Cerebral magnetic resonance imaging in children with prenatal drug exposure

Structural and functional aspects of the opioid-exposed brain

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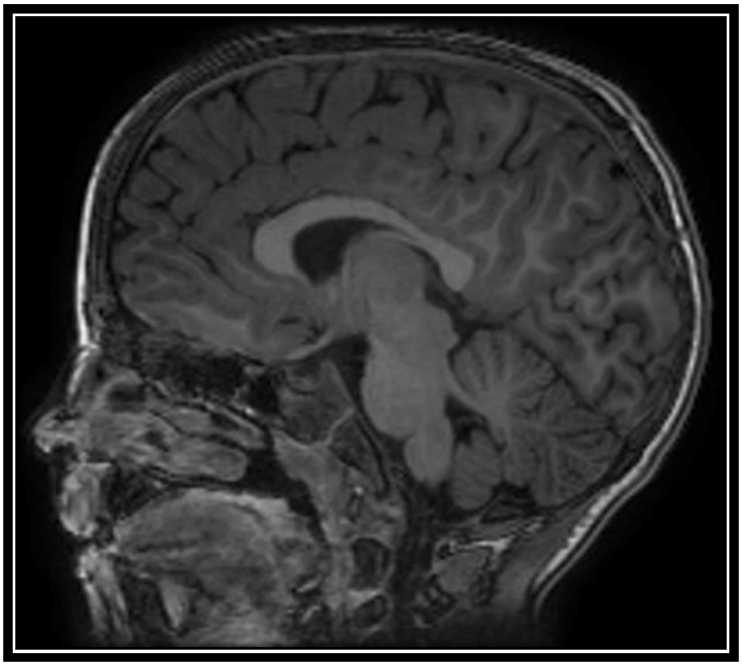
Preface

In this thesis, advanced brain imaging methods based on magnetic resonance imaging (MRI) were used to investigate brain structure and function in children with prenatal drug exposure. As the existing knowledge of possible associations between prenatal opioid exposure and future brain alterations was very limited, and opioid exposure was common in the sample recruited for the study, the main focus of the work has been to elucidate these associations.



Pilot scan of my oldest son Jona at the age of seven.

"Not everything that counts can be counted,
and not everything that can be counted counts."
Albert Einstein

Eivind Sirnes Bergen, October 2017

List of abbreviations

ADHD	Attention-deficit/hyperactivity disorder
BOLD	Blood-oxygen-level-dependent
CI	Confidence interval
DTI	Diffusion tensor imaging
EPI	Echo planar imaging
FAS	Fetal alcohol syndrome
FASD	Fetal alcohol spectrum disorder
fMRI	Functional magnetic resonance imaging
FWE	Family wise error
HR	Hazard ratio
ICV	Intracranial volume
MNI	Montreal Neurological Institute
MRI	Magnetic resonance imaging
NAS	Neonatal abstinence syndrome
OMT	Opioid maintenance treatment
OR	Odds ratio
SD	Standard deviation
SES	Socio economic status

Scientific environment

This thesis is part of the PhD program at the Department of Clinical Medicine, University of Bergen. However, the main scientific environment was located at the Department of Child and Adolescent Psychiatry, in collaboration with the Department of Radiology, the Department of Pediatrics, and Centre for Clinical Research, all at Haukeland University Hospital. Important contributions have also been provided by researchers affiliated at Great Ormond Street Hospital for Children, London, UK, and University of California, San Diego, US.

This PhD project is based on a cohort of prenatally drug-exposed children recruited from the Pediatric Department, Haukeland University Hospital, included in a larger follow-up study, initiated by Alcohol and Drug Research Western Norway (KoRFor), and led by the pediatrician and child psychiatrist Professor Irene B Elgen. She has been my co-supervisor and the principal investigator of the study, while Associate Professor Stein Magnus Aukland, an experienced pediatric radiologist and senior researcher of the project, has been my main supervisor.

Acknowledgments

First of all, I would like to thank the sources of financial support for the study, Alcohol and Drug Research Western Norway (KoRFor) and the Western Norway Health Authority. This support made it possible for me to put a substantial part of my time and effort into this project. I am also grateful to the Department of Child- and Adolescent Psychiatry and to the Department of Research, Division of Psychiatry, Haukeland University Hospital, as my employer during this time. However, without all the participating families, there would have been no study. I am therefore greatly thankful to all children, parents and foster-parents taking the time to participate in the study.

The scientific content of the work would not have been possible without all my co-workers and supervisors. I am truly grateful to all the participants in this collaborative scientific endeavor.

Initially I was invited into the project by Professor Irene B. Elgen. I am grateful for the way you introduced me to the world of research, although frustrating and hard to grasp at first, your enthusiasm and encouragements as my co-supervisor, always made me eager to move forward into this brave new world. Realizing that the project was based on magnetic resonance imaging (MRI), a field full of confusing abbreviations and complicated technical terms, I was more than lucky to have Associate Professor Stein Magnus Aukland, an experienced pediatric radiologist, as my main supervisor. I am especially grateful for the way you always managed to find time to discuss our analyses, results and manuscripts, in the middle of your busy work schedule. Thank you! My way into functional MRI was made possible by my last two co-supervisors, Hilde S. Gundersen and Silja T. Griffiths. I want to express my deep gratitude for all your time and effort spent on this project. Hilde, always last minute, but always full of enthusiasm and confident about our next scientific breakthrough. I thank you for keeping me from giving up on our many complicated analyses. Silja, I appreciate your kind and well-structured feedback on my writing and your continuous supportive attitude.

I have been privileged to have several talented co-authors in addition to my excellent team of supervisors. First, I want to thank Kling Chong who analyzed all structural scans. Next, I want to thank Leif Oltedal and Hauke Bartsch, who helped me carry out the FreeSurfer analyses. Leif, I am especially grateful for fruitful discussions, crucial for the scientific content of the morphometric study. Finally, I wish to thank Professor Geir Egil Eide for helping me out with the statistics.

The practical part of the work would not have been possible without a web of contributors. I would like to mention Sidsel Bruarøy for her invaluable help in organizing the MRI examinations. Thank you. I would also like to thank all the MRI radiographers for their crucial role in data collection. Roger Brandon, Christel Jansen, Turid Irene Randa, Eva Øksnes, and Trond Martin Øvreaas, your abilities to make anxious children feel comfortable and safe in the scanner really impressed me. Then, of course, I wish to thank the Department of Radiology, Haukeland University Hospital, for the use of the 3T MRI scanner. My fellow PhD-student Lisbeth B. Sandtorv also deserves mentioning and gratitude for her contributions in the recruitment process of the families and for important scientific discussions.

My own mental wellbeing during this period has been supported by my good friend, psychologist and fellow PhD-student Arne Kodal. It has been an absolute privilege to share coffee breaks, PhD-courses, frustrations and triumphs with you.

Last, but not least, I would like to thank my family. Thank you for not giving up on me during this research period. At times, I have been working too much, often coming home late for dinner, and even when I have finally shown up, my mind has been stuck in my research. Catharina, you are the love of my life, Jona, Cecilie and Sondre, you make me proud. I owe you everything!

Abstract

Background: Over the last few decades brain imaging studies have made important contributions to our understanding of how prenatal drug exposure can impact normal brain development. The teratogenic potential of alcohol has been most widely studied, with growing evidence of structural and functional brain alterations in prenatally exposed children. However, the current knowledge on possible detrimental effects of drugs other than alcohol is still limited, and effects of prenatal opioids in particular, have only been explored in a few small-scale brain imaging studies.
Aims: The overall aim was to investigate associations between prenatal drug exposure and later brain structure and function in children. The specific aims were to investigate gross anatomical brain changes after prenatal drug exposure and associations between prenatal opioids and morphometric and functional brain characteristics.
Materials and methods: A hospital-based sample of 43 school-aged children with prenatal alcohol-, opioid- or polysubstance exposure and 43 sex- and age-matched unexposed controls underwent cerebral magnetic resonance imaging (MRI). All MRI scans were evaluated by an expert pediatric neuroradiologist blinded to the participants' backgrounds. In children with confirmed exposure to opioids, volumetric brain characteristics were compared to controls. Brain activation patterns and performance on a working memory-selective attention task were compared between opioid-exposed and unexposed children using functional MRI (fMRI).
Results: No association between prenatal drug exposure and gross structural brain changes was seen by means of expert visual analysis of cerebral MRI scans. Reduced regional brain volumes were found in prenatally opioid-exposed children compared to their matched controls. Functional imaging revealed impaired task performance and increased blood-oxygen-level-dependent (BOLD) activation in prefrontal cortical areas during the most cognitive demanding versions of the working memory-selective attention task in the opioid-exposed group as compared to unexposed controls.
Conclusions: Cerebral MRI is probably of limited value in the clinical assessment of children with histories of prenatal drug exposure in a hospital setting, where

polysubstance exposure and unspecified drug exposure is a common feature. Adverse effects of opioids on the developing fetal brain may explain the associations between prenatal opioids and brain alterations in children as seen by structural and functional MRI in this study. However, the sample was small and inevitably confounding factors were difficult to account for. Thus, further research is needed to explore the causal nature of these findings and to elucidate the functional consequences of the observed brain alterations in the opioid-exposed group.

List of papers

I **Cerebral Magnetic Resonance Imaging in Children With Prenatal Drug Exposure: Clinically Useful?**
Sirnes E, Elgen IB, Chong WK, Griffiths ST, Aukland SM
Clinical Pediatrics. 2017 Apr; 56(4):326-332.

II **Brain morphology in school-aged children with prenatal opioid exposure: A structural MRI study**
Sirnes E, Oltedal L, Bartsch H, Eide GE, Elgen IB, Aukland SM
Early Human Development. 2017 Mar - Apr; 106-107:33-39.

III **Functional MRI in prenatally opioid-exposed children during a working memory-selective attention task**
Sirnes E, Griffiths ST, Aukland SM, Eide GE, Elgen IB, Gundersen H
Submitted manuscript

1. Introduction

1.1 Prenatal drug exposure

1.1.1 Definition and prevalence

The unborn child could potentially be exposed to a variety of drugs when a pregnant woman suffers from a substance use disorder. The term "prenatal drug exposure" does not have a well-established or strict definition. In this thesis "prenatal drug exposure" will be used to describe in utero exposure to drugs of abuse, including licit drugs like alcohol and nicotine, and illicit drug use (e.g., amphetamines, cocaine, cannabis, and opioids). Since the main-focus of the thesis is on prenatal opioid exposure, opioid medication given to pregnant women as part of opioid maintenance treatment (OMT) will also be included in the term "prenatal drug exposure", although opioids in this setting should not be considered drugs of *abuse*.

The exact number of children with histories of prenatal drug exposure is unknown. In the most recent report from the National Survey on Drug Use and Health, an annual survey in the United States, 4.7 % of pregnant women reported use of illicit drugs, 13.9 % used tobacco products and 9.3% used alcohol.[1] Global estimates hardly exist. However, taking recent global trends of drug use in the general population into account [2] several million children worldwide are each year most likely exposed to drugs in utero according to the above outlined broad definition of the term. Potential negative effects of such exposure should therefore be an important concern for health care providers, policy makers, and researchers.

When it comes to prenatal opioid exposure, recent reports on the prevalence of neonatal abstinence syndrome (NAS), a common consequence of prenatal opioid exposure, indicate a worldwide increase in the number of children exposed to opioids in utero.[3-5] The prevalence of opioid abuse or dependence during pregnancy was found to be 0.39% in a large American register study.[6] In Norway 30-60 children (approximately 0.05–0.1% of all births) are born annually to mothers included in

OMT,[7] while the number of children with other opioid exposures (incl. heroin) is unknown.

1.1.2 Possible consequences for the child

Historical perspectives: As a research field the delineation of child outcomes after prenatal drug exposure is quite new. The specific pattern of malformations, growth restriction and developmental delay seen after heavy prenatal alcohol exposure, now known as Fetal Alcohol Syndrome (FAS), was first described by Drs. Smith and Jones in 1973.[8] However, the observation of fetal malformations associated with maternal alcoholism was not new. As described by Smith and Jones in their review of historical evidence, the link between maternal drinking and faulty development of the offspring could be traced all the way back to the early Greek and Roman mythology.[9] Early reports of devastating fetal effects of maternal cocaine use led to the terms "crack baby" and "crack kid" in the early 1970s.[10] However, many of the findings once thought to be specific effects of in utero cocaine exposure were later shown to be explained by other factors like the quality of the child´s environment and exposure to other drugs.[11] This clearly demonstrates some of the complexity in the research on possible effects of prenatal drug exposure. A large body of literature has unequivocally revealed detrimental effects of prenatal alcohol, which is now frequently cited as the most common preventable cause of intellectual disability.[12] However, the history of the purported "crack baby" phenomenon points out the need of rigorous methodological considerations to avoid jumping into conclusions about causal mechanisms.

Prenatal effects - congenital anomalies: The teratogenic potential of prenatal alcohol is well known, with a series of facial malformations established as one of the hallmarks of the diagnosis of FAS.[12] Gross structural abnormalities of the brain, like microcephaly and agenesis of the corpus callosum, have also been reported in children with prenatal alcohol exposure.[13] Maternal smoking during pregnancy has been linked to increased risk of several structural malformations, but in general there has been a failure of replication and conflicting results.[14] Prenatal cocaine exposure

was initially reported to be associated with increased risk for malformations, still these associations have not been replicated in larger, prospective, well controlled studies.[15] Some recent studies have reported an increased risk of birth defects after prenatal opioid exposure.[16,17] However, there is no consistent literature to support any causal link between prenatal exposure to cocaine, amphetamines, cannabis or opioids and birth defects.[18-21]

Perinatal effects: Maternal use of both licit and illicit drugs has been linked to increased risk of a large range of adverse pregnancy outcomes, including stillbirth, intrauterine growth restriction, and preterm birth.[22,23] However, the impact of specific drugs on these outcomes relative to the contribution of myriads of interconnected risk factors like poor prenatal care, stress, and poor maternal nutrition is unclear.[24] Still, there is unequivocal evidence for impaired fetal growth caused by maternal tobacco and alcohol use during pregnancy.[18,25] Prenatal cocaine exposure has also been associated with impaired fetal growth in several large, well-controlled studies.[26,27] Both amphetamines [19,20] and opioids [6,28,29] have been associated with low birth weight and preterm birth, whereas maternal marijuana was not found to be an independent risk factor for intrauterine growth restriction or preterm birth in a recent meta-analysis.[30] Heavy maternal cigarette smoking was shown to have a larger individual impact on birth weight than both alcohol and illicit drug use in a recent, prospective study on pregnancy outcomes and substance abuse.[31]

Withdrawal symptoms in the neonate have been reported after prenatal exposure to several different licit and illicit drugs.[32] However, the most severe symptoms clearly appear in the opioid-exposed neonate, commonly recognized as the neonatal abstinence syndrome (NAS).[33] The variable clinical manifestations of NAS involve excitability of the central nervous system, autonomic dysregulation, gastrointestinal, and respiratory symptoms.[34] The occurrence and severity of NAS after prenatal opioid exposure has been shown highly variable, and seem to be affected by a complex interplay between several risk factors including exposure to non-opioid drugs and genetic factors affecting opioid metabolism.[33,35,36]

Although withdrawal symptoms are not commonly reported in neonates after prenatal exposure to psychostimulants (cocaine, amphetamines), tobacco, cannabis, or alcohol,[18,19] neonatal or infant neurobehavioral abnormalities have been found in all these groups.[18] Abnormalities like altered muscle tone, irritability and signs of stress suggesting early self-regulatory problems have been associated with prenatal exposure to cocaine, amphetamines, marijuana and opioids.[19,37,38] Finally, prenatal drug exposure has been associated with increased risk of sudden infant death syndrome.[25,39]

Long-term effects on growth and somatic health: Maternal alcohol consumption during pregnancy is associated with impaired childhood growth, and evidence of postnatal growth restriction is one of the diagnostic criteria used in guidelines for the diagnosis of FAS.[40] While alcohol effects on early growth seem to persist through childhood, there has been no consistent literature to show impaired later growth after prenatal exposure to other drugs.[15,18] However, impaired intrauterine growth may place these children at risk for adverse health outcomes in later life. Maternal smoking during pregnancy has been linked to both childhood obesity and diabetes.[25] Among other somatic health problems, an increased risk of visual problems, particularly strabismus and nystagmus, has been repeatedly reported after prenatal drug exposure.[41,42] Several recent studies have pointed out a possible link between prenatal opioid exposure in particular and impaired visual functioning.[43-46]

Long-term effects on cognition and behavior: Heavy maternal alcohol consumption during pregnancy is associated with neuropsychological difficulties in affected children ranging from subtle learning and/or behavioral problems to severe intellectual impairment.[12,47] A variety of behavioral and cognitive difficulties, including deficits in visuospatial functioning, memory and learning, attention, self-regulation, executive functioning and motor skills are commonly seen.[47] There is convincing evidence that detrimental effects of alcohol on the developing fetal brain underlay these neurobehavioral problems, with proposed mechanisms of neurotoxicity supported by extensive animal- and cell culture research.[48-50] A wide range of neuropsychological impairments have also been associated with prenatal

exposure to drugs other than alcohol, however, a far more limited number of studies exist to answer whether these impairments are caused by specific drugs.[18,23] The possible neurobehavioral long-term effects of prenatal cocaine exposure have been investigated in several large, well-controlled, prospective studies.[15] There has been no consistent pattern of general cognitive impairment, but even after adjustment for numerous confounding variables prenatal cocaine exposure has been associated with deficits related to attention, executive functioning, language and behavior.[51-53] In "The Infant Development, Environment, and Lifestyle" (IDEAL) study possible long-term effects of prenatal amphetamines were explored for the first time in a large, prospective cohort.[19] Subtle cognitive and behavioral deficits were found in the amphetamine-exposed group.[54,55] Tobacco and marijuana have both been associated with behavioral and cognitive impairments in prenatally exposed children and adolescents.[56-58] Prenatal opioid exposure has been associated with several neuropsychological difficulties in children, particularly attention problems.[59,60] As possible opioid effects on brain development in prenatally exposed children is one of the main topics of this thesis, studies looking specifically into neurocognitive and behavioral consequences of prenatal opioid exposure will be covered in some more detail in the next section.

To sum up this section, extensive work has demonstrated that prenatal alcohol exposure causes a broad range of adverse developmental effects, commonly described under the umbrella term fetal alcohol spectrum disorders (FASD).[12,61] In a recent review, The American Academy of Pediatrics stated that "*There is no known absolutely safe quantity, frequency, type, or timing of alcohol consumption during pregnancy*."[12] However, to what extent low to moderate alcohol intake during pregnancy is causal to cognitive and behavioral impairments observed in exposed children is still debated.[62] When it comes to possible consequences for the child of prenatal exposure to drugs other than alcohol, the research base is more limited. There is growing evidence of negative effects on both short- and long-term outcomes, but still conclusions about causal relationships should be made with caution.[18,23] In general more subtle, neurocognitive deficits are reported in these groups, in contrast to the marked impairments caused by prenatal alcohol exposure.

