Development of infants of drug dependent mothers. *J. Child Psychol. Psychiatry* 31, 911–920.
- Ball, G., Counsell, S.J., Anjari, M., Merchant, N., Arichi, T., Doria, V., Rutherford, M.A., Edwards, A.D., Rueckert, D., Boardman, J.P., 2010. An optimised tract-based spatial statistics protocol for neonates: applications to prematurity and chronic lung disease. *NeuroImage* 53, 94–102.
- Ball, G., Boardman, J.P., Arichi, T., Merchant, N., Rueckert, D., Edwards, A.D., Counsell, S.J., 2013. Testing the sensitivity of tract-based spatial statistics to simulated treatment effects in preterm neonates. *PLoS One* 8, e67706.
- Blesa, A., Serag, A., Wilkinson, A.G., Anblagan, D., Telford, E.J., Putaky, R., Sparrow, S.A., Macnaught, G., Sempke, S.I., Bastin, M.E., Boardman, J.P., 2016. Parcellation of the healthy neonatal brain into 107 regions using atlas propagation through intermediate time points in childhood. *Front. Neurosci.* 10, 220.
- Boardman, J.P., Waller, A., Ball, G., Takousis, P., Krishnan, M.L., Hughes-Carre, L., Aljabar, P., Serag, A., King, C., Merchant, N., Srinivasan, L., Froguel, P., Hajnal, J., Rueckert, D., Counsell, S., Edwards, A.D., 2014. Common genetic variants and risk of brain injury after preterm birth. *Pediatrics* 133, e1655–1663.
- Bogen, D.L., Perel, J.M., Helzel, J.C., Hanusa, B.H., Romkes, M., Nukui, T., Friedman, C.R., Wisner, K.L., 2013. Pharmacologic evidence to support clinical decision making for peripartum methadone treatment. *Psychopharmacology* 225, 441–451.
- Brijikij, W., El-Rida El-Masri, A., Wald, J.T., Lanzino, G., 2017. Prevalence of developmental venous anomalies increases with age. *Stroke* 48, 1997–1999.
- Chen, H.H., Chiang, Y.C., Yuan, Z.F., Kuo, C.C., Lai, M.D., Hwang, T.W., Ho, L.K., Chen, S.T., 2015. Buprenorphine, methadone, and morphine treatment during pregnancy: behavioral effects on the offspring in rats. *Neuropsychiatr. Dis. Treat.* 11, 609–618.
- Counsell, S.J., Ball, G., Edwards, A.D., 2014. New imaging approaches to evaluate newborn brain injury and their role in predicting developmental disorders. *Curr. Opin. Neurol.* 27, 166–175.
- Duerden, E.G., Guo, T., Dooliba, L., Chakravarty, M.M., Chau, V., Poskitt, K.J., Symes, A., Grunau, R.E., Miller, S.P., 2016. Midazolam dose correlates with abnormal hippocampal growth and neurodevelopmental outcome in preterm infants. *Ann. Neurol.* 79, 548–559.
- Frank, D.A., McCarten, K.M., Robson, C.D., Mischick, M., Cabral, H., Park, H., Zuckerman, B., 1999. Level of in utero cocaine exposure and neonatal ultrasound findings. *Pediatrics* 104, 1101–1105.
- Hans, S.L., Jeremy, R.J., 2001. Postneonatal mental and motor development of infants exposed in utero to opioid drugs. *Infant Mental Health Journal* 22, 15.
- Hant, R.W., Tzioumi, D., Collins, E., Jeffery, H.E., 2008. Adverse neurodevelopmental outcome of infants exposed to opiate in-utero. *Early Hum. Dev.* 84, 29–35.
- Jamson, L.M., DiPietro, J.A., Velez, M., Elko, A., Knauer, H., Kivlighan, K.T., 2009. Maternal methadone dosing schedule and fetal neurobehaviour. *J. Matern. Fetal Neonatal Med.* 22, 29–35.