1.1.3 Prenatal opioid exposure and neuropsychological impairments

Important knowledge gaps still remain regarding possible neurobehavioral, long-term consequences of prenatal opioid exposure. The literature is sparse, especially when it comes to follow-up beyond infancy and preschool age. In the following section, some important studies and findings to date are presented.

Several small studies were conducted in the 70's and 80's, mainly on children born to methadone maintained mothers. As reviewed by Kaltenbach, these early studies did not show any convincing opioid effect on behavioral outcomes when prenatally exposed children were compared to unexposed children from similar socio economic disadvantaged, high-risk populations.[63] The important impact of postnatal social and environmental factors on neurodevelopment has later been emphasized in several studies on children born to heroin using mothers by Ornoy et al.[59,64,65] In school-aged children general intellectual development was shown to be influenced to a large extent by postnatal environment, as cognitive impairment was found in children living in low socio economic environments, regardless of prenatal drug exposure.[59] However, high rates of attention-deficit/hyperactivity disorder (ADHD) were found among children with prenatal opioid exposure, also in those adopted into high socio economic status (SES) families.[59] Similar high rates of attention problems/ADHD in prenatally opioid-exposed children have been reported in several more recent studies.[60,66-68]

In the Maternal Life Style Study, a large prospective, multisite study of a high-risk population, prenatal opioid exposure was not associated with mental, motor, or behavioral deficits in infants through three years of age after controlling for birth weight and environmental risks, including other drug exposures.[69] In 2008 Hunt et al. concluded that there was consistent evidence of neurodevelopmental impairment throughout early childhood in prenatally opioid-exposed infants in a cross-sectional study and review of 14 previously published studies.[70] A recent systematic review and meta-analysis on neurobehavioral consequences of in utero opioid exposure in infants and preschool children showed no significant impairments for behavioral,

psychomotor or cognitive outcomes in exposed children compared to non-exposed controls.[71] However, an error in data entry was uncovered after publication, and in the repeated, corrected analyses significant impairments with small effect sizes were found in opioid-exposed children for all the included neurobehavioral outcomes.[72]

A national, population-based cohort of 38 children born to mothers included in OMT and 36 comparison children from a low-risk population have been followed from birth in a Norwegian, longitudinal study.[73,74] Overall, scores within the normal range on cognitive and behavioral measures in preschool children have been reported from this study, but reduced cognitive performance and more behavioral problems in opioid-exposed children as compared to unexposed controls.[75,76] The importance of postnatal environmental factors for child development has been demonstrated in several publications from this study. Maternal psychosocial stress, rather than prenatal exposure to OMT medications was shown to predict child behavioral problems,[76] language-related cognitive development was linked to mother-child interaction,[75] and impaired executive functioning in the exposed group was mainly associated with lower maternal employment rate and education.[77] However, some aspects of higher cognitive functioning and subtle alterations in the attention system appeared influenced by prenatal opioid exposure.[75,78]

There has been a lack of studies exploring possible long-term effects of prenatal opioid exposure in school-aged children and adolescents. In a recent population-based, registry linkage-study, children in New South Wales, Australia who had been diagnosed with NAS (n = 2234) were compared to controls (n = 4330), matched for gestational age, gender, and SES.[79] A history of NAS was strongly associated with poor and deteriorating high school performance. Detrimental opioid effect on the fetal brain was discussed by the authors as one possible contributing factor to explain impaired school performance.

In Norway, a sample of children born to mothers with opioid and polysubstance abuse problems during pregnancy has been followed from birth to early adulthood. High rates of attention problems and impaired cognitive function have been reported

in these children, even in adoptive/foster children living in stable family situations from an early age, and thus with minimal postnatal risk.[80,81] At age 17–21 years opioid- and polysubstance exposure was associated with lower cognitive abilities when exposed youth were compared to a non-exposed control group.[82] No firm conclusions regarding specific opioid effects can be drawn from these studies, as opioid exposure was inextricably associated with other environmental risks, as discussed by the authors.[81,82]

In summary, firm conclusions about the influence of prenatal opioids on long-term cognitive and behavioral development in exposed children, cannot be made based on the existing literature. However, subtle neurocognitive deficits and attention problems have been repeatedly reported in this group, and opioid induced brain changes could possibly contribute to some of these difficulties.

1.1.4 Methodological challenges in clinical studies exploring prenatal drug effects

An important challenge in all human studies on possible long-term developmental consequences of prenatal drug exposure is the complex nature of human development, influenced by a web of interconnected factors. Some of these factors, with possible impact on developmental outcomes in prenatally drug-exposed populations, are illustrated in Figure 1. Although complex statistical modelling can take into account possible mediating, moderating or confounding factors regarding the relationship between drug exposure and developmental outcome, several unknown or unmeasurable factors still remain. Randomized controlled trials could overcome this problem, but it would obviously be unethical and practically impossible to randomize prenatal drug exposure. Consequently, human studies have limited ability to make inferences with confidence about causal mechanisms behind developmental impairments observed after prenatal drug exposure. Conclusions about causality should not be solely made based on associations between prenatal exposures and later developmental outcomes. Even in the case of consistently reported associations across different populations, like the well-established association between maternal smoking during pregnancy and childhood ADHD, these

associations are not necessarily causal. Several recent studies have called into question the assumption that prenatal tobacco exposure causes ADHD.[83,84] In summary, the potential harmful developmental effects of prenatal drug exposure must be evaluated in the context of other biological, psychosocial and environmental factors with impact on the developmental outcomes of interest. Prenatal exposure may represent a biological risk that could be moderated by several other influences like genetic factors and postnatal environmental factors, as discussed in a model for neurobehavioral teratology by Minnes et al.[85] As long as several of these factors remain unknown, conclusions about specific drug effects should be interpreted with caution.

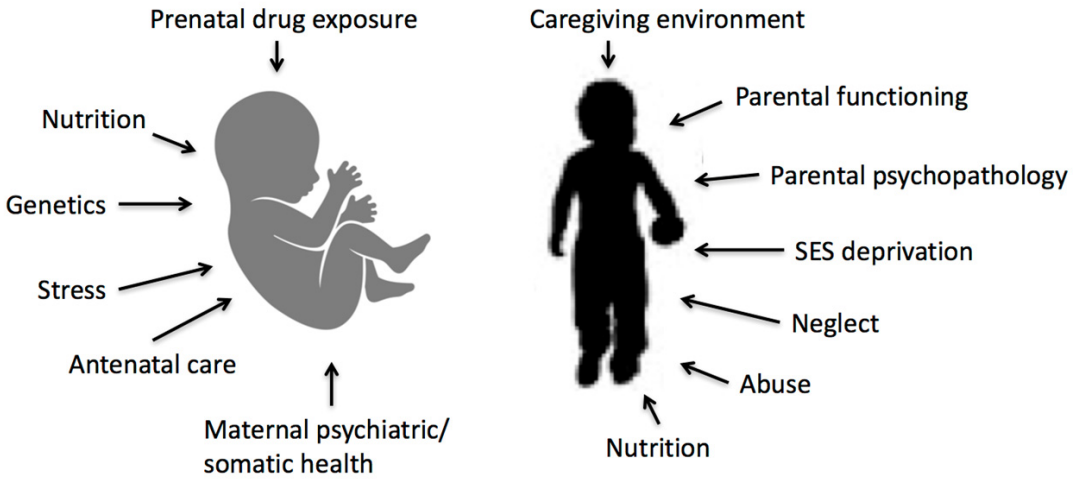


Figure 1 *Examples of prenatal and postnatal factors with possible impact on developmental outcomes in prenatally drug-exposed children.*

Animal studies on prenatal drug exposure can control environmental and genetic risk factors by random assignment of exposure. Such studies have made important contributions to our understanding of potential drug effects, pointing out plausible biological causal relationships.[49] However, the translational potential of animal research is always limited by interspecies differences. Lack of pharmacokinetic

reference data in animals is an important limitation,[86] and animal models have limited ability to elucidate complex aspects of human development.

All observational human studies exploring possible consequences of prenatal drug exposure are to some extent influenced by uncertain and unreliable measures of prenatal drug exposure. Most studies relay on some kind of maternal self-report of drug use during pregnancy. Information is often obtained after pregnancy, and recall bias could consequently be introduced. Even in prospective studies using structured interviews to assess drug exposure, underreporting may occur, due to the punitive social and legal implications of disclosure. If toxicology tests are used at birth (mother or infant urine toxicology screens and/or infant meconium and hair analyses), these tests cannot tell anything about exposure in first trimester of pregnancy, total exposure during pregnancy or drug dose, although these factors could be of importance for adverse drug effects.

Negative drug effects on the developing fetal brain may be subtle and not easily recognized until later in development, when e.g. language problems and poor academic achievement show up. Therefore, well controlled longitudinal studies with appropriate, sensitive measures are needed to investigate such effects. Results from longitudinal neuroimaging indicate that early life factors, like birth weight, impact brain structure and cognition for the entire life course,[87] and cerebral magnetic resonance imaging (MRI) stands out as a useful tool to study how prenatal factors impact brain development. As discussed in the next sections, a growing body of evidence from MRI studies has pointed out possible detrimental drug effects on the developing fetal brain. However, associations between prenatal drug exposure and later brain alterations, as seen by MRI, do not need to be causal, and several knowledge gaps remain, especially when it comes to possible brain alterations after prenatal opioid exposure.

1.2 Neuroimaging after prenatal drug exposure

1.2.1 MRI-based neuroimaging

Over the last few decades, advances in neuroimaging methods based on magnetic resonance imaging (MRI) have made important contributions to our understanding of the living growing human brain.[88,89] MRI is a safe method to study the developing brain without the use of ionizing radiation, and thus particularly well suited for research purposes. Structural MRI allows for high-resolution images of the brain to be obtained within the time frame of a few minutes. In addition to the study of gross anatomy, automated computerized methods enable researchers to analyze and compare morphometric measures like regional volumes and cortical thickness across different groups and across time. Diffusion tensor imaging (DTI) is an MRI technique were diffusion of water molecules within the brain can be used as an indirect measure of white matter structure and integrity. In functional MRI (fMRI) the so called blood-oxygen-level-dependent (BOLD) contrast is used as an indirect measure of neuronal activity. The BOLD contrast is based on the paramagnetic state of deoxygenated hemoglobin and reflects changes in blood oxygenation detected as a change in the MRI signal.[90] Since the first human fMRI studies were published in 1992,[91,92] fMRI has been applied to almost every aspect of brain science, and is by far the most frequently used imaging technique to study human brain function.[93] Overall, MRI-based neuroimaging has been crucial for the current understanding of the normal brain. In addition, MRI has been a useful tool in the study of developmental changes, like ADHD,[94,95] and brain injury, like the preterm brain.[96] The knowledgebase of normal brain development is fast growing. However, precise growth trajectories of several anatomical brain measures are still incompletely understood.[97]

1.2.2 MRI studies of prenatally drug-exposed children

Neuroimaging studies have also made important contributions to our understanding of how prenatal drug exposure can impact normal brain development, in particular by elucidating the teratogenic potential of alcohol, showing both structural and functional brain alterations in prenatally alcohol-exposed populations.[13,98] Reduction

of the overall brain size has been the most consistent finding in children with FASD.[13] Several macrostructural abnormalities, like malformations of the corpus callosum and cerebellar atrophy, have been reported in prenatally alcohol-exposed children.[99-101] Using advanced methods have allowed more subtle brain changes to be found throughout multiple brain regions.[13] Specific patterns of changes have suggested certain structures like the basal ganglia,[102] corpus callosum,[103] and cerebellum[104] to be especially vulnerable to the adverse effect of alcohol.[50] The knowledgebase of possible brain changes in children prenatally exposed to drugs other than alcohol is still quite limited. Existing studies tend to be based on small samples with numerous confounding variables difficult to account for. However, there is growing evidence that prenatal exposure to tobacco, amphetamines and cocaine is associated with structural and functional brain changes in children.[105,106] In the first structural MRI study on prenatally methamphetamine-exposed children, published in 2004, Chang et al. reported reduced subcortical brain volumes in the exposed group.[107] After that study prenatal methamphetamine has been associated with reduced regional brain volumes linked to attention processing deficits,[108] altered brain activation patterns (fMRI),[109] and recently alterations in white matter developmental trajectories (DTI).[110] For cocaine-exposed children a variety of gross structural abnormalities were reported in early imaging studies.[106] More recent studies have reported subtle changes, like alterations of regional patterns of striatal morphology in prenatally cocaine-exposed adolescents.[111] However, there have been some conflicting results. Avants et al.[112] reported reduced caudate volumes after prenatal cocaine exposure, whereas no differences in caudate volumes were found between prenatally cocaine-exposed and control children in a sample of 40 adolescents derived from the Maternal Life Style study.[113] In two recent, well controlled studies with large populations ($n > 100$), alterations in cortical morphology in children with prenatal exposure to tobacco and/or marijuana were shown.[114,115] Polysubstance exposure is common in populations of prenatally drug-exposed children. Results from a volumetric MRI study of children with prenatal exposure to alcohol, tobacco, cocaine and marijuana suggested that these substances may act cumulatively during gestation to exert long lasting effects on brain volumes.[116] The

dopamine-rich basal ganglia seem to be particularly vulnerable to possible harmful effects of prenatal exposure across several different drugs, and it has been suggested that drugs of abuse share a specific profile of developmental neurotoxicity.[106] Prenatal opioid exposure has been associated with reduced regional brain volumes and alterations in white matter tracts in preliminary studies.[117,118] However, research on possible brain alterations after prenatal opioid exposure is scarce, and the few existing studies will be reviewed in more detail in the next section.

To sum up this section, there is growing evidence for both structural and functional brain alterations in prenatally drug-exposed children. However, it is not clear whether cerebral MRI is useful in the clinical assessment of these children, as MRI findings ranging from gross anatomical abnormalities to subtle morphometric changes or normal imaging have been reported across different groups of prenatally drug-exposed children.

1.3 MRI studies of children with prenatal opioid exposure

Current knowledge of possible brain changes after prenatal opioid exposure is based on a few small-scale samples. MRI studies investigating specifically the association between in utero opioid exposure and later brain changes up to December 2016 are summarized in Table 1. In 2007 Kahila et al. published results from a pilot study with cerebral MRI scans of seven neonates prenatally exposed to buprenorphine and several other drugs.[119] Upon expert visual analyses all scans were interpreted as normal, and no further analyses were performed. Volumetric cerebral characteristics of children with prenatal opioid exposure have been explored in two previous studies. Walhovd et al. included 14 school-aged children born to mothers with histories of heroin and polysubstance abuse during pregnancy and 14 unexposed controls in a volumetric MRI study.[117] Volume reductions in various brain measures were reported in the exposed group, including reduced total brain volumes. In analyses restricted to a subgroup of 10 children exposed to opioids, pallidum and putamen volumes appeared especially reduced. Analyses of cortical thickness revealed thinner cortex in the exposed group in the anterior cingulate and orbitofrontal cortical areas.

Morphometric brain alterations were to some extent related to cognitive and behavioral difficulties in the exposed group. The other study to explore volumetric characteristics of opioid-exposed children was a pilot study by Yuan et.al, where 16 neonates, mostly born to methadone- or buprenorphine maintained mothers, were examined with cerebral MRI.[120] Reduced total brain and basal ganglia volumes were found in the opioid-exposed neonates. An important limitation of that study was the lack of a control group. Volumes from manual segmentation of MRI scans were compared to published population values. Of note, the referred normal value for neonatal basal ganglia volume was based on a very small sample (n =12) of healthy term-born neonates in a study on brain maturation in preterm infants.[121] Indication of altered structural integrity of white matter after prenatal opioid exposure has been reported in two small studies using DTI.[118,122]

To summarize, there is circumstantial evidence that prenatal opioids can affect the developing fetal brain, with reports of reduced volumes, especially of the basal ganglia, cortical thinning, and altered white matter characteristics in prenatally opioid-exposed groups. Such brain changes may contribute to neuropsychological difficulties reported in these groups. However, firm conclusions cannot be made, due to small samples and effect sizes, and inevitable confounding factors difficult to account for. Overall, there is no compelling evidence for an increased risk of gross structural brain changes in opioid-exposed groups, although a link between maternal opioid use during pregnancy and neonatal stroke has been suggested in case reports.[123] To date, no previous study has examined brain activation patterns associated with prenatal opioid exposure using fMRI.

Table 1 Studies examining the association between prenatal opioid exposure and cerebral MRI findings up to December 2016

Study	Sample	Control group	Main results
Kahila et al. 2007 [119]	Infants (0-2 months) exposed to buprenorphine, all exposed to tobacco and benzodiazepines (*n* = 7)	No	All cerebral MRI scans normal (expert visual analysis)
Walhovd et al. 2007 [117]	14 children (9–14 years) exposed to polysubstance abuse, subgroup of 10 children born to mothers reporting heroine as their main drug of choice	14 unexposed, healthy, children (9–10 years) from a low-risk population	No cerebral pathology found by visual inspection of MRI scans. Reduced brain volumes and cortical thinning in the exposed group. Pallidum and putamen especially reduced in the opioid-exposed group (automated computerized segmentation)
Walhovd et al. 2010 [122]	Same population as Walhovd et al. 2007	-	Altered white matter characteristics in the exposed group, also when analyses were restricted to the heroine-exposed subgroup (DTI)
Walhovd et al. 2012 [118]	13 infants (0-2 months) born to methadone maintained mothers, 11/13 medically treated for NAS	7 unexposed controls (randomly identified from hospital delivery bookings)	Higher mean diffusivity in the exposed group, suggesting altered maturation of cerebral connective tracts (DTI)
Yuan et al. 2014 [120]	16 neonates with prenatal opioid exposure (methadone/ buprenorphine/ other opioids incl. heroine), most exposed to multiple drugs	No	All scans structurally normal (visual inspection). Whole brain and basal ganglia volumes reduced compared to published population values (manual segmentation)

Abbreviations: DTI, diffusion tensor imaging; MRI, magnetic resonance imaging; NAS, neonatal abstinence syndrome.

2. Aims

The overall aim of the study was to investigate associations between prenatal drug exposure and brain structure and function in school-aged children.

The specific aims were:

I. To investigate brain changes detectable by expert visual inspection of cerebral MRI scans in children with prenatal drug exposure and unexposed controls.

II. To investigate brain morphology in children with prenatal opioid exposure. Based on prior research, we hypothesized that prenatal opioid exposure would be associated with reduced volumes of the basal ganglia.

III. To investigate brain activation patterns in children with prenatal opioid exposure during a working memory-selective attention task. We hypothesized that prenatally opioid-exposed children would show impaired task performance with corresponding differences in blood-oxygen-level-dependent (BOLD) activation as compared with unexposed controls.

3. Materials and methods

3.1 Study design

Observational study with cross-sectional MRI data from a hospital-based population of prenatally drug-exposed children and unexposed controls derived from the general population.

3.2 Study population

3.2.1 Drug-exposed group

The prenatally drug-exposed group included in the present study was derived from a larger group of children included in a clinical follow-up study of children referred to the pediatric department at Haukeland University Hospital, between 1997 and 2012, due to prenatal drug exposure. Children were identified as prenatally drug-exposed if they had been admitted to the neonatal department due to maternal drug use, in most cases treated for withdrawal symptoms, or if they were referred to a pediatric neurologist at a later age with a medical history of prenatal drug exposure and symptoms of attention and/or behavioral problems. A total of 70 out of these children were in the age range of 10–14 years and hence eligible for the chosen MRI protocol. Forty-three out of 70 (61%) children consented to participate. Details on inclusion/exclusion of drug-exposed children into the final study populations of the different papers are shown in Figure 2. Among the 27 nonparticipants in the MRI study, 19 were included in other parts of the clinical follow-up. For these children information about type of drug exposure and intelligence quotient (assessed by Wechsler Intelligence Scale for Children, fourth edition and Wechsler Preschool and Primary Scale of Intelligence-R) was available, and did not differ significantly from what was found in the participating group.

Information regarding drug exposure was based on history without toxicology testing. Given the presence of heavy substance abuse, detailed information about the

frequency or amounts of drugs used during pregnancy was not readily available for all participants. However, children were only included in the study if prenatal drug exposure could be confirmed, either in medical records (obstetric or pediatric) or by information from their mother.

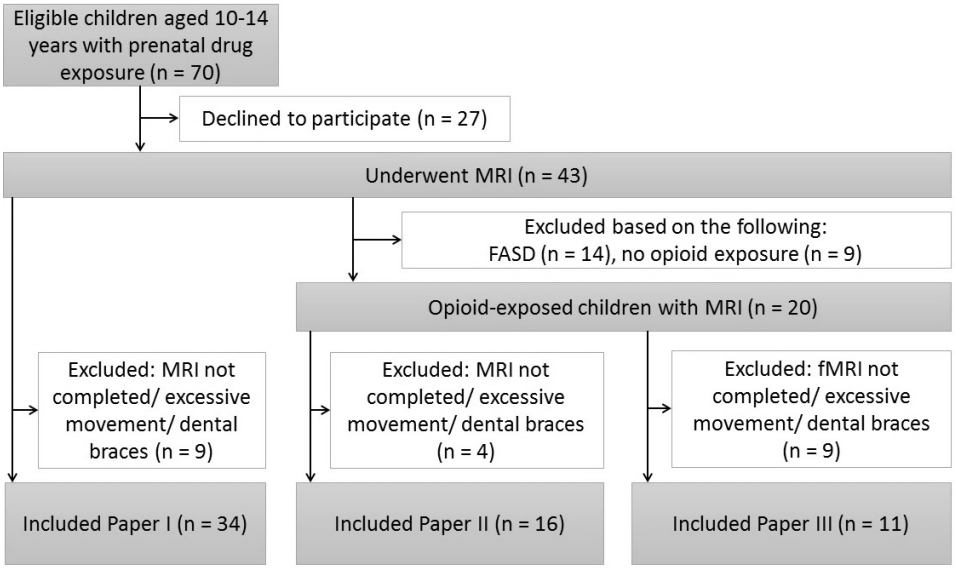


Figure 2 *Flow chart showing the inclusion/exclusion of drug-exposed groups into the final study populations of paper I–III.*

3.2.2 Control group

For each drug-exposed child included in the study, the first child of the same gender born at Haukeland University Hospital on the same date, with a birth weight above 3000 g, was invited to serve as the matched control. If they declined, the family of the next child born on the same date (or the nearest date) was approached. Forty-tree controls were recruited. According to questionnaires filled out by their parents, none of the included controls were exposed to prenatal medication or substance abuse.

3.2.3 Population paper I

All 43 drug-exposed and 43 unexposed controls recruited to the study were included in paper I. MRI scans of acceptable quality for analyses were obtained in 34 (19

males) drug-exposed and 40 (23 males) control children. There were 32 complete pairs of exposed children and controls matched 1:1 for sex and age. In two matched pairs, there was no measurement for the control, and for eight pairs there were missing MRI data for the exposed child. Mean age at scan (SD)/ range was 142 (12.8)/ 116–163 months and 141 (13.5)/ 116–165 months in the exposed/ control groups respectively.

3.2.4 Population paper II

As the aim of paper II was to investigate possible impact of prenatal opioid exposure on brain morphology, only children where prenatal opioid exposure could be confirmed were included. Children with FASD were excluded due to the well-established effects of alcohol on brain volumes in this group.[13] After subsequent and appropriate exclusions, 16 prenatally opioid-exposed children with MRI images considered to be of acceptable quality for the volumetric analyses were included (Figure 2). MRI data for three of the 16 originally matched controls were missing (movement artifacts/ no scan). Before further image processing, these three "missing" controls were replaced by available sex- and age-matched controls originally recruited for children with FASD/children exposed to drugs other than opioids. Thus, the final sample for paper II consisted of 16 children with prenatal opioid exposure and 16 1:1 sex- and age-matched unexposed controls.

Table 2 Sample characteristics paper II

Variable, *statistic*	Opioid-exposed (*n* = 16)	Controls (*n* = 16)	*p*
Males, *n (%)*	9 (56)	9 (56)	-
Age at scan (months), *mean (SD)*	143.6 (12.2)	143.6 (12.8)	-
Head circumference (cm), *mean (SD)*	54.2 (1.9)	54.8 (1.7)	0.402
ADHD, *n (%)*	11 (69)	1 (6)	0.002
Birth weight (g), *mean (SD)*	3026 (470)	3665 (430)	0.001
Reported NAS, *n (%)*	10 (63)	-	-

Abbreviations: ADHD, attention-deficit/hyperactivity disorder; NAS, neonatal abstinence syndrome; SD, standard deviation; *p* = *p*-values for group difference (controls vs. exposed) from paired *t*-test (continuous variables) and McNemar's test (categorical variables).

3.2.5 Population paper III

In paper III 20 children with confirmed prenatal opioid exposure, without known exposure to heavy maternal alcohol consumption were included (Figure 2). For these opioid-exposed children included, only 17 controls were successfully recruited, hence 20 exposed children and 17 control children underwent functional MRI. Nine opioid-exposed and five control children were excluded from analyses due to abortion of the fMRI-protocol by the child, head movement artifacts or dental braces distorting the images. Thus, the final sample for paper III consisted of 11 prenatally opioid-exposed children and 12 unexposed controls. Although the two study groups were primarily 1:1 matched for sex and age, the groups were treated as independent in our analyses, as matching was disrupted by appropriate exclusions of more than one third of the participants. Response logging failed for one participant (unexposed control). As in scanner observational data revealed appropriate task performance, data from this participant was still included in the analyses of the BOLD fMRI data, while analyses of task performance were run with $n = 11+11$.

Table 3 Sample characteristics paper III

Variable, *statistic*	Opioid-exposed (*n* = 11)	Controls (*n* = 12)	*p*
Males*, n (%)*	6 (55)	6 (50)	0.84
Age at scan (months), *mean (SD)*	146.1 (13.3)	146.0 (10.6)	0.99
Head circumference (cm), *mean (SD)*	54.9 (1.4)	54.5 (1.7)	0.55
Left handedness, *n (%)*	0 (0)	1 (8)	0.52
ADHD, *n (%)*	7 (64)	1 (8)	0.01
Birth weight (g), *mean (SD)*	2956 (520)	3545 (431)	0.01
Reported NAS, *n (%)*	6 (55)	-	-

Abbreviations: ADHD, attention-deficit/hyperactivity disorder; NAS, neonatal abstinence syndrome; SD, standard deviation; *p* = *p*-values for group difference (controls vs. exposed) from independent *t*-test (continuous variables) and Fisher's exact test with mid-p correction (dichotomous variables).

3.3 Measures

3.3.1 MRI data acquisition

MRI protocol: Structural and functional images were acquired without sedation on a GE Signa Excite HD 3.0 Tesla (Milwaukee, WI, USA) MRI scanner at Haukeland University Hospital during the period of January to June 2014. The anatomical examination included a high-resolution, three-dimensional, T1-weighted structural image, collected sagittally using a fast spoiled gradient recovery sequence (Inversion time (TI) = 500 ms; repetition time (TR) = 8 ms; echo time (TE) = out of phase; flip angle 11°; 256 × 256 matrix; field of view (FOV) = 256 mm; slice thickness 1.0 mm, voxel size 1×1×1 mm), and an axial T2-weighted sequence (TE = 100 ms; TR = 3000 ms; slice thickness 0.8 mm). Functional images were collected axially using an Echo Planar Imaging (EPI) sequence with the following parameters: TR = 3000 ms, TE = 30 ms, flip angle 90°, 128 × 128 matrix, FOV = 220 mm, no. of slices 38, slice thickness 3 mm with 0.5 mm skip, voxel size 1.72 × 1.72 × 3.5 mm. Fourteen EPI scans per 8 blocks, arranged in a task - rest - task manner, making a total of 112 scans, were analyzed for each of the four conditions (five initial dummy scans were discarded before data analysis). Total scan time was approximately 45 min.

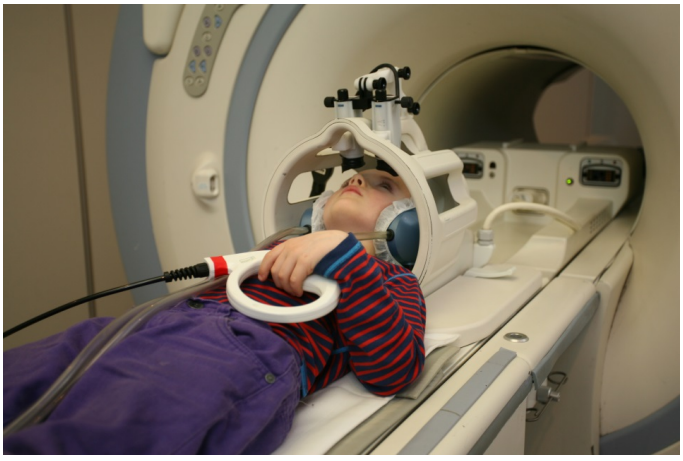


Figure 3 *Scanner environment showing the LCD-goggles and response button used during fMRI (Illustration from pilot scan).*

Participants were provided with ear plugs and headphones, and foam padding was placed around their heads to reduce noise and head movement. LCD-googles were used to present the task during functional imaging and a cartoon during structural scans. During the functional imaging the stimulus sequences were presented via the E-prime software (Psychology Software Tools, Inc.). The participants were instructed to respond to certain target stimuli by pressing a button held in their dominant hand (Figure 3). When the button was pressed, the response time was recorded using the E-prime software.

fMRI task: A combined working memory-selective attention task, previously used in a study of extremely preterm children.[124] The task was based on two well-known neuropsychological tests; the n-back test for working memory [125] and the Stroop color word test for selective attention.[126] The combination of the two tests consisted of visual presentations of different color-words written in conflicting ink color, presented one by one. The words RED, BLUE, GREEN, and YELLOW, each written in the three incongruent colors (e.g. red written in blue, green, or yellow) were presented sequentially through LCD-goggles mounted on the head coil. The words were written in Norwegian, the native language of all participants. The child was asked to respond when either the word or the ink color of the word matched the one presented either one- or two stimuli backwards in the presentation sequence, yielding four different experimental conditions (word 1-back, word 2-back, color 1-back, color 2-back). A schematic illustration of the stimulus set-up is given in Figure 4. The four experimental conditions were presented in a pseudorandom order to avoid any order effects. A block design with alternating ON and OFF blocks was used, with four ON blocks, for which a sequence of 16 stimuli were presented, and four OFF blocks with a blank screen in each of the four experimental conditions. In each ON block tree to five target stimuli were randomly presented. Each stimulus was presented for 2.25 s, followed by a blank interval of 0.3 s. All participants were introduced to the procedure through a short computer program test sampling all four experimental conditions, and effort was made to be sure the instructions were comprehended before entering the scanner.

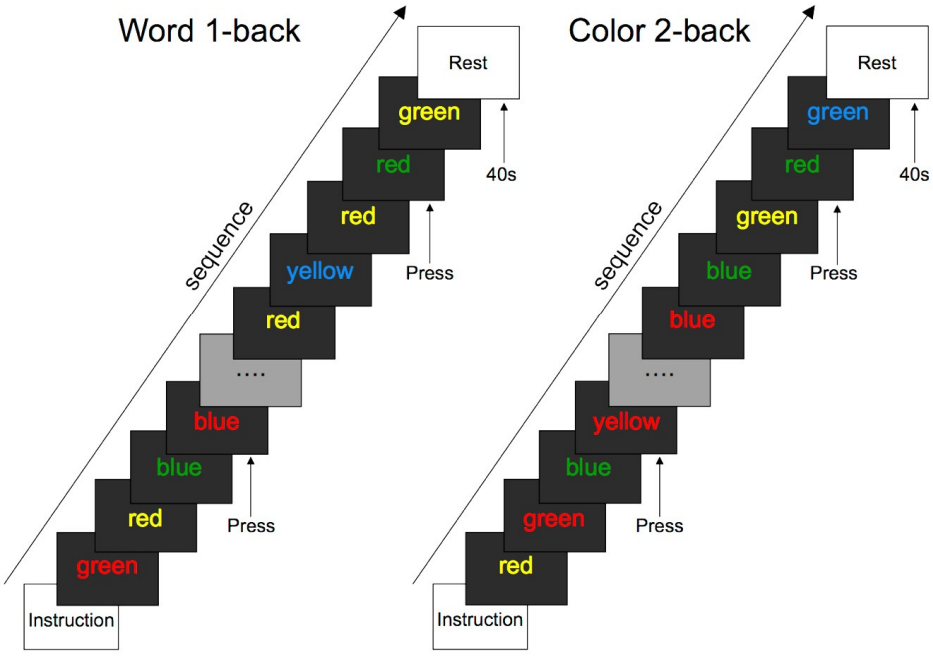


Figure 4 *Schematic illustration of the fMRI task presentation: In the word 1-back task the participants were instructed to press the response button when the written word presented was the same as the word presented one screen back in the presentation sequence. In the color 2-back task, the response was based on the ink color of the word being the same as the one presented two screens back in the presentation sequence (modified from Griffiths et al.[127]).*

3.3.2 Paper I: Visual evaluation of cerebral MRI scans

The scans were evaluated by an experienced pediatric neuroradiologist who was blinded to the background of the participants. After exclusion of examinations with pronounced artifacts (movement and/or dental braces), pathology was recorded in terms of I) reduced volume of the cerebellum, II) reduced thickness of the corpus callosum, III) pathology in the basal ganglia, IV) presence and extent of dilation of the lateral ventricles and V) presence of focal white matter pathology. Each parameter was scored as being either normal or as displaying mild or moderate/severe pathology. The assessment of each of these parameters was subjective. In addition to these five MRI parameters, other pathology was recorded, but not graded. Further, an

additional variable, termed *any pathology*, was interpreted as positive if the images showed MRI pathology in any of the five previously mentioned parameters.

3.3.3 Paper II: Volumetric measures from T1-weighted scans

Examinations with pronounced artifacts due to movement and/or dental braces were excluded. A quality score was given to all included scans, based on the grade of movement artifacts, by an experienced radiologist blinded to the participants' background (1 = no motion; 2 = very little; 3 = some; 4 = marked). There was no difference in this quality score between the two matched groups (Wilcoxon signed rank test: $Z = -1.70$; $p = 0.12$). The three-dimensional volumes were corrected for scanner gradient field non-linearities to reduce variance that could be caused by varying head placement within the gradient field among participants.[128] Brain volume measures were obtained from the automated processing pipeline of FreeSurfer version 5.3 (http://surfer.nmr.mgh.harvard.edu/). This automated processing includes whole brain segmentation with automated labelling of neuroanatomical structures.[129] Total intracranial volume (ICV) was estimated according to the method described by Buckner et al.[130] The quality of the subcortical segmentations was evaluated by use of a semi-automated approach (http://enigma.ini.usc.edu/). In addition, for volumes with the highest quality score for movement artifacts, careful visual inspection of the segmentations was performed. None of the inspected volumes were excluded from further analysis due to segmentation error.

3.3.4 Paper III: Image processing (fMRI)

Image processing and data analysis were performed using the SPM12 software package revision 6470 (Welcome Trust Center for Neuroimaging, London, UK) and Matlab version 9.0 (MathWorks Inc., Natick, MA). Default preprocessing routines, as implemented in SPM12, were followed. To adjust for variations caused by head movement EPI-scans in each of the four experimental conditions were realigned. Participants with head movement >5mm (translation) were excluded from further analyses. Co-registration of the T1-weighted structural scan to the mean EPI-scan in each of the four experimental conditions was performed, with subsequent

segmentation of the structural scan, providing normalization parameters used to normalize the EPI-scans to Montreal Neurological Institute (MNI) space (resized voxels 3 × 3 × 3 mm). Finally, the EPI-scans were smoothed with a Gaussian kernel of 8 mm. Visual inspection of all EPI-scans was performed to assure quality. Two participants were excluded in case of signal drop out due to dental braces. Individual participant first-level fixed effect analyses were performed on the ON-OFF block contrasts for the four experimental conditions, creating four contrast images per person. These images were subjected to second-level random effect analyses using the general linear model, as implemented in SPM12.

3.3.5 Additional measures

Somatic growth parameters (height, weight, and head circumference) were obtained prior to MRI scanning. Background and clinical characteristics were obtained from medical records and/or questionnaires filled in by parents or foster parents.

3.4 Statistics

Descriptive statistics were reported using the mean and standard deviations (SD) as well as counts and percentages. For demographic and clinical variables, differences between the 1:1 matched groups (paper I and II) were tested with the paired *t*-test or McNemar's test, as appropriate. Otherwise, the independent *t*-test (continuous variables) or Fisher's exact test with mid-p correction[131] (dichotomous variables) were used. All significance tests were two-sided, and a significance level of 5% was set. Statistical analyses were performed using IBM SPSS Statistics version 23 and Stata version 14.0 (Stata Corp. College Station, TX), except for the analyses of BOLD fMRI data that were performed using SPM12.

3.4.1 Paper I

For group comparisons of MRI findings, any degree of pathology in each of the five MRI parameters (reduced volume of the cerebellum, thinning of the corpus callosum, pathology in the basal ganglia, dilatation of the lateral ventricles, and presence of

focal white matter pathology) and the variable termed *any pathology* were considered categorical variables. Logistic regression of pathology (not normal/normal) on drug exposure/control using generalized estimating equations to adjust for the matching was performed. Risk estimations were expressed as odds ratios (OR) with 95% confidence intervals (CI).

3.4.2 Paper II

Group differences in volumetric brain measures were tested using a linear mixed model to take into account the dependency of observations from matched pairs.[132] Brain volumes were entered as dependent variables, with random effect of matching. Firstly, a hypothesis-driven analysis was performed with the volume of the basal ganglia as the dependent variable. Secondly, explorative analyses were performed with the additional brain volumes from the automated segmentation as dependent variables, using the same model. Estimated ICV and birth weight and were entered as covariates in all analyses (with the exception of the analysis of differences in ICV that was only adjusted for birth weight). Finally, analyses were repeated and adjusted for the presence of ADHD. Since there were no hypotheses of differential effects in the two cerebral hemispheres, the sum of the left and right volumes was used for paired structures.

3.4.3 Paper III

fMRI task performance: For each target stimuli time to correct response (0–2.25 s) was recorded. To allow for both response accuracy and reaction time to be modeled simultaneously, time to correct task response was analyzed using Cox proportional hazards modeling. If there was not a correct answer, the time to response was considered to be censored as opposed to uncensored when the correct answer was obtained. As each child responded to multiple target stimuli, a frailty term for child was included.[133] Altogether 1430 observations (65 targets × 22 children) were included in these analyses. Results were reported using the hazard ratio (HR) with 95 % CI. A HR > 1 is interpretable as a greater instant probability of a correct answer. The model was used to assess possible group differences in task performance. Other

variables possibly influencing task performance were difficulty level (4 different experimental conditions) and birth weight. All children performed the same tasks, so by the design experimental condition was independent of exposure group and was not adjusted for in the models. Birth weight may be a mediator of the possible opioid effect on task performance and analyses were done without and with birth weight as an additional covariate to study any mediating effect. Finally, interactions between group and respectively birth weight and difficulty level were tested.

fMRI BOLD activation: Within-group activation patterns for the opioid-exposed and control groups were modeled using one-sample *t*-tests, and two-sample independent *t*-tests were used to determine between-group differences. To account for multiple comparisons a cluster-extent, random field theory based family wise error (FWE) corrected threshold at $p < 0.05$ was used to define significant activations in all analyses, with a primary cluster-defining threshold at $p < 0.001$. Anatomical location of significantly activated clusters was identified using Anatomical Automatic Labeling.[134]

3.5 Ethical considerations

The project was approved by the Regional Ethics Committee for Medical Research in Western Norway (REK-Vest 2010/3301). Written consent was obtained from parents or foster parents and Child Welfare Services, as appropriate, for all participants. Written consent was also obtained from all children above the age of 12 years, and verbal consent from participants younger than 12 years.

4. Results

4.1 Paper I

Cerebral MRI scans of acceptable quality for analyses were obtained in 34/43 (79%) drug-exposed children and 40/43 (93%) unexposed controls. With few exceptions (among children of mothers in OMT and children with FASD) exposure to more than one drug was reported in the exposed group. Most of the drug-exposed children (65%) were exposed to opioids and various illicit drugs and were categorized as opioid- and polysubstance exposed. Twelve children (35%) with reports of heavy maternal alcohol use during pregnancy fulfilled the criteria for FASD and were categorized as alcohol-exposed.

Expert visual analysis of MRI scans revealed similar frequencies of pathology in all groups (Figure 5). Overall pathological findings categorized as *any pathology* were recorded in 35% of the drug-exposed children versus 33% of the controls (OR: 1.08; 95% CI: 0.36, 3.25). There were no statistically significant differences in the risk estimates of pathology in drug-exposed children compared to controls. No pathology was identified in the basal ganglia. Off note, reduced cerebellar volume was recorded in 25% of the alcohol-exposed children versus 3% of the controls. However, subgroups based on major drug exposure were too small for meaningful statistical analysis of group differences. All the pathological brain findings were categorized as mild, except for moderate/severe thinning of corpus callosum and ventricular dilatation reported in one drug-exposed child. The alcohol-exposed group had lower head circumference compared to controls (mean difference: 2.18 cm; 95% CI: 0.84, 3.51; $p = 0.004$). Otherwise there were no statistically significant group differences in the somatic growth parameters.

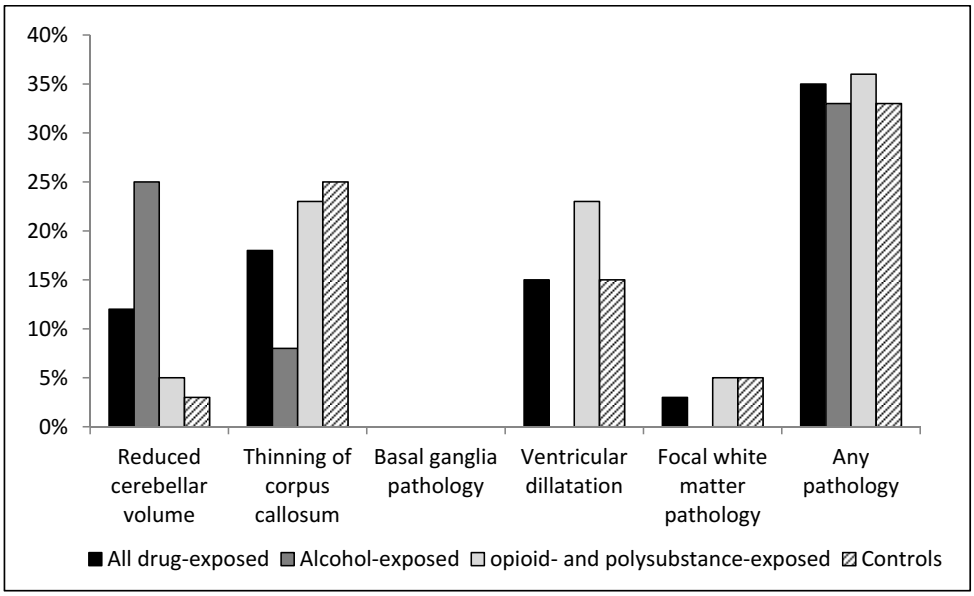


Figure 5 *Structural MRI findings in 34 prenatally drug-exposed children and 40 sex- and age-matched controls (expert visual analysis).*

4.2 Paper II

The study population of paper II included seven children born to mothers undergoing OMT and nine children born to mothers with a history of heroin use during pregnancy. Most of these children (69%) were also exposed to one or more non-opioid drugs. Exposure to benzodiazepines was reported in (50%), cannabis in (25%), amphetamines in (25%), and alcohol in (6%) opioid-exposed children. There was a high prevalence of ADHD in the exposed group (69%), while only one child (6%) in the control group was diagnosed with ADHD. All children in the exposed group either lived in foster care or were adopted.

The combined volume of the basal ganglia (accumbens + caudate + putamen + pallidum) was reduced in the opioid-exposed group. There were no statistically significant group differences in global brain measures (total brain, cortical gray matter and cerebral white matter). The analyses adjusted for ICV and birth weight revealed 6.5% smaller basal ganglia (difference of 1.60 ml; 95% CI: 0.20, 3.01 ml; p = 0.027), 9.2% smaller caudate (difference of 0.75 ml; 95% CI: 0.03, 1.46 ml; p =

0.042), 7.6% smaller thalamus (difference of 1.21 ml; 95% CI: 0.10, 2.32 ml; p = 0.035), and 10.3% smaller cerebellar white matter (difference of 3.00 ml; 95% CI: 0.57, 5.44 ml; p = 0.018) in opioid-exposed children compared to their matched controls. The estimated group differences were mainly unchanged when analyses were adjusted for ADHD, in addition to ICV and birth weight adjustments.

4.3 Paper III

In the opioid-exposed group included in the final study population of paper III there were four children born to mothers undergoing OMT and seven children born to mothers using heroin during pregnancy. Additional exposure to non-opioid drugs (benzodiazepines, cannabis, and amphetamines) was reported in 8/11 opioid-exposed children. The prevalence of ADHD was 64% in the exposed group versus 8% in the control group. Like in paper II all opioid-exposed children included were adopted or lived in foster care.

Task performance: The opioid-exposed group showed impaired task performance compared to controls, with an unadjusted HR of controls vs. exposed = 1.46 (95 % CI: 1.04 to 2.06; p = 0.030). However, this group difference was no longer significant when the model was adjusted for birth weight. As expected there were significant differences in task performance between the four difficulty levels, with slower responses and fewer correct answers in the more cognitive demanding 2-back tasks (p < 0.001). There were no significant interactions between group and respectively difficulty level or birth weight.

fMRI activation patterns: Analyses of BOLD activation patterns revealed activated clusters including prefrontal and parietal cortical areas in both opioid-exposed and control children across the four experimental conditions. In the most cognitive demanding conditions (color 2-back and word 2-back tasks) more widespread, diffuse activations were found in the exposed group (Figure 6). The between-group analyses showed increased activation in prefrontal cortical areas in the exposed group as compared to the unexposed control group in both 2-back tasks (Figure 7). One significant cluster in the left prefrontal cortex including left precentral gyrus and superior and middle frontal gyrus showed increased activation in the word 2-back task, whereas increased prefrontal activation in left and right middle frontal gyrus were found in the color 2-back condition.

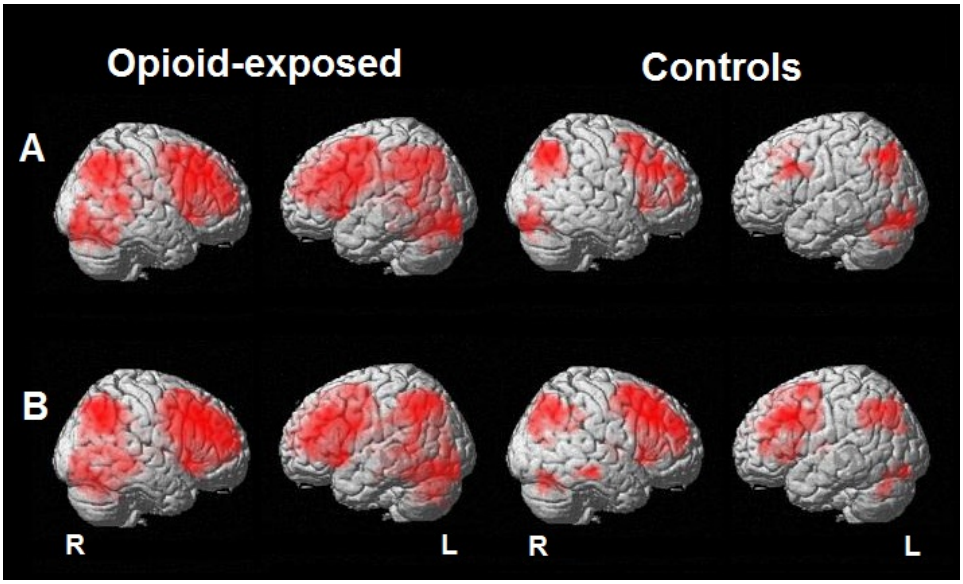


Figure 6 *Within-group analyses in opioid-exposed children (n=11) and controls (n=12) A = word 2-back; B = color 2-back. Clusters of activation that survived corrections for multiple comparisons with a cluster-extent based threshold at family wise error (FEW) corrected p < 0.05 are shown overlaid on a single subject Montreal Neurological Institute template. Abbreviations: L, left; R, right.*

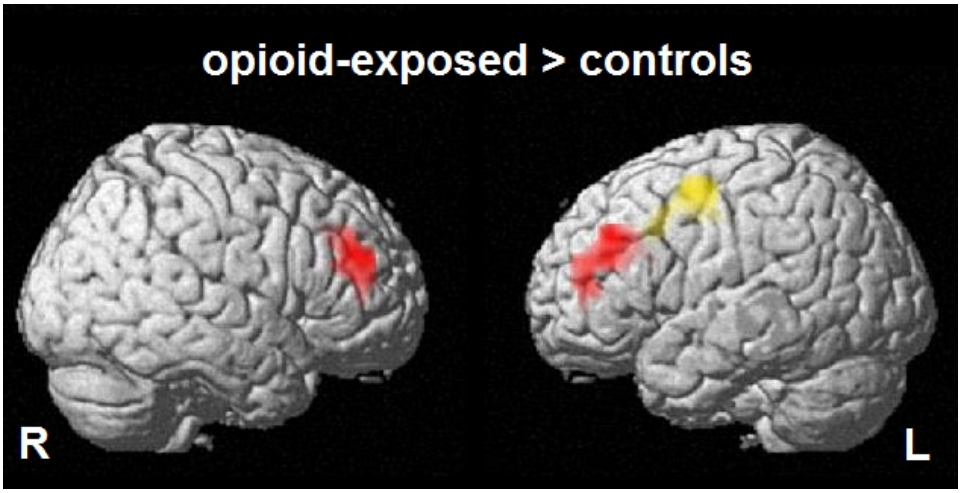


Figure 7 *Areas with increased activation in the opioid-exposed group. Surface rendering of activated clusters from between-group analyses that survived corrections for multiple comparisons with a cluster-extent based threshold at family wise error (FEW) corrected p < 0.05 on a single subject Montreal Neurological Institute template. Yellow = word 2-back; Red = color 2-back. Abbreviations: L, left; R, right.*

5. Discussion

Brain alterations associated with prenatal drug exposure were not seen by the means of simple visual analysis of cerebral MRI scans form a hospital-based population of school-aged children with prenatal exposure to various licit and illicit drugs and sex- and age-matched unexposed controls. In children prenatally exposed to opioids morphometric analyses revealed volume reductions of the basal ganglia and several other regional brain measures compared to their matched controls. Impaired task performance and increased BOLD activation in prefrontal cortical areas was found in children with prenatal opioid exposure during a combined working memory-selective attention task as compared with unexposed controls.

5.1 Metodological considerations

In the following sections strengths and limitations of the study will be discussed to assess the generalizability and the validity of the results.

5.1.1 Study design

An observational study design is an important limitation when it comes to the interpretation of the results, as it precludes firm conclusions about causality. The associations between prenatal drug exposures and later brain changes as seen by structural and functional MRI of our study population are not necessarily causal. Possible effect of prenatal drug exposure on brain structure and function cannot be distinguished from those of several known and unknown factors differing between the exposed and control groups, a common challenge in all observational studies of drug-exposed children.[135] Furthermore, cross-sectional MRI data cannot provide exact information on the developmental course of the brain alterations seen, and whether these findings represent permanent changes or alterations of normal growth trajectories.

5.1.2 Study population

The inclusion of a hospital-based population of children with prenatal drug exposure in the present study has implications for the generalizability of the results, as it could have introduced a selection bias. One would expect behavioral and/or attention problems to be more frequent in our hospital-based population than in the general population of prenatally drug-exposed children, as many of the children included in the study were referred to the hospital-based on these problems in addition to a medical history of prenatal drug exposure. If behavioral and/or attention problems are related to structural and/ or functional brain changes due to prenatal drug exposures, one would expect such brain changes to be more frequent or pronounced in a hospital-based population. On the other hand, we do not know for certain if some of the most impaired children eligible for the study refused to participate, as we have no information regarding neuropsychological function or type of drug exposure for eight out of the 27 nonparticipants in the study. Furthermore, behavioral and/or attention problems could be related to brain alterations that are not affected by prenatal drug exposure. In this case brain alterations, as seen by MRI in our study, could all be explained by the fact that we compare a group of children with a high prevalence of ADHD and related problems with a healthy low-risk group. In this case ADHD could be a confounder, as discussed in 5.1.5.

The results from paper I could probably be extended to other hospital-based populations of prenatally drug-exposed children where polysubstance exposure and unspecified drug exposure is a common feature. However, extending our results from the opioid-exposed groups in paper II and III to other groups of children with prenatal opioid exposure is not without issues, as selection bias could have influenced the positive associations between opioid-exposure and aberrant brain structure and function that were found.

The recruitment of our unexposed control group, based on date of birth, is a strength of the study, compared to other methods of recruitment, like advertisements or use of classmates which could have also introduced selection bias. The use of 1:1 sex- and

age-matched controls is an important strength, in particular in paper II, since changes in volumetric brain measures show heterogeneous sex- and age-related trajectories, including curvilinear trajectories, which make these factors difficult to control in statistical modeling.[136,137]

5.1.3 "Mapping" the risk factor: Prenatal drug exposure

Prenatal drug exposure was assessed by history without toxicology testing. Information was based on drug history from the mother or medical records (obstetric or pediatric) for the exposed groups and questionnaires filled in by the mothers for the controls. This could have introduced recall bias and rater bias with potential underreporting, or misclassification. Although reports on maternal drug use during pregnancy could be biased, we find it unlikely that any child included in the study was misclassified as prenatally drug-exposed. In paper II and III children were only included if prenatal opioid exposure, from maternal use of heroin or from opioids given as part of OMT, could be confirmed by information form their mother or in medical records. We find it unlikely that any of these children were misclassified as opioid-exposed, as opioids were the main "drug of choice" for all the mothers of these children. We also find it unlikely that any child in the control group was misclassified as unexposed, as all mothers in this group refused any use of medication or substance abuse during pregnancy. However, detailed information about type, frequency or amount of all drugs used during pregnancy, was not readily available for all participants in the exposed group. In addition, our study lacked reliable information about prenatal smoking. Thus, information regarding additional exposure to non-opioid drugs in the opioid-exposed groups must be considered uncertain. This is an important limitation of our study, as this uncertain degree of exposure to non-opioid drugs restricted our possibility to control potential confounding effects on the associations between prenatal opioids and brain alterations in our statistical modeling (paper II and III), as discussed in 5.1.5.

5.1.4 MRI measures

In the following section the different MRI-based outcome measures will be discussed, focusing on the choice of measures, limitations and reliability.

Paper I: To assess cerebral MRI in a clinical context we used expert visual evaluation of the MRI scans. Although more advanced methods could have revealed more detailed information, simple visual analysis of MRI scans still represents the common method used in everyday clinical practice. The evaluation was done by a very experienced pediatric neuroradiologist. The fact that this rater was blinded to the participants' background regarding prenatal drug exposure is an important strength, reducing possible rater bias. Only one expert evaluating the scans may be considered a weakness, as substantial inter-rater differences have been reported in studies using visual analysis of cerebral MRI scans.[138] Differences in the observers' choice of threshold between normal and abnormal may explain most of this inter-rater variability, in studies with more than one observer. As long as this threshold between normal and abnormal is equal in all the groups under study, group comparisons, like the present study, will not be influenced by such inter-rater disagreements. However, our ability to compare frequencies of cerebral changes detected in the drug-exposed group in paper I to similar changes reported in other populations is limited. Our finding of MRI pathology in more than 30% of the drug-exposed and unexposed groups, could also call into question the appropriateness of the term "MRI pathology" used to describe these findings.