- Jones, H.E., Kaltenbach, K., Heil, S.H., Stine, S.M., Coyle, M.G., Arria, A.M., O'Grady, K.E., Selby, P., Martin, P.R., Fischer, G., 2010. Neonatal abstinence syndrome after methadone or buprenorphine exposure. *N. Engl. J. Med.* 363, 2320–2331.
- Konijnsbergen, C., Melinder, A., 2015. Visual selective attention is impaired in children prenatally exposed to opioid agonist medication. *Eur. Addict. Res.* 21, 63–70.
- Leuchter, R.H., Gui, L., Poncet, A., Haggmann, C., Lodygensky, G.A., Martin, E., Koller, B., Darque, A., Bucher, H.U., Huppi, P.S., 2014. Association between early administration of high-dose erythropoietin in preterm infants and brain MRI abnormality at term-equivalent age. *JAMA* 312, 817–824.
- Mactier, H., Shipton, D., Dreyden, C., Tappin, D.M., 2014. Reduced fetal growth in methadone-maintained pregnancies is not fully explained by smoking or socio-economic deprivation. *Addiction* 109, 482–488.
- McCarthy, J.J., Leamon, M.H., Willis, N.H., Salo, R., 2015. The effect of methadone dose regimen on neonatal abstinence syndrome. *J. Addict. Med.* 9, 105–110.
- McCarthy, J.J., Leamon, M.H., Finnegan, L.P., Fanshender, C., 2017. Opioid dependence and pregnancy: minimizing stress on the fetal brain. *Am. J. Obstet. Gynecol.* 216, 226–231.
- McGlone, L., Mactier, H., 2015. Infants of opioid-dependent mothers: neurodevelopment at six months. *Early Hum. Dev.* 91, 19–21.
- McGlone, L., Mactier, H., Hamilton, R., Bradnam, M.S., Boulton, R., Borland, W., Hepburn, M., McCulloch, D.L., 2008. Visual evoked potentials in infants exposed to methadone in utero. *Arch. Dis. Child.* 93, 784–786.
- McGlone, L., Hamilton, R., McCulloch, D.L., Boulton, R., Bradnam, M.S., Weaver, L.T., Mactier, H., 2013a. Neonatal visual evoked potentials in infants born to mothers prescribed methadone. *Pediatrics* 131, e857–863.
- McGlone, L., Mactier, H., Hasan, H., Cooper, G., 2013b. In utero drug and alcohol exposure in infants born to mothers prescribed maintenance methadone. *Arch. Dis. Child. Fetal Neonatal Ed.* 98, F542–544.
- McGlone, L., Hamilton, R., McCulloch, D.L., MacKinnon, J.R., Bradnam, M., Mactier, H., 2014. Visual outcome in infants born to drug-misusing mothers prescribed methadone in pregnancy. *Br. J. Ophthalmol.* 98, 238–245.
- O'Gorman, R.L., Bucher, H.U., Held, U., Koller, B.M., Huppi, P.S., Haggmann, C.F., 2015. Tract-based spatial statistics to assess the neuroprotective effect of early erythropoietin on white matter development in preterm infants. *Brain* 138, 388–397.
- Porter, E.J., Counsell, S.J., Edwards, A.D., Allsop, J., Azopardi, D., 2010. Tract-based spatial statistics of magnetic resonance images to assess disease and treatment effects in perinatal asphyxial encephalopathy. *Pediatr. Res.* 68, 205–209.
- Robinson, S.E., Guo, H., Maher, J.R., McDowell, K.P., Kunko, P.M., 1996. Postnatal methadone exposure does not prevent prenatal methadone-induced changes in striatal cholinergic neurons. *Brain Res. Dev. Brain Res.* 95, 118–121.
- Rosen, T.S., Johnson, H.L., 1985. Long-term effects of prenatal methadone maintenance. *NIDA Res. Monogr.* 59, 73–83.
- Salzweid, A.P., Grewen, K.M., Vachet, C., Gerig, G., Lin, W., Gao, W., 2015. Prenatal drug exposure affects neonatal brain functional connectivity. *J. Neurosci.* 35, 5860–5869.