Paper II: To investigate cerebral volumetric characteristics, volumetric measures were obtained from T1-weighted MRI scans using FreeSurfer. Volumetric measures from FreeSurfer have been validated against manual segmentation[129] and the method has been widely used in previous pediatric studies.[114,139,140] Head motion during MRI acquisition can affect the automated volumetric estimates.[141] It is however, unlikely that head motion artifacts could explain the observed group differences in volumetric brain measures, as there was no significant difference between exposed and unexposed children in the quality score given to the MRI scans based on the grade of

movement artifacts. To avoid introducing rater bias, no corrections were performed by manual editing after the semi-automated quality control of the segmentations.

Paper III: Prenatal opioids have been consistently associated with high rates of attentional problems and ADHD in exposed children,[59,60,66-68,78] also in studies trying to account for the impact of genetic vulnerabilities and postnatal environmental influences.[59,66] Executive dysfunction is regarded a key factor in the complex neuropsychology of ADHD,[142] and impaired executive functions have been demonstrated in children with prenatal opioid exposure.[77] Working memory and selective attention are executive functions crucial for normal cognitive function, and most likely implicated in the neurodevelopmental impairments reported in prenatally opioid-exposed children. On this basis, an executive function task combining working memory and selective attention was chosen.

To investigate possible group differences in brain activation patterns related to working memory and selective attention we compared the BOLD task-rest contrasts across groups. An important limitation that applies to all BOLD fMRI studies is the fact that the BOLD contrast is an indirect measure of neuronal activation. Regional changes in cerebral blood flow detected as a change in the MRI signal by means of changes in blood oxygenation are used as a proxy for measuring the activity of neurons.[93] To which extent the differences in the BOLD signal between our study groups represents actual differences in neuronal activation as compared to other possible underlying mechanisms, like altered vascularization, therefore remains unknown.

The reliability of BOLD fMRI data, particularly in children, is an important issue for this thesis. In general, BOLD activation maps have been shown to be fundamentally the same in normal children older than 8 years and adults.[143] Image processing and analysis using the SPM12 software package (or earlier versions) has been widely used in previous pediatric samples.[127,144-146] Typical whole brain fMRI analyses using voxel-based methods, like the present study, include more than 50.000 voxels, resulting in numerous statistical tests. Consequently, corrections for multiple

comparisons are important in all statistical analyses of fMRI data. Cluster-extent based thresholding was used in the present study.[147] An important advantages of this method include increased sensitivity to group differences in small samples, as compared to more stringent methods, like voxel-level corrections using the Bonferroni correction.[147] The effectiveness of cluster-extent based thresholding to correct for multiple comparisons in fMRI studies has recently been called into question,[148] but the problem of inflated false positive rates, was mainly shown for more liberal primary cluster defining thresholds than the one used in our study ($p <$ 0.001). Using a more conservative method, like voxel-wise corrections, would decrease the risk of Type I errors (i.e. false alarms). However, at the same time the risk for Type II errors (i.e. missing true effects) would increase. The small sample and the explorative nature of the present study could justify a focus on the avoidance of Type II errors, as advocated by Lieberman and Cunningham in a highly cited paper.[149] The use of a cluster-extent based threshold to correct for multiple comparisons also precludes inferences about specific anatomical regions within significant clusters to be made with confidence.[147] If an activated cluster is large, one can only infer that there is signal somewhere within the cluster. Consequently, detailed discussion and comparisons of the anatomical localization of BOLD activations could not be performed based on our analyses.

Task related BOLD activation patterns in our sample included parietal and prefrontal cortical areas. This finding supports the reliability of our results, as activation in these areas has been a common finding in fMRI studies of working memory and selective attention.[150-152]

5.1.5 Confounding

An extraneous variable that correlates both with the independent variable (i.e. prenatal opioid exposure), and the dependent variable (i.e. MRI-based outcome measure) under investigation could be a confounder. If the statistical modeling does not account for the confounder, a spurious association between the dependent variable and the independent variable will arise. In the present study, several possible

confounders in the relationship between prenatal opioids and brain measures obtained by MRI in paper II and III should be considered. The ability to account for several of these confounders in our statistical modeling was restricted by the small sample sizes and uncomplete or non-existing measures for some factors. Important possible confounders are discussed in the following section.

Genetic factors: Twin studies have shown high heritability for subcortical brain measures,[153,154] and brain activation as seen by fMRI has been shown to be under substantial genetic control.[155] However, specific genetic variants influencing these brain measures are largely unknown.[155,156] Our study had no measure suitable to control the potential influence of genetic vulnerabilities on volumetric or functional brain measures.

Socio economic disparities: There is growing evidence from neuroimaging studies that early adverse experiences shape the developing brain.[157] Structural and functional brain alterations associated with increased risk for cognitive and emotional maladjustments have been reported in children reared in maltreating family environments.[157] Low SES has been associated with increased BOLD response in prefrontal cortical areas in the context of poorer performance on an executive function task.[158] Family income and parental education have been linked to differences in brain structure,[159] and reduced volumes of the striatum have been associated with childhood maltreatment.[160] However, structural alterations in the amygdala and hippocampus have been most widely studied in children form low SES backgrounds, while alterations in basal ganglia regions have not typically been observed.[157,161] In the present study no specific measurement of SES was obtained. All the opioid-exposed children included in paper II and III lived in stable family situations (either in foster care or adopted). Nonetheless, social and environmental differences between our study groups could have influenced structural and/or functional brain measures.

ADHD: Two thirds of the opioid-exposed children included in paper II and III were diagnosed with ADHD. The question as to whether ADHD could be a confounder to

the observed associations between prenatal opioids and aberrant brain structure and function is therefore crucial for the interpretation of the results. However, the answer to this question is not straight forward. If associations between prenatal opioids and structural and functional brain alterations as seen in paper II and III are causal, these brain alterations could be mediators of possible opioid effects on the occurrence of ADHD. This would imply that adjusting our analyses for ADHD could potentially mask these causal effects of opioids leading to reduced brain volumes or altered brain function. On the other hand, both inherited and non-inherited factors are shown to contribute to ADHD, and no single risk factor is neither necessary nor sufficient to explain the disorder.[162,163] Reduced brain volumes have been repeatedly reported in children with ADHD [94,164,165] however, these brain alterations are most likely unrelated to prenatal drug exposure in most cases. The estimated group differences in brain volumes found in paper II were mainly unchanged after adjustment for ADHD in addition to ICV and birth weight adjustments. Decreased BOLD activation in prefrontal cortical areas has been a consistent finding in numerous fMRI studies on children with ADHD across several different cognitive tasks.[95,166] Contrary to this, we found increased prefrontal activation in our opioid-exposed group. No adjustments for ADHD were made in paper III, and the sample was too small for meaningful statistical analysis of subgroups.

Exposure to non-opioid drugs: Due to the uncertain degree of exposure to non-opioid drugs and the small sample, convincing statistical adjustments for possible confounding effects could not be performed. Possible influence of non-opioid drugs on the outcome measures in paper II and III could therefore not be quantified or ruled out. However, no children with FASD or known prenatal exposure to heavy maternal alcohol use were included in the studies of opioid-exposed children. The lack of data on prenatal smoking is a limitation to our study. However, we find it unlikely that prenatal smoking could explain reduced volumes of the basal ganglia in paper II, as no volume reductions of deep gray matter structures were found in a recent prospective study including 113 children prenatally exposed to tobacco.[114] Very few studies have examined possible effects of prenatal smoking on brain activation

patterns. In a study of young adults with prenatal nicotine exposure greater activity in several brain regions, including the middle frontal gyrus were found compared to non-exposed controls, despite similar performance on a working memory task.[167] However, contrary to our results in opioid-exposed children, Bennet et al. found reduced prefrontal BOLD response in a sample of 7 tobacco-exposed 12-year-olds during working memory.[146]

Birth weight: The unexposed controls were only included in the study if they had a normal birth weight (above the 10th percentile). In accordance with previous studies we found significantly lower birth weights in the opioid-exposed groups compared to controls in paper II and III, as preterm birth and low birth weight have been associated with prenatal opioids in several studies.[6,28,29] However, it is difficult to know whether to conceptualize birth weight as a confounder or as a mediator. If prenatal opioids have a direct, causal effect on birth weight, low birth weight could be a mediator of possible opioid effects on the outcome variables in paper II and III. Reports of lower birth weight associated with higher dose of methadone or buprenorphine at delivery in opioid maintained pregnant women could support this.[29,168,169] There is also evidence from animal research to support a causal effect of opioids on fetal growth.[170] However, there are myriads of associated risk factors, like poor maternal nutrition, maternal infections, and co-exposure to non-opioid drugs, and it is still unclear if opioids have a direct, causal effect on human fetal growth.[171] Reduced brain volumes have been found in preterm and low birth weight children.[172] Influence of birth weight on volumetric brain measures has even been shown within the normal birth weight range in healthy term-born children.[139] In paper II birth weight was included as a covariate in all analyses of brain volumes. The group differences shown in these analyses therefore indicate effect of opioids on brain volumes beyond the possible effects mediated by low birth weight. However, it is likely that the birth weight adjustment actually removed some of the possible effect of opioids on brain volumes. We did not attempt to adjust the between-group analyses of BOLD fMRI data for birth weight. We find it unlikely that the selection of a low birth weight group should explain the increased prefrontal activation seen in our opioid-exposed group, as decreased BOLD activation has been a consistent finding in

fMRI studies of preterm and low birth weight groups, including one study using the same fMRI paradigm as the one used in the current study.[127] Birth weight has also been associated with neuropsychological functioning, including executive functions.[173] The group difference in task performance found in paper III was no longer significant after birth weight adjustment. Birth weight as a mediator of the possible effect of opioid exposure on task performance could explain this finding.

5.1.6 Statistical power

Pre-study power calculations were not performed. Overall, a small sample size may have reduced our power to detect significant group differences, in particular in paper II and III. However, the number of prenatally opioid-exposed children included in the analyses of structural MRI in paper II (n = 16) was comparable to number of opioid-exposed children included in previous studies, reporting significant differences between opioid-exposed and unexposed groups (n = 10–16).[117,120] The number of prenatally opioid-exposed children included in analyses of BOLD response in paper III (n =11) was also similar to numbers of exposed children included in previous fMRI studies of children with prenatal exposure to alcohol, amphetamines, and tobacco (n = 7–23) reporting group differences in BOLD response during working memory.[145,146,174,175]

5.2 Discussion of the results

5.2.1 Paper I

Gross structural brain abnormalities after prenatal drug exposure: The frequency of cerebral MRI pathology, as seen by expert visual analysis of the MRI scans, did not differ between the prenatally drug-exposed children and the unexposed controls. No brain changes specific to prenatal drug exposure was revealed by the means of simple visual analysis of cerebral MRI scans as the exposed children and their controls shared the same MRI findings. Comparing these results to previous studies is challenging, as most studies focus on specific drug effects, most often using more advanced quantitative methods, like computerized volumetric analyses.[105,106]

Although subgroups based on type of drug exposure were too small for meaningful statistical testing of potential group differences in paper I, the drug-exposed group was split into alcohol-exposed and opioid- and polysubstance exposed. Similar frequencies of MRI pathology were found in all groups. The literature is diverging regarding visual analysis of cerebral MRI scans of children with prenatal alcohol exposure. Gross structural abnormalities in up to 100% alcohol-exposed children have been reported,[101] while other studies report such anomalies in less than 10%.[176,177] Our findings could support brain changes in alcohol-exposed children being mostly subtle and not detectable on simple visual analysis of MRI scans. The cerebellum seems to be among brain structures especially vulnerable to the adverse effects of prenatal alcohol, with several studies reporting atrophy or hypoplasia.[99,100] Reduced volume of the cerebellum in 25% alcohol-exposed children in our study could support this. Although several gross structural brain alterations have been reported in children prenatally exposed to drugs other than alcohol,[106,123] most studies on prenatal opioids, the most common drug in our sample, have reported no gross brain anomalies.[117,119] Overall, the results from our expert visual analyses of cerebral MRI scans suggest that most brain changes associated with prenatal drug exposure in the growing body of imaging literature seem to be limited to subtle changes. However, subtle structural brain alterations may have important functional consequences.

Feasibility of MRI examinations in prenatally drug-exposed populations: In a hospital-based sample of children with heterogeneous drug exposure an increased risk of a wide spectrum of cognitive and behavioral problems ranging from severe mental retardation to attention problems and hyperactivity could be expected.[18] These factors might influence the feasibility of performing a diagnostic MRI, without the use of sedation or general anesthesia. Cerebral MRI scans of acceptable quality for analyses were obtained in 34/43 (79%) of the drug-exposed children included in our study. In comparable studies, MRI scans were successfully obtained in 75 – 88% prenatally drug-exposed children of the same age range.[116,117,178] However, comparing rates of scanning success is difficult, due to a wide range of different imaging protocols and differences in inclusion- and exclusion criteria among studies of drug-exposed

children. Overall, cerebral MRI seems to be feasible in most children with prenatal drug exposure at the age of 10-14 years, despite their wide range of cognitive and behavioral problems.

5.2.2 Paper II

Volumetric characteristics of the prenatally opioid-exposed brain: Analyses adjusted for ICV and birth weight revealed 6.5% smaller basal ganglia in prenatally opioid-exposed children compared to their matched controls. Further analyses also showed reduced thalamus and cerebellar white matter in the exposed group.

Largely, these results support previous neuroimaging studies on prenatally opioid-exposed populations. Only two previous studies examined specifically the association between prenatal opioid exposure and volumetric brain measures in children,[117,120] both of which reported reduced basal ganglia volumes. Among the individual basal ganglia nuclei, only the difference in caudate volume reached statistical significance in our analyses. In the study by Walhovd et al. volume reduction in various brain measures, including total brain volume were reported in the opioid- and polysubstance exposed group.[117] Restricting their analyses to the subgroup exposed to opioids, only pallidum, putamen and lateral ventricles remained significantly reduced after adjustment for ICV.[117] In the pilot study by Yuan et.al both basal ganglia volumes and total brain volumes were reduced in the group of neonates exposed to gestational opioids.[120] No measurements of individual basal ganglia nuclei were obtained in that study. Unequal influences of possible confounders, including exposure to various non-opioid drugs, could possibly explain some of the discrepancies between the results in previous studies and our findings. The use of a sex- and age-matched control group in our study should be regarded an improvement in methodology, compared to previous studies. The finding of reduced basal ganglia volumes in the exposed group was based on our primary hypothesis. Further analyses must be regarded explorative, and differences found in these secondary analyses would not survive statistical corrections for multiple comparisons. Consequently, the finding of reduced volume of thalamus and cerebellar white matter should be

interpreted with caution. However, these findings suggest more widespread, albeit subtle effects of prenatal opioids on brain volumes, in line with the results form Walhovd et al.[117]

Several possible interpretations of reduced regional brain volumes in the opioid-exposed group in paper II are plausible. As already discussed, the observed effect of prenatal opioids on regional brain volumes could be epiphenomena of prenatal opioid exposure, explained by confounding factors and selection bias. However, detrimental effects of opioids on the developing fetal brain could not be ruled out as an explanation of reduced regional brain volumes in the exposed group. An abundance of data from animal and cell culture studies demonstrating adverse effects of opioids on brain development could support this.[48,179] Possible mechanisms underlying a reduction in neuroanatomical volumes after prenatal opioid exposure include increased apoptosis of neurons and glia cells,[180] decreased dendrite length and branching,[181] altered cell migration,[182] reduced neurogenesis,[183] and alterations of growth factors.[184] It has been demonstrated that opioids readily cross the human placenta to enter the fetal bloodstream.[185,186] Both opioid receptors and opioid ligands are expressed in the fetal brain, and there is growing evidence for the endogenous opioid system as a regulator of neurogenesis, with inhibitory effects of opioids.[187] Thus, interference with this system by maternal opioid use could alter the normal maturation process of the developing brain.

The functional consequences of reduced regional brain volumes in the opioid-exposed group in paper II could not be addressed by the present study. However, the caudate, thalamus and cerebellum are all crucial parts of the neurobiological circuits involved in regulation of attention.[188] Subtle attentional deficits mediated by reduced caudate volumes have been suggested in an MRI study of prenatally methamphetamine-exposed children.[108] It is thus tempting to speculate that changes in the basal ganglia as a consequence of prenatal opioid exposure could contribute to attention deficits in children exposed to opioids in utero. However, Walhovd et al. did not find any correlation between basal ganglia volumes and attention problems as

assessed by the Child Behavior Checklist in their sample of opioid- and polysubstance exposed children.[117]

5.2.3 Paper III

Working memory, selective attention and BOLD activation after prenatal opioid exposure: In the two most cognitive demanding versions of the working memory-selective attention task, increased BOLD activation in prefrontal cortical areas was found in the opioid-exposed group as compared to controls. The exposed group showed impaired task performance, although these group differences lost significance when analyses were adjusted for birth weight.

To our knowledge, there are no previous fMRI studies examining possible associations between prenatal opioid exposure and brain activation patterns. However, similar to our finding in the opioid-exposed group, increased prefrontal BOLD activation during working memory tasks has been reported in several fMRI studies of children with histories of heavy prenatal alcohol exposure.[145,175,189,190] In these studies varying degrees of behavioral differences between alcohol-exposed children and unexposed controls have been found. Less efficient task related networks or compensation for other less active regions have been suggested as an explanation to increased activation in alcohol-exposed children.[175,190] Similar compensatory mechanisms could possibly explain our finding of increased prefrontal activation in the opioid-exposed group. On the other hand, several studies have reported working memory deficits with corresponding lower brain activation including prefrontal areas in groups of children with prenatal exposure to alcohol and methamphetamine.[109,191] In a group of adolescents with prenatal exposure to cocaine and/or heroin subtle attentional challenges related to a reduced BOLD activity in frontal and cerebellar regions were found.[192] Despite the fact that most of the opioid-exposed children included in paper III were diagnosed with ADHD (64%), increased prefrontal BOLD activation was found, in contrast to the prefrontal hypoactivity that has consistently been reported in children with ADHD.[95,166] It is tempting to speculate that these differences could reflect different neural correlates of ADHD in opioid-

exposed and non-exposed groups. However, our sample was too small to allow for comprehensive statistical analyses of subgroups, and future studies are needed to elucidate these possible differences.

The opioid-exposed group performed poorer on the executive function task used in our experiment compared to controls. This is in line with previous reports of impaired executive function in children with prenatal opioid exposure.[77] Although a causal link between prenatal opioids and ADHD is not established, increased risk of attention problems and ADHD has been repeatedly reported in prenatally opioid-exposed groups.[59,60,66] It is therefore not surprising that impaired executive function was found in the opioid-exposed group, as most of these children were diagnosed with ADHD, and executive dysfunction is associated with ADHD.[142] However, group differences in task performance were in general small, and were no longer significant after adjustment for birth weight.

The relationship between BOLD activation patterns, task performance and neuropsychological abilities is complex, and not fully understood. Previous studies have suggested that those with better neuropsychological abilities required fewer neural recourses to perform a working memory task.[193] In line with this, better task performance in the control group of the present study was related to decreased BOLD activation.

Like in paper II, there are several inevitable confounding factors difficult to account for in the study sample of paper III. The observed effect of prenatal opioids on brain activation patterns and performance on the executive function task could therefore be explained as epiphenomena of prenatal opioid exposure. However, adverse effects of prenatal opioids on the developing fetal brain may also have influenced brain function in the exposed group. Results from animal and cell culture studies could support this. Opioids can affect several neurotransmitters in the developing brain, and alterations in neurotransmission could possibly interfere with cognitive development in areas like memory, executive function, and attention.[194] In animal models both cognitive and behavioral effects of prenatal opioid exposure have been

demonstrated.[195,196] The endogenous opioid system has been shown to be crucial in the control of oligodendrocyte function and myelination.[197] Interference with this system by maternal opioid use could thus alter the normal myelination process. Signs of altered myelination have also been found in DTI studies of prenatally opioid-exposed children.[118,122] In a rat model, prenatal morphine has been shown to reduce both cortical thickness and the number of neurons in the developing frontal cerebral cortex.[170] Cortical thickness after prenatal opioid exposure has only been assessed in one human study, wherein Walhovd et al. found thinner cortex in prefrontal areas in school-aged children with prenatal opioid exposure.[117] In summary, several biological plausible mechanisms of opioid effects on the developing fetal brain may have contributed to the functional brain alterations found in our sample of opioid-exposed children.

6. Conclusions

This thesis revealed no increased risk of gross structural brain abnormalities in a hospital-based population of children with prenatal drug exposure, as there were no significant group differences in the frequency of brain changes on cerebral MRI scans detectable by expert visual analyses between drug-exposed children and sex- and age-matched unexposed controls.

In prenatally drug-exposed children with confirmed exposure to opioids, from maternal use of heroin or opioids given as part of OMT, subtle alterations in volumetric brain measures and changes in brain activation patterns as seen by fMRI were found.

An association between reduced basal ganglia volumes and prenatal opioid exposure was found, as reduced regional brain volumes, including reduced volumes of the basal ganglia, were found in exposed children compared to their matched controls. There were no group differences in global brain measures (total brain, cortical gray matter and cerebral white matter). The functional consequences of regional brain volume reductions could not be addressed by the present study.

An association between prenatal opioid exposure and impaired executive functioning with corresponding aberrant BOLD activation was found. The opioid-exposed group showed increased BOLD activation in prefrontal cortical areas during the most cognitive demanding versions of a working memory-selective attention task as compared to unexposed controls. Increased activation in the opioid-exposed group could represent compensatory mechanisms to less efficient task related networks.

However, these results should be interpreted with caution, as the sample was small, and several inevitable confounding factors exist.

7. Implications and future perspectives

Cerebral MRI is probably of limited value in the clinical assessment of children with histories of prenatal drug exposure in a hospital setting, where polysubstance exposure and unspecified drug exposure is a common feature. Based on paper I cerebral MRI should therefore not be recommended as part of a routine examination of these children unless otherwise clinically indicated.

Research into possible long-term effects of prenatal opioid exposure is scarce, with a pressing need for longitudinal follow-up studies of exposed children. MRI-based brain imaging should be included in future studies to elucidate potential detrimental opioid effects on the developing fetal brain. Adverse effects of opioids may explain the associations between prenatal opioid exposure and brain alterations in children as seen by structural and functional MRI in paper II and III. However, further research is needed to explore the causal nature of these findings and to elucidate the functional consequences of the observed brain alterations.

First of all, replication of our findings in larger populations better suited to account for potential confounding factors, especially co-exposure to non-opioid drugs, would be of great value. Preferably, population-based studies should be performed, as selection bias could have influenced the results from our hospital-based population. Furthermore, thorough neuropsychological examinations of future study populations would be beneficial to investigate structure-function relationships. To explore associations between prenatal opioids, brain structure, brain function, and ADHD, future studies would benefit form including unexposed control groups both with and without ADHD.

To inform clinicians about optimal treatment of pregnant women with opioid use disorders and their children, knowledge about potential adverse effects of opioids used as part of OMT on fetal brain development is needed. The samples of opioid-exposed children included in paper II and III were too small to allow for comprehensive statistical analyses of subgroups, like comparing children exposed to

OMT medication with those exposed to heroin. Consequently, larger MRI-studies of children born to mothers included in OMT during pregnancy are warranted, to study specifically potential effects of OMT medication on fetal brain development. Population-based studies with longitudinal design would be best suited to investigate such effects. If possible, samples large enough to compare MRI measures across groups exposed to different dosing of OMT medication should be included. A possible dose-response relationship could then be explored by comparing brain measures in children born to mothers who taper their OMT medication and children born to mothers on stable or increased doses through pregnancy.

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Brain morphology in school-aged children with prenatal opioid exposure: A structural MRI study

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ABSTRACT

Background: Both animal and human studies have suggested that prenatal opioid exposure may be detrimental to the developing fetal brain. However, results are somewhat conflicting. Structural brain changes in children with prenatal opioid exposure have been reported in a few studies, and such changes may contribute to neuropsychological impairments observed in exposed children.
Aim: To investigate the association between prenatal opioid exposure and brain morphology in school-aged children.
Study design: A cross-sectional magnetic resonance imaging (MRI) study of prenatally opioid-exposed children and matched controls.
Subjects: A hospital-based sample ($n = 16$) of children aged 10–14 years with prenatal exposure to opioids and 1:1 sex- and age-matched unexposed controls.
Outcome measures: Automated brain volume measures obtained from T1-weighted MRI scans using FreeSurfer.
Results: Volumes of the basal ganglia, thalamus, and cerebellar white matter were reduced in the opioid-exposed group, whereas there were no statistically significant differences in global brain measures (total brain, cerebral cortex, and cerebral white matter volumes).
Conclusions: In line with the limited findings reported in the literature to date, our study showed an association between prenatal opioid exposure and reduced regional brain volumes. Adverse effects of opioids on the developing fetal brain may explain this association. However, further research is needed to explore the causal nature and functional consequences of these findings.

1. Introduction

The effects of prenatal drug exposure are difficult to determine in clinical studies due to a web of interconnected risk factors [1]. Increased risk of neuropsychological dysfunction, such as impaired executive functions and attention problems, has been widely reported in observational studies of children with prenatal opioid exposure [2–5]. However, such studies cannot isolate the effect of prenatal opioid exposure. In some studies, neurodevelopmental differences between exposed and unexposed children have been explained by social and environmental risk factors and low birth weight [3,6].

An abundance of data from animal and cell culture studies have demonstrated adverse effects of opioids on brain development [7]. Morphine has been shown to induce apoptosis in human neurons and microglia in vitro [8], and increased neural apoptosis has been linked to deficits in learning and memory after prenatal heroin exposure in mice [9]. In addition, decreased dendrite length and branch number in cortical neurons, decreased cortical thickness, altered myelin formation, and decreased neurogenesis have been reported after prenatal opioid exposure in rodents [10–13]. Harlan and Song found altered survival and/or migration of neurons in opioid-exposed rat embryos, with the most pronounced effect in the striatum [14]. It has been demonstrated that opioids readily cross the human placenta to enter the fetal bloodstream [15,16]. What remains unclear is whether negative effects of opioids on the developing human brain contribute to the neuropsychological impairments observed in prenatally exposed children.

Abbreviations: ADHD, attention-deficit/hyperactivity disorder; FASD, fetal alcohol spectrum disorders; ICV, intracranial volume; IQ, intelligence quotient; MRI, magnetic resonance imaging; NAS, neonatal abstinence syndrome; OMT, opioid maintenance treatment.

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Over the past few decades, brain imaging studies have helped improve our understanding of how prenatal drug exposure can impact normal brain development, particularly by elucidating the teratogenic effects of alcohol [17,18]. Moreover, two longitudinal studies on adults have suggested that opioids can induce structural brain changes [19,20], with volumetric changes in reward- and pain-related brain regions observed after 1 month of daily morphine administration in patients with chronic low back pain. Reduced volumes of the nucleus accumbens in heroin-dependent patients and of the amygdala in prescription opioid-dependent patients have also been reported in cross-sectional studies [21,22]. However, there is a paucity of brain imaging studies in children with prenatal opioid exposure [23].

Current knowledge of possible brain changes in children with intrauterine exposure to opioids is based on a few small-scale samples. In a recent pilot study, 16 neonates with prenatal opioid exposure showed reduced total brain and basal ganglia volumes, compared to population values [24]. Altered white matter characteristics and reduced neuroanatomical volumes, particularly in the pallidum and putamen, were found in a group of ten school-aged children prenatally exposed to opioids [25,26]. However, strong conclusions from these studies cannot be drawn, due to small sample and effect sizes and also due to important confounders that were difficult to account for.

The aim of the present study was to investigate brain morphology in a sample of prenatally opioid-exposed children using magnetic resonance imaging (MRI). Based on prior research, we hypothesized that prenatal opioid exposure would be associated with reduced volumes of the basal ganglia. Our study is the first study on prenatally opioid-exposed children to include a sex- and age-matched control group, which is an important improvement in methodology, compared to previous studies.

2. Material and methods

2.1. Participants

2.1.1. Opioid-exposed group

This study sample was derived from a larger group of children with prenatal drug exposure who were referred to the pediatric department at Haukeland University Hospital in Bergen, Norway, between 1997 and 2012. Children were identified as prenatally drug-exposed if they had been admitted to the neonatal department due to maternal drug use, in most cases treated for withdrawal symptoms, or if they were referred to a pediatric neurologist at a later age with a medical history of prenatal drug exposure and symptoms of attention and/or behavioral problems. A total of 70 children, aged 10–14 years, with prenatal exposure to alcohol, illicit drugs, or opioids given as part of opioid maintenance treatment (OMT) were invited to undergo an MRI examination, as previously described [27]. For the present study cases where exposure to opioids was not confirmed, whether from heroin abuse or from opioids given as part of OMT, were excluded. Children with fetal alcohol spectrum disorders (FASD) were also excluded due to the well-established effects of alcohol on brain volumes in this group [18]. Details on inclusion/exclusion are given in Fig. 1. Of the initial 43 children who consented to participate and underwent MRI scanning, after subsequent and appropriate exclusions, 16 prenatally opioid-exposed children with MRI images considered to be of acceptable quality were included. Nine of these 16 children had been admitted to the neonatal department due to maternal drug use, and seven had been referred to a pediatric neurologist at a later age. Reports from earlier follow-up of these 16 children showed average intelligence quotient (IQ) scores [mean 107.7, standard deviation (SD) 13.6, range 82–130], as assessed by Wechsler Intelligence Scale for Children, fourth edition and Wechsler Preschool and Primary Scale of Intelligence-R.

2.1.2. Matched control group

For each drug-exposed child included in the study, the first child of the same gender born at Haukeland University Hospital on the same date, with a birth weight above the 10th percentile (≥3000 g), was invited to serve as the matched control. If they declined, the next child born on the same date (or the nearest date) was contacted. According to questionnaires filled out by their mothers, none of the included controls were exposed to prenatal medication or substance abuse. Since MRI data for three of the 16 originally matched controls were missing, before further image processing, these three "missing" controls were replaced by available sex- and age-matched controls originally recruited in a similar manner for children with FASD/children exposed to drugs other

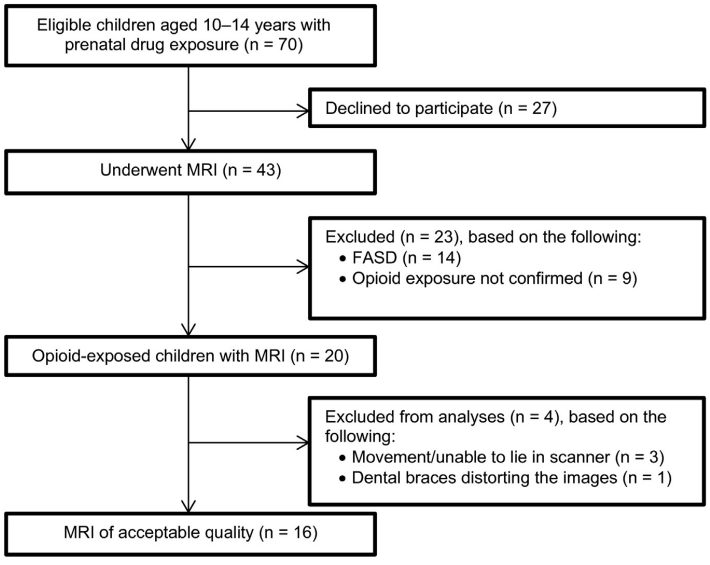

Fig. 1. Flow chart showing the inclusion/exclusion of prenatally opioid-exposed children.

than opioids. Thus, the final sample for this study consisted of 16 children exposed to opioids in utero and 16 sex- and age-matched controls.

2.2. Prenatal drug exposure

Information about drug exposure was based on history, but given the presence of heavy substance abuse, detailed information about the frequency or amount of opioids used and the type of other drugs used during pregnancy was not readily available. However, children were only included in the study if prenatal opioid exposure could be confirmed, either in medical records (obstetric or pediatric) or by information from their mother.

2.3. MRI data acquisition

MRI scanning was performed, without sedation, using a GE Signa Exite HD 3.0 Tesla (Milwaukee, WI, USA) MRI scanner. The MRI protocol included a sagittal T1-weighted, three-dimensional (3D) fast spoiled gradient recovery sequence (TI = 500 ms; TE = out of phase; slice thickness 1.0 mm; scan time 10:34 min) and an axial T2-weighted sequence (TE = 100 ms; TR = 3000 ms; slice thickness 0.8 mm; scan time 5:40 min).

2.4. Evaluation of magnetic resonance images: structural measures

All MRI scans (both T1- and T2-weighted) were inspected by an experienced pediatric neuroradiologist. No major structural abnormalities were found [27]. Scans with pronounced artifacts due to movement and/or dental braces were excluded, as shown in Fig. 1. Of note, even minimal head motions during MRI acquisition can affect the automated volumetric estimates and may lead to underestimation [28]. A quality score was given to all included scans, based on the grade of movement artifacts, by an experienced radiologist blinded to the participants' background (1 = no motion; 2 = very little; 3 = some; 4 = marked). There was no difference in the quality score between the two matched groups (Wilcoxon signed rank test: $Z = -1.70$; exact p value = 0.12). In addition, T1 3D volumes were corrected for scanner gradient field non-linearities to reduce variance that could be caused by varying head placement within the gradient field among participants [29]. Brain volume measures were obtained from the automated processing pipeline of FreeSurfer (version 5.3; http://surfer.nmr.mgh.harvard.edu/). This automated processing includes segmentation of the subcortical white matter and deep gray matter volumetric structures and automated parcellation of the cerebral cortex [30,31]. Total intracranial volume (ICV) was estimated according to the method described by Buckner et al. [32]. Volumetric measures from FreeSurfer have been validated against manual segmentation and the method has been widely used in previous pediatric studies [30,33–36]. The quality of the subcortical segmentations was evaluated by use of a semi-automated approach (http://enigma.ini.usc.edu/). In addition, for volumes with the highest quality score for movement artifacts, careful visual inspection of the segmentations was performed. None of our study cases were excluded from analysis due to segmentation error.

2.5. Additional measures

Somatic growth parameters (height, weight, and head circumference) were obtained prior to MRI scanning. Background and clinical characteristics were obtained from medical records and/or questionnaires filled out by parents or foster parents.

2.6. Statistical analyses

Descriptive statistics were reported using the mean and standard deviations (SD). For demographic and clinical variables, differences between the 1:1 matched groups were tested with the paired t-test or McNemar's test, as appropriate. Group differences in volumetric brain measures were tested using a linear mixed model to take into account the dependency of observations from matched pairs [37]. Brain volumes were entered as dependent variables, with random effect of matching. Firstly, a hypothesis-driven analysis was performed with the volume of the basal ganglia as the dependent variable. Secondly, explorative analyses were performed with the additional brain volumes from the automated segmentation as dependent variables, using the same model. Birth weight and estimated ICV were entered as covariates in all analyses (with the exception of the analysis of differences in ICV that was only adjusted for birth weight). Finally, analyses were repeated and adjusted for the presence of attention-deficit/hyperactivity disorder (ADHD). Since there were no hypotheses of differential effects in the two cerebral hemispheres, the sum of the left and right volumes was used for paired structures. All significance tests were two-sided, and a significance level of 5% was set. Statistical analyses were performed using IBM SPSS Statistics version 23.

2.7. Ethics

The project was approved by the Regional Ethics Committee for Medical Research in Western Norway (REK-Vest 2010/3301). Written consent was obtained from parents or foster parents and Child Welfare Services, as appropriate, for all participants. Written consent was also obtained from all children above the age of 12 years, and verbal consent from participants younger than 12 years.

3. Results

3.1. Sample characteristics

Demographic and clinical characteristics of the study sample are shown in Table 1. Somatic growth parameters obtained prior to MRI scanning, including head circumference, did not differ between the two groups. Birth weight was lower in the opioid-exposed group. There was a high prevalence of ADHD in the exposed group (69%), compared to the control group (6%). All opioid-exposed children included in the study either lived in foster care or were adopted. Seven children in the exposed group were born to mothers undergoing OMT, whereas nine children were born to mothers with a history of heroin abuse during pregnancy. Prenatal exposure to drugs other than opioids was reported in 11 of 16 (69%) opioid-exposed children: benzodiazepines in eight (50%), cannabis in four (25%), amphetamines in four (25%), and alcohol in one (6%). Ten children in the exposed group were reported to have symptoms of neonatal abstinence syndrome (NAS), of whom seven had been medically treated for these symptoms.

3.2. Neuroanatomical volumes

Brain volumes obtained from automated segmentation and results from linear mixed model analyses, used to test differences in brain volumes between the two groups, are shown in Table 2. The combined volume of the basal ganglia (accumbens + caudate + putamen + pallidum) was significantly reduced in the opioid-exposed group in the analysis adjusted for estimated ICV and birth weight. Among the individual basal ganglia nuclei, only the difference in caudate volume reached statistical significance. Furthermore, the volumes of the thalamus and cerebellar white matter were reduced, whereas there were no differences in ICV or global brain measures (total brain, cerebral cortex, cerebral white matter volumes). Relative to controls, exposed children had 6.5% smaller basal ganglia, 9.2% smaller caudate, 7.6% smaller thalamus, and 10.3% smaller cerebellar white matter (differences calculated from estimated marginal means). Fig. 2 shows individual differences in basal ganglia volumes for the 16 matched pairs of exposed children and controls. Of note, a smaller volume in the exposed child was found in 13 of the 16 matched pairs. When analyses were adjusted for ADHD, in addition to ICV and

Table 1
Sample characteristics for 16 prenatally opioid-exposed children and their sex- and age-matched controls.

Variable, *statistic*	Exposed group ($n = 16$)	Range	Control group ($n = 16$)	Range	p
Males, *n (%)*	9 (56)	n/a	9 (56)	n/a	–
Age at scan (months), *mean (SD)*	143.6 (12.2)	116–160	143.6 (12.8)	116–160	–
Birth weight (g), *mean (SD)*	3026 (470)	2330–4010	3665 (430)	3070–4380	0.001
Head circumference (cm), *mean (SD)*	54.2 (1.9)	49.0–57.0	54.8 (1.7)	51.5–58.2	0.402
Height (cm), *mean (SD)*	151 (12.1)	121–169	150 (9.3)	130–167	0.811
Weight (kg), *mean (SD)*	41.2 (11.5)	22.8–64.5	45.4 (10.7)	29.3–68.8	0.223
ADHD, *n (%)*	11 (69)	n/a	1 (6)	n/a	0.002

Abbreviations: ADHD, attention-deficit/hyperactivity disorder; n/a, not applicable; SD, standard deviation; $p = p$ values for group difference (controls vs. exposed) from paired *t*-test (continuous variables) and McNemar's test (categorical variables).

birth weight adjustments, statistically significant group differences were found in volumes of the basal ganglia ($p = 0.023$), thalamus ($p = 0.019$), and cerebellar white matter ($p = 0.019$), whereas the difference in caudate volume lost statistical significance using this model ($p = 0.056$). Estimated group differences were marginally larger after this adjustment, with reduced volumes in the exposed group, but with wider 95% confidence intervals (data not shown). We attempted to control for the presence of poly-drug exposure in our sample. The opioid-exposed group was split into two: (I) children without reported exposure to drugs other than opioids ($n = 5$) and (II) children with additional exposure to one or more non-opioid drugs ($n = 11$). We obtained estimated group differences in the expected direction, with smaller volumes for the basal ganglia, caudate nucleus, thalamus, and cerebellar white matter in both exposed groups, as compared to controls, but these group differences were not statistically significant. Details about these analyses and results are shown in Supplementary Table 1.

4. Discussion

Results from this study support our hypothesis for an association between reduced basal ganglia volumes and prenatal opioid exposure, as analyses adjusted for ICV and birth weight revealed 6.5% smaller basal ganglia in exposed children, compared to their matched controls. Furthermore, results showing reduced thalamus and cerebellar white matter volumes observed in the exposed group also suggest more widespread, albeit subtle, group differences.

Although evidence for structural brain changes in children prenatally exposed to substance abuse is accumulating [17], only two previous studies examined specifically the association between prenatal opioid exposure and volumetric brain measures in children [24,25], both of which reported reduced basal ganglia volumes. A third study included preschool children born to women using opioid and/or poly-drugs who were hospitalized and detoxified during pregnancy. No significant neuroanatomical differences were found between these children and a non-risk comparison group [38], although the authors speculated that reduced drug exposure as a result of detoxification could have influenced the neuroanatomical volumes. Only one of these previous studies included school-aged children born to mothers with opioid dependency. In this study Walhovd et al. reported a volume reduction in various brain measures, including total brain volume in the opioid-exposed group [25]. After adjustment for ICV, only the pallidum, putamen, and lateral ventricle volumes remained significantly reduced. Unequal influence of possible confounders that are difficult to account for in such small samples, including exposure to various other drugs, could possibly explain some of the discrepancies between the results from these studies and our findings.

Possible mechanisms underlying a reduction in neuroanatomical volumes after prenatal opioid exposure, as suggested by cell culture and animal studies, include increased apoptosis of neurons and glia cells, decreased dendrite length and branching, altered cell migration, and reduced neurogenesis [8,10,13,14]. Both opioid receptors and opioid ligands are expressed in the fetal brain, and there is growing evidence for the endogenous opioid system as a regulator of neurogenesis, with inhibitory effects of opioids [39]. Findings from a recent study, in which μ-opioid receptor knockout mice displayed regional increases in the gray matter, could support this [40]. The endogenous opioid system

Table 2
Brain volumes with estimated group differences in 16 prenatally opioid-exposed children and 1:1 sex- and age-matched controls.

Volume (ml)[a)]	Exposed group ($n = 16$) Mean	SD	Range	Control group ($n = 16$) Mean	SD	Range	Mean difference[b)]	95% CI	p
ICV	1521.48	148.23	[1119.48, 1726.12]	1525.41	128.54	[1322.62, 1713.91]	−80.32	(−208.62, 47.98)	0.207
Total brain	1203.23	126.69	[886.75, 1350.00]	1250.09	97.13	[1085.32, 1427.09]	24.66	(−22.10, 71.43)	0.285
Cerebral cortex	558.55	62.35	[441.66, 24.93]	585.78	44.50	[515.10, 660.73]	11.50	(−22.52, 45.52)	0.488
Cerebral WM	428.55	58.62	[276.70, 520.68]	437.35	46.34	[353.08, 522.48]	−0.78	(−23.50, 21.94)	0.944
Basal ganglia[c)]	23.10	2.40	[19.46, 27.19]	24.77	2.64	[19.72, 29.57]	1.60	(0.20, 3.01)	**0.027**
Accumbens	1.25	0.20	[0.94, 1.63]	1.40	0.25	[1.06, 1.85]	0.05	(−0.13, 0.23)	0.539
Caudate	7.40	1.16	[5.58, 10.13]	8.08	0.99	[6.41, 9.90]	0.75	(0.03, 1.46)	**0.042**
Putamen	11.40	1.36	[9.48, 13.53]	12.16	1.36	[9.51, 14.85]	0.82	(−0.14, 1.77)	0.090
Pallidum	3.06	0.41	[2.35, 3.71]	3.13	0.46	[2.16, 4.08]	0.18	(−0.15, 0.51)	0.274
Thalamus	14.71	1.95	[10.88, 16.98]	15.73	1.57	[13.78, 19.02]	1.21	(0.10, 2.32)	**0.035**
Hippocampus	8.35	0.93	[5.72, 9.65]	8.98	0.76	[7.44, 10.39]	0.51	(−0.07, 1.09)	0.079
Amygdala	2.95	0.35	[2.35, 3.59]	3.26	0.41	[2.50, 4.40]	0.20	(−0.11, 0.52)	0.196
Brainstem	19.01	2.67	[14.21, 22.92]	19.49	1.47	[16.57, 21.87]	0.53	(−0.63, 1.70)	0.353
Cerebellar WM	26.39	2.77	[20.85, 33.21]	28.82	3.22	[23.10, 34.13]	3.00	(0.57, 5.44)	**0.018**
Cerebellar cortex	119.11	11.10	[95.59, 140.00]	121.88	9.25	[107.59, 142.09]	1.98	(−4.62, 8.59)	0.536
Lateral ventricles	8.53	4.79	[2.48, 15.55]	9.39	5.61	[2.53, 24.04]	1.85	(−2.35, 6.05)	0.368
3rd ventricle	0.76	0.27	[0.30, 1.37]	0.84	0.32	[0.48, 1.60]	0.02	(−0.25, 0.29)	0.891
4th ventricle	1.42	0.65	[0.64, 3.12]	1.67	0.66	[0.97, 3.30]	0.15	(−0.49, 0.79)	0.636

Abbreviations: CI, confidence interval; ICV, intracranial volume; SD, standard deviation; WM, white matter; a) for paired structures total volumes (left + right) are shown; b) estimated mean difference between the groups (positive values indicate a smaller volume in the exposed group) with 95% CI and p values (bold: $p < 0.05$) from linear mixed model analyses, with brain volume as dependent variable, matched pairs as random effect variable, and ICV and birth weight as covariates; c) basal ganglia = accumbens + caudate + putamen + pallidum.

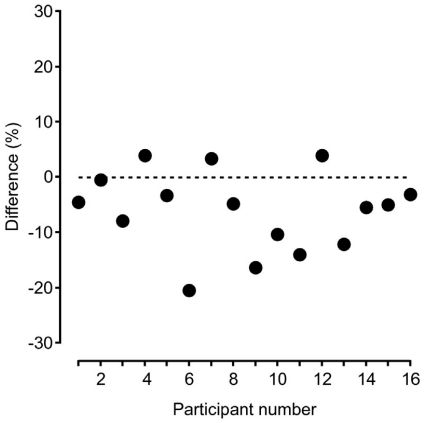

Fig. 2. Individual differences in basal ganglia volumes from 1:1 matched controls for the 16 prenatally opioid-exposed children, shown as % of control values. A negative value indicates a smaller volume in an exposed child. All basal ganglia volumes were normalized to the intracranial volumes.

has also been shown to be crucial in the control of oligodendrocyte function and myelination, and interference with this system by maternal opioid use could alter the normal maturation process of the developing brain [11].

Although via different mechanisms, all drugs of abuse increase dopamine release in the nucleus accumbens [41]. Alterations of dopaminergic systems in the fetal brain could consequently be induced by all these drugs, and dopamine has been shown to play an important role in normal brain development [42]. The dopamine-rich basal ganglia seem to be among the brain structures particularly vulnerable to the adverse effects of prenatal alcohol exposure [18,34,35]. Prenatal exposure to both amphetamine and cocaine has been associated with reduced volumes of basal ganglia nuclei in children [43,44]. Results from a volumetric MRI study in children with intrauterine exposure to cocaine, alcohol, tobacco, and marijuana suggested that these drugs may act cumulatively during gestation to exert long-lasting effects on brain volumes [45]. Taken together with our results, all these findings suggest that several drugs of abuse share a specific profile of neurotoxicity during brain development.

Previous studies have described an association between maternal opioid abuse and dependency and an increased risk of premature birth and impaired fetal growth [46,47]. Reduced brain volumes have been reported in children born prematurely/with low birth weight and in term-born children with intrauterine growth restriction [48,49]. Even in healthy term-born children, an association between birth weight and morphometric brain characteristics has been shown [36]. Interestingly, our results indicate effects of opioids on brain volumes beyond the possible effects mediated by low birth weight, as birth weight was included as a covariate in our analyses.

Whether reduced regional brain volumes in opioid-exposed children relate to neuropsychological impairments could not be addressed by the present study. However, the basal ganglia, previously considered to have largely motor functions, seem to be important for a wide range of cognitive and behavioral functions [50]. The caudate, thalamus, and cerebellum are all crucial parts of the neurobiological circuits involved in regulating attention [51]. In youth with prenatal alcohol exposure, reduced volumes of the caudate nuclei have been shown to predict decreased cognitive control and verbal learning performance, and smaller basal ganglia volumes have been associated with lower IQ scores in patients with FASD [35,52]. Subtle attentional deficits mediated by reduced caudate volumes have been suggested in children with prenatal exposure to methamphetamine [43]. In our study, given that the exposed group had average IQ scores, we believe an association between reduced basal ganglia volumes in this group and a clinically significant decrease in general cognitive abilities would be unlikely. However, possible associations with more subtle neurocognitive impairments and attention deficits should be investigated in future studies.

An important strength of our study is the inclusion of a sex- and age-matched control group, since changes in volumetric brain measures show heterogeneous sex- and age-related trajectories, including curvilinear trajectories, which make these factors difficult to control in statistical modeling [53]. Another strength is the recruitment of controls based on the time of birth, compared to other methods of control recruitment, including the use of advertisements or recruiting classmates which could introduce potential selection bias.

A limitation of our study is that our cross-sectional design precludes firm conclusions about causality, even if a causal relationship between prenatal opioid exposure and reduced brain volumes is plausible. The effect of prenatal opioid exposure cannot be distinguished from those of several known and unknown factors differing between the exposed and control groups. Some of the most obvious factors include genetic vulnerabilities and psychosocial and lifestyle factors associated with maternal substance abuse. Twin studies have shown high heritability for subcortical brain measures [54]. However, part of the variance in these measures also seems to be explained by environmental influences. Both parental education and family income have been linked to differences in brain structure [55], and childhood maltreatment has been associated with altered brain morphology, including reduced volumes of the striatum [56]. In our study sample, all opioid-exposed children lived in stable family situations (either in foster care or adopted). Nonetheless, social and environmental differences between our study groups could possibly explain some of the observed differences in brain volumes. Future studies would benefit from including control groups better matched for factors like living conditions, family income, and parental education.

When studying a hospital-based population, one might expect children with the most severe cognitive and behavioral problems to be selected. If these problems were related to structural brain changes due to prenatal opioid exposure, one would expect a higher frequency of such brain changes in a hospital-based population. Therefore, extending our results to other groups of children with prenatal opioid exposure is not without issues, as it is possible selection bias might have contributed to the observed differences.

Reduction in basal ganglia volumes has been a consistent finding in children with ADHD [57,58]. This could raise the question of whether all the differences observed in our study could be explained by the fact that we compared a group of children with a high prevalence of ADHD and its related problems with a healthy, low-risk control group. However, estimated group differences were mainly unchanged in our analyses adjusted for ADHD. Although ADHD is highly heritable [59], several non-inherited factors, including prenatal substance abuse, have also been implicated as risk factors [60]. A recent study including opioid-dependent parents and their children reported higher rates of ADHD among the children than among their parents [61]. Higher rates of ADHD among children born to opioid-dependent mothers, compared with children of opioid-dependent fathers, suggested that prenatal opioid exposure might lead to an increase in the rate and severity of ADHD [61]. It is tempting to speculate that changes in the basal ganglia as a consequence of prenatal opioid exposure contribute to ADHD and its related problems in children exposed to opioids in utero. This would imply that adjusting for ADHD could potentially mask this causal effect of opioids leading to reduced basal ganglia volumes. In future research, inclusion of unexposed control groups both with and without ADHD, together with neuropsychological assessment, would help to elucidate the associations between basal ganglia volumes, ADHD, and prenatal opioid exposure.

Like in previous studies, most opioid-exposed children in our study sample were exposed to multiple drugs. As information about drug exposure was based on history without toxicological testing, the amount of polysubstance exposure is likely to be underestimated [62]. The possibility to control for exposure to drugs other than opioids in our statistical modeling was restricted by the small sample and the uncertain degree of exposure to non-opioid drugs. Therefore, possible influence of drugs other than opioids cannot be ruled out. However, in our study, only children with confirmed exposure to opioids were included and children with FASD were excluded. In addition, our study lacked reliable data for prenatal smoking. A recent prospective study including 113 children prenatally exposed to tobacco demonstrated an association between prenatal smoking and altered brain morphology, although volumes of deep gray matter structures were not reduced, compared to controls, after adjustment for total brain volume [33].

Finally a small sample size may have reduced our power to detect significant differences, and the sample was too small for comprehensive statistical analyses of subgroups (e.g. children to mothers undergoing OMT). The finding of reduced basal ganglia volumes in the exposed group was based on our primary hypothesis. Other group differences should be interpreted with caution, as differences found in these secondary analyses would not survive statistical corrections for multiple comparisons.

5. Conclusion

In line with the limited findings reported in the literature to date, our study demonstrated an association between prenatal opioid exposure and reduced regional brain volumes in children. Adverse effects of opioids on the developing fetal brain may explain this association. This could have implications for how opioid-dependent pregnant women should be treated in the best interests of both the mother and her unborn child. However, further research is needed to explore the causal nature of this association and to elucidate the functional consequences of the observed brain alterations.

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Conflict of interest statement

None declared.

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III

Functional MRI in prenatally opioid-exposed children during a working memory-selective attention task

Running title: fMRI after prenatal opioid exposure

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Category of study: Clinical research

ABSTRACT

Background: Opioid induced cerebral changes may contribute to neuropsychological difficulties, like attention problems, frequently reported in prenatally opioid-exposed children. Reduced regional brain volumes have been shown after prenatal opioid exposure, but no study to date has explored the possible impact of prenatal opioids on brain activation patterns.

Methods: A hospital-based sample of prenatally opioid-exposed school-aged children (n = 11) and unexposed controls (n = 12) underwent functional Magnetic Resonance Imaging (fMRI) during a combined working memory-selective attention task. Within-group- and between-group analyses of blood-oxygen-level-dependent (BOLD) activation were performed using the SPM12 software package and group differences in task performance were analyzed using Cox proportional hazards modeling.

Results: Overall, similar patterns of task related parietal and prefrontal BOLD activations were found in both groups. The opioid-exposed group showed impaired task performance, and during the most cognitive demanding versions of the working memory-selective attention task, increased activation in prefrontal cortical areas was found in the opioid-exposed group compared to controls.

Conclusion: Our findings suggest that prenatal opioids affect later brain function, visible through changes in BOLD activation patterns. However, results should be considered preliminary until replicated in larger samples better suited to control for potential confounding factors.

Introduction

Recent studies on the prevalence of neonatal abstinence syndrome (NAS), a common consequence of prenatal opioid exposure, indicate a worldwide increase in the number of children exposed to opioids in utero (1). Consequences of prenatal opioid exposure beyond NAS are still debated (2,3) and the research base is scarce, especially when it comes to possible long term effects (4). A web of interconnected risk factors complicates the interpretation of repeatedly reported suboptimal neurocognitive outcomes in prenatally opioid-exposed children (5-7).

Both cognitive and behavioral effects of prenatal opioid exposure have been demonstrated in animal models (8). Possible mechanisms underlying altered brain function, as suggested by cell culture and animal studies, include increased apoptosis of neurons and glia cells (9), altered neuronal differentiation (10), and altered myelination (11). Both opioid receptors and opioid ligands are expressed in the fetal brain, and there is growing evidence of the endogenous opioid system as a regulator of neurogenesis, with inhibitory effects of opioids (12). Opioids can affect several neurotransmitters in the developing brain, and alterations in neurotransmission could possibly interfere with cognitive development in areas like memory, executive function, and attention (13). However, it is still unclear whether negative effects of opioids on the developing fetal brain contribute to the neuropsychological impairments observed in prenatally exposed children.

Results from a recent longitudinal brain imaging study suggested that several early life factors have an impact on brain and cognition for the entire life course (14). Neuroimaging studies have made important contributions to our understanding of how prenatal drug exposures can affect normal brain development, and evidence of brain structures and patterns of functional activation being altered in exposed children is accumulating (15). However, very few studies have investigated possible brain alterations

after prenatal opioid exposure. Structural brain changes in opioid-exposed children have been reported in a few small-scale samples, including one report on ten children born to mothers with histories of heroin abuse during pregnancy that showed subtle alterations in structures involved in frontal-striatal circuitry (16). Confounding factors difficult to account for in such small samples preclude firm conclusions, but some of the changes could still be linked to attentional difficulties in the exposed group (16). To date, no studies have examined possible effects of prenatal opioid exposure on brain activation patterns.

The aim of the present study was to investigate brain activation patterns in school-aged children with prenatal opioid exposure using functional magnetic resonance imaging (fMRI). In children with prenatal drug exposure very high rates of attention-deficit/hyperactivity disorder (ADHD) have been reported, regardless of the type of drug exposure (17), and increased risk of attention problems and ADHD has been widely reported in prenatally opioid-exposed groups (7,18,19). Associations between attention problems and prenatal opioid exposure have also been found in studies trying to account for the impact of genetic vulnerabilities and postnatal environmental influences (7,18). Executive dysfunction is regarded a key factor in the complex neuropsychology of ADHD (20), and impaired executive functions have been demonstrated in children with prenatal opioid exposure (21). In the present study, a task combining working memory and selective attention was chosen. These are executive functions crucial for normal cognitive function, and most likely implicated in the neurodevelopmental impairments reported in prenatally opioid-exposed children. We hypothesized that prenatally opioid-exposed children would show impaired task performance with corresponding differences in blood-oxygen-level-dependent (BOLD) activation as compared with unexposed controls.

Methods

Participants

The opioid-exposed group was derived from a larger group of children with prenatal drug exposure referred to the pediatric department at Haukeland University Hospital in Bergen, Norway, between 1997 and 2012. A total of 70 children, aged 10–14 years, identified as prenatally drug-exposed, were invited to undergo an MRI examination, as previously described (22). Children were identified as prenatally drug-exposed if they had been admitted to the neonatal department due to maternal drug use, in most cases treated for withdrawal symptoms, or if they were referred to a pediatric neurologist at a later age with a medical history of prenatal drug exposure and symptoms of attention and/or behavioral problems. Among the initial 43 children who agreed to participate, 20 children were exposed to opioids, either from heroin abuse or from opioids given as part of opioid maintenance treatment (OMT), and were therefore included in the present study. Information regarding exposure was based on history, but given the presence of heavy substance abuse, detailed information about the frequency or amounts of opioids and the type of other drugs used during pregnancy was not readily available. However, children were only included in the study if prenatal opioid exposure could be confirmed, either in medical records (obstetric or pediatric) or by information from their mother.

Sex- and age-matched unexposed controls were recruited based on date of birth as described in a previously published article (22). For the 20 opioid-exposed children included, only 17 controls were successfully recruited, hence 20 exposed children and 17 control children underwent MRI-scanning. Eight children (five opioid-exposed and three controls) were excluded from analyses due to abortion of the fMRI-protocol by the child. In addition, scans from two opioid-exposed children and two controls had to be excluded due to head movement artifacts (> 5 mm translation in any of the four experimental conditions) and scans from two opioid-exposed children were excluded due to dental braces distorting the images.

Thus, the final sample for this study consisted of 11 prenatally opioid-exposed children and 12 unexposed controls. Response logging failed for one participant (unexposed control) during fMRI. As in scanner observational data revealed appropriate task performance, data from this participant was still included in the analyses of the BOLD fMRI data, while analyses of task performance were run with $n = 11+11$.

All structural images were inspected by an experienced pediatric neuroradiologist. No major structural abnormalities were found. Somatic growth parameters (height, weight, and head circumference) were obtained prior to MRI scanning. Background and clinical characteristics were obtained from medical records and/or questionnaires filled in by parents or foster parents. Reports from earlier follow-up of the 11 children in the opioid-exposed group showed mean intelligence quotient (IQ) score of 110.6 (SD: 13.9, median: 111, range: 82–130), as assessed by Wechsler Intelligence Scale for Children, fourth edition and Wechsler Preschool and Primary Scale of Intelligence-R.

The project was approved by the Regional Ethics Committee for Medical Research in Western Norway (REK-Vest 2010/3301). Written consent was obtained from parents or foster parents and Child Welfare Services, as appropriate, for all participants. Written consent was also obtained from all children above the age of 12 years, and verbal consent from participants younger than 12 years.

fMRI-task

A working memory-selective attention task combining the n-back task and the Stroop color word task was used (23,24). The protocol of the present study has been used earlier by our group in a study on extremely preterm children. See ref. (25) for the complete description of the procedure. In short, the words RED, BLUE, GREEN, and YELLOW, each written in the three incongruent colors (e.g. red written in blue, green, or yellow) were presented

sequentially through LCD goggles mounted on the head coil. The participants were asked to press a response key when either the word or the ink color of the word matched the one presented either 1- or 2-stimuli backwards in the presentation sequence, yielding four different experimental conditions (word 1-back, word 2-back, color 1-back, color 2-back). These four experimental conditions were presented in a pseudorandom order to avoid any order effects. A block design with alternating ON and OFF blocks was used, with four ON blocks and four OFF blocks in each of the four conditions. In each ON block, three to five target stimuli were randomly presented within a sequence of 16 stimuli in total, each presented for 2250 ms. All participants were introduced to the procedure through a short computer program test sampling all four research conditions, and effort was made to be sure the instructions were comprehended before entering the scanner.

MRI data acquisition

Structural and functional images were acquired on a GE Signa Excite HD 3.0 Tesla (Milwaukee, WI, USA) MRI scanner. A high-resolution three-dimensional T1-weighted structural image was collected sagittally for co-registration with functional data using a fast spoiled gradient recovery sequence (TR = 8 ms; TE = out of phase; FA 11°; 256 × 256 matrix; FOV = 256 mm; slice thickness 1.0 mm). Functional images were collected axially using an Echo Planar Imaging (EPI) sequence with the following parameters: TR = 3000 ms, TE = 30 ms, FA 90°, 128 × 128 matrix, FOV = 220 mm, no. of slices 38, slice thickness 3 mm with 0.5 mm skip, voxel size 1.72 × 1.72 × 3.5 mm. Fourteen EPI scans per 8 blocks, arranged in a task - rest - task manner, making a total of 112 scans, were analyzed for each of the four conditions (five initial dummy scans were discarded before data analysis). Total scan time was approximately 45 min.

Data analysis

Sample characteristics and task performance

For descriptive statistics, the mean and SD is reported, as well as counts and percentages. Although the two study groups were primarily 1:1 matched for sex and age, the groups were treated as independent in our analyses, as matching was disrupted by appropriate exclusions of more than one third of the participants as described in the "Participants" section. Comparisons of continuous variables and dichotomous variables between groups were done with Gosset's independent *t*-test and Fisher's exact mid-p test respectively. During fMRI, the participants were instructed to respond to certain target stimuli, and time to correct answer was recorded. To allow for both response accuracy and reaction time to be modeled simultaneously, time to correct task response was analyzed using the Cox proportional hazards model. For each target stimuli, time to correct answer was registered, with maximal response time (2250 ms) registered if a correct response was not obtained. If there was not a correct answer, the time to response was considered to be censored as opposed to uncensored when the correct answer was obtained. Altogether 1430 observations were included in these analyses (65 target stimuli × 22 children). As each child responded to multiple target stimuli a frailty term for child was included. Primary exposure of interest was a group variable coded 0 for opioid-exposed children and 1 for controls. The results are reported using the hazard ratio (HR) with 95 % CI, e.g. a HR > 1 means a greater instant probability of a correct answer for a control than an exposed child. Other variables possibly influencing task performance were difficulty level (4 different experimental conditions) and birth weight. All children performed the same tasks, so by the design experimental condition was independent of exposure group and was not adjusted for in the models. Birth weight may be a mediator of the effect of exposure on task performance and analyses were done without and with birth weight as an additional covariate to study any mediating effect. Interactions between group and

respectively birth weight and difficulty level were tested. All significance tests were two-sided, and a significance level of 5 % was set. Analyses were performed using IBM SPSS Statistics version 23 and Stata version 14.0 (Stata Corp. College Station, TX).

fMRI-data

Image processing and data analysis were performed using the SPM12 software package revision 6470 (Welcome Trust Center for Neuroimaging, London, UK) and Matlab version 9.0 (MathWorks Inc., Natick, MA). Default preprocessing routines, as implemented in SPM12, were followed for realignment of EPI-scans and co-registration of the T1-weighted structural scan to the mean EPI-scan in each of the four experimental conditions. Subsequent segmentation of the structural scan was performed, providing normalization parameters used to normalize the EPI-scans to Montreal Neurological Institute (MNI) space (resized voxels 3 × 3 × 3 mm). Finally, the EPI-scans were smoothed with a Gaussian kernel of 8 mm. Visual inspection of all EPI-scans was performed to assure quality.

Individual participant first-level fixed effect analyses were performed on the ON-OFF block contrasts for the four experimental conditions, creating four contrast images per person. These images were subjected to second-level random effect analyses using the general linear model, as implemented in SPM12. Within-group activation patterns for the opioid exposed and control groups were modeled using one-sample *t*-tests, and two-sample independent *t*-tests were used to determine between-group differences. To account for multiple comparisons a cluster-extent, random field theory based family wise error (FWE) corrected threshold at $p < 0.05$ was used to define significant activations in all analyses, with a primary cluster-defining threshold at $p < 0.001$. Anatomical location of significantly activated clusters was identified using Anatomical Automatic Labeling (26).

Results

Sample characteristics

Demographic and clinical characteristics of the 11 prenatally opioid-exposed children and the 12 unexposed controls included in the final sample are shown in Table 1. Of note, there was a high prevalence of ADHD in the exposed group (64 %), compared to the control group (8 %). All opioid-exposed children included in the study either lived in foster care or were adopted. Four children in the exposed group were born to mothers undergoing OMT, whereas seven children were born to mothers with a history of heroin abuse during pregnancy. Prenatal exposure to drugs other than opioids was reported in 8 of 11 (72 %) opioid-exposed children: benzodiazepines in six (55 %), cannabis in three (27 %), and amphetamines in three (27 %). Six children in the exposed group were described with symptoms of NAS in the newborn period.

Task performance

Results from Cox proportional hazards models, used to analyze task performance, are presented in Table 2. The opioid-exposed group responded slower with fewer correct answers than the control group, as shown in Figure 1, with an unadjusted hazard ratio (HR) of control vs. exposed = 1.46 (95 % CI: 1.04 to 2.06; $p = 0.030$). Adjusting the model for birth weight revealed no significant group difference (HR = 1.29; 95 % CI: 0.90 to 1.83; $p = 0.164$). Children with birth weight in the range 3000 – 4000 g performed better with faster correct responses compared to children in the lowest birth weight group (< 2500 g). No significant interaction between group and birth weight was found. As expected, there were significant differences between the four experimental conditions ($p < 0.001$), with lowest ratio of correct responses in the more cognitive demanding 2-back tasks (Table 2). However, the interaction between group and difficulty level (experimental condition) was not significant ($p = 0.170$).

fMRI activation patterns

Overall, the within-group analyses showed similar, bilateral prefrontal and parietal areas of BOLD activation in both groups, but more widespread, diffuse activation in the exposed group in the more cognitive demanding conditions (word 2-back and color 2-back tasks). In the exposed group, only one large cluster, expanding widespread, bilateral cortical areas survived corrections for multiple comparisons in each of these 2-back tasks. Figure 2 shows within-group activation patterns for all four tasks (word 1-back, word 2-back, color 1-back, and color 2-back) for the two groups. The corresponding MNI coordinates for peak voxel activations for the significant clusters are listed in Table 3.

Results from the between-group analyses revealed increased activation in the exposed group in both 2-back conditions, whereas there were no significant group differences in the easier 1-back conditions. There were no areas where the control group showed increased activation relative to exposed children in any of the four experimental conditions (control minus exposed contrasts). In Figure 3 clusters with significant group differences are shown, with corresponding peak voxel activations listed in Table 4. In the word 2-back condition one significant cluster in the left prefrontal cortex including left precentral gyrus and superior and middle frontal gyrus showed increased activation in the exposed group. Increased bilateral prefrontal activations in the exposed group were found in two clusters including left and right middle frontal gyrus in the color 2-back condition.

Discussion

Results from this first fMRI study on prenatally opioid-exposed children showed increased BOLD activation in prefrontal cortical areas in the exposed group as compared to unexposed controls during the most cognitive demanding versions of a working memory-

selective attention task. In both groups, task related activation patterns included parietal and prefrontal cortical areas previously shown to be important in working memory and selective attention tasks (23,27,28), a finding that supports the validity of our results. The opioid-exposed group showed impaired task performance, but this group difference was no longer significant when analyses were adjusted for birth weight.

Aberrant brain activation patterns in children have been reported in fMRI studies exploring possible effects of maternal use of alcohol, cocaine, amphetamines, and tobacco on the developing fetal brain (15,29). However, to date, no study has examined specifically if prenatal opioid exposure affects brain activation patterns. In studies on prenatally drug-exposed children, heavy prenatal alcohol exposure has been most widely reported, also when it comes to brain imaging studies (15). Several of these studies have shown increased prefrontal BOLD response in alcohol-exposed groups during working memory tasks, but with varying degrees of behavioral differences (30,31). Less efficient task related networks or compensation for other less active regions have been suggested as an explanation to these findings in alcohol-exposed children (30,31). Similar compensatory mechanisms could possibly explain our finding of increased prefrontal activation in the opioid-exposed group. On the other hand, Astley et al. (32) found significant working memory deficits in children with fetal alcohol spectrum disorders (FASD) with corresponding lower brain activation in extended prefrontal and parietal regions, particularly on the most cognitive demanding tasks. The authors have discussed a possible “ceiling effect”, where the capacity of compensatory mechanisms to less efficient networks is surpassed, as an explanation to the inability of the FASD group to increase activity in response to increasing cognitive load (32). Impaired behavioral performance on a working memory task with corresponding decreased prefrontal activation has also been reported in children exposed to methamphetamine (33). In young adults with histories of prenatal marijuana exposure fMRI studies have shown increased brain

activation across several different executive function tasks, but unlike our findings in opioid-exposed children, this increased activation has been consistently located in posterior brain regions (34).

Decreased activation in prefrontal cortical areas has been a consistent finding in numerous fMRI studies on children with ADHD across several different cognitive tasks (35). Contrary to this, we found increased prefrontal activation in our opioid-exposed group, despite the fact that most of these children were also diagnosed with ADHD (64 %). It is therefore tempting to speculate that these differences could reflect different neural correlates of ADHD in opioid-exposed and non-exposed groups. However, our sample was too small to allow for comprehensive statistical analyses of subgroups, and future studies including control groups with and without ADHD would be needed to elucidate these possible differences.

Consistent with earlier reports of impaired executive function in children with prenatal opioid exposure (21), the exposed group in the present study performed poorer on the executive function task compared to controls, with slower response and fewer correct answers. However, the group difference found in the unadjusted model was no longer significant when analyses were adjusted for birth weight. Birth weight as a mediator of the possible effect of opioid exposure on task performance could explain this finding. The exposed group in the present study had significantly lower birth weight compared to controls, and prenatal opioid exposure has been associated with increased risk of preterm birth and low birth weight (2). However, there are myriads of associated risk factors, and it is still unclear if opioids have a direct, causal effect on these birth outcomes (3). It is therefore difficult to know as to whether birth weight should be conceptualized as a mediator or a confounder. A larger sample size or a more cognitive demanding task (e.g. a 3-back task) could possibly reveal more convincing group differences in task performance. However, previous studies

(25), in scanner observations, and pilot scans performed prior to our study, have indicated that the 2-back tasks used were quite difficult for children in the chosen age range.

An abundance of data from animal and cell culture studies have demonstrated adverse effects of opioids on brain development that could possibly underlay altered brain activation patterns in prenatally exposed children (9-11). Interestingly both animal and human data have demonstrated reduced thickness of frontal cortical areas after prenatal opioid exposure (16,36). In animal models, the endogenous opioid system has been shown to be crucial in the control of oligodendrocyte function and myelination (11), and interference with this system by maternal opioid use could alter the normal maturation process of the developing brain. There is also circumstantial evidence suggesting that opioids can alter myelination in prenatally exposed children (37). Incomplete myelination may result in poorer conduction efficiency and thus less efficient neural function in related networks.

There are several limitations to the present study. The results should therefore be considered preliminary and conclusions made with caution. First of all, an observational study design precludes firm conclusions about causality to be made, even if a causal relationship between prenatal opioid exposure and altered BOLD activation is plausible. The possible effect of prenatal opioid exposure on brain development cannot be distinguished from those of several known and unknown factors differing between the exposed and control groups, a challenge in all studies on drug-exposed children. Some of the most obvious factors include genetic vulnerabilities and psychosocial and lifestyle factors associated with maternal substance abuse. The impact of many of these factors on brain activation patterns is largely unknown. However, some factors, like parental socio-economic status, have been associated with altered BOLD activation, including increased prefrontal activation found in socioeconomic disadvantaged children (38). In our study sample, all opioid-exposed children lived in stable family situations (either in foster care or adopted). Nonetheless, social and

environmental differences between our study groups could influence some of the observed differences. Future studies would benefit from including control groups better matched for factors like living conditions, family income, and parental education.

A small sample size may have reduced our power to detect significant, but subtle group differences, and the ability to account for possible confounders. Most children in the opioid-exposed group in our study were exposed to multiple drugs. Information regarding exposure to non-opioid drugs was based on history without toxicology testing, and exposure may thus have been underestimated. Due to this uncertain degree of exposure and the small sample size, we were not able to control for exposure to non-opioid drugs in our statistical modeling. Therefore, possible influence of drugs other than opioids cannot be ruled out. However, only children with confirmed exposure to opioids and no children with known exposure to heavy maternal alcohol consumption were included. In addition, our study lacked reliable data for prenatal smoking. Very few studies have examined possible effects of prenatal tobacco using fMRI. Contrary to our findings, Bennett et al. (29) found greater prefrontal activation in their unexposed control group whereas tobacco-exposed children showed greater activation in inferior parietal regions during an n-back working memory task.

Due to the small sample size and the risk of masking possible opioid effects that were mediated by low birth weight, we did not attempt to adjust the between-group analyses of BOLD fMRI data for birth weight. However, we find it unlikely that the selection of a low birth weight group should explain the increased prefrontal activation seen in our opioid-exposed group, as decreased BOLD activation has been a consistent finding in fMRI studies of preterm and low birth weight groups, including one study using the same fMRI paradigm as the one used in the current study (25).

The use of a cluster-extent based threshold to correct for multiple comparisons in our study precludes inferences about specific anatomical regions within significant clusters to be

made with confidence (39). Even with a primary cluster defining threshold of $p < 0.001$, some of the activated clusters were large, spanning several anatomical regions. The anatomical labels for peak-voxel activation listed in tables should therefore be interpreted with caution, as one cannot infer that all these peaks were truly activated, but only that there was significant signal somewhere within each cluster. Detailed discussion and comparisons of the anatomical localization of BOLD activations could therefore not be performed based on our results. However, our main finding of increased activation in the exposed group was restricted to relatively small clusters that could be localized in prefrontal cortical areas with confidence. The effectiveness of cluster-extent based thresholding to correct for multiple comparisons in fMRI studies has recently been called into question (40), but the problem of inflated false positive rates, was mainly shown for more liberal primary cluster defining thresholds than the one used in the present study ($p < 0.001$). To investigate differences in activation patterns in greater detail, larger samples, allowing for voxel-vise correction methods are needed.

The generalizability of our results is also limited, as the hospital-based sample of prenatally opioid-exposed children included in the study represents a subset of the exposed population. However, the signs of opioid effects on brain function in our sample warrants further research, and if possible population-based samples should be included in future studies.

Finally, it should be acknowledged that fMRI use level of oxygenated blood as a proxy for measuring the activity of neurons. The extent to which differences in the BOLD