- Salzweid, A.P., Grewen, K.M., Goldman, B.D., Gao, W., 2016. Thalamicocortical functional connectivity and behavioral disruptions in neonates with prenatal cocaine exposure. *Neurotoxicol. Teratol.* 56, 16–25.
- Smith, S.M., Nichols, T.E., 2009. Threshold-free cluster enhancement: addressing problems of smoothing, threshold dependence and localisation in cluster inference. *NeuroImage* 44, 83–98.
- Smith, S.M., Jenkinson, M., Johansen-Berg, H., Rueckert, D., Nichols, T.E., Mackay, C.E., Watkins, K.E., Ciccarelli, O., Cader, M.Z., Matthews, P.M., Behrens, T.E., 2006. Tract-based spatial statistics: voxelwise analysis of multi-subject diffusion data. *NeuroImage* 31, 1487–1505.
- United Nations Office of Drugs and Crime, 2017. World Drug Report 2017. United Nations publication, Sales No. E.17.XI.6. United Nations.
- Vestal-laborde, A.A., Echenroeder, A.C., Bigbee, J.W., Robinson, S.E., Sato-Bigbee, C., 2014. The opioid system and brain development: effects of methadone on the oligodendrocyte lineage and the early stages of myelination. *Dev. Neurosci.* 36, 409–421.
- Villar, J., Cheikh Ismail, L., Victora, C.G., Ohuma, E.O., Bertino, E., Altmann, D.G., Lambert, A., Papageorgiou, A.T., Carvalho, M., Jaffer, Y.A., Geavett, M.G., Purwar, M., Frederick, L.O., Noble, A.J., Pang, R., Barros, F.C., Chumlea, C., Bhatta, Z.A., Kennedy, S.H., 2014. International standards for newborn weight, length, and head circumference by gestational age and sex: the Newborn Cross-sectional Study of the INTERGROWTH-21st Project. *Lancet* 384, 857–868.
- Walhovd, K.B., Watts, R., Amlien, I., Woodward, L.J., 2012. Neural tract development of infants born to methadone-maintained mothers. *Pediatr. Neurol.* 47, 1–6.
- Whitham, J.N., Spurrier, N.J., Sawyer, M.G., Baghurst, P.A., Taplin, J.E., White, J.M., Gordon, A.L., 2010. The effects of prenatal exposure to buprenorphine or methadone on infant visual evoked potentials. *Neurotoxicol. Teratol.* 32, 280–288.
- Wilson, G.S., 1989. Clinical studies of infants and children exposed prenatally to heroin. *Ann. N. Y. Acad. Sci.* 562, 183–194.
- Wilson, G.S., Desmond, M.M., Wait, R.B., 1981. Follow-up of methadone-treated and untreated narcotic-dependent women and their infants: health, developmental, and social implications. *J. Pediatr.* 98, 716–722.
- Witmann, B.K., Segal, S., 1991. A comparison of the effects of single- and split-dose methadone administration on the fetus: ultrasound evaluation. *Int. J. Addict.* 26, 213–218.
- Wong, C.S., Lee, Y.J., Chiang, Y.C., Fan, L.W., Ho, L.K., Tien, L.T., 2014. Effect of prenatal methadone on reinstated behavioral sensitization induced by methamphetamine in adolescent rats. *Behav. Brain Res.* 258, 160–165.
- Woodward, L.J., Anderson, P.J., Austin, N.C., Howard, K., Inder, T.E., 2006. Neonatal MRI to predict neurodevelopmental outcomes in preterm infants. *N. Engl. J. Med.* 355, 685–694.
- Zedler, B.K., Mann, A.L., Kim, M.M., Amick, H.R., Joyce, A.R., Murrelle, E.L., Jones, H.E., 2016. Buprenorphine compared with methadone to treat pregnant women with opioid use disorder: a systematic review and meta-analysis of safety in the mother, fetus and child. *Addiction* 111, 2115–2128.
- Zelson, C., Lee, S.J., Casalino, M., 1973. Neonatal narcotic addiction. Comparative effects of maternal intake of heroin and methadone. *N. Engl. J. Med.* 289, 1216–1220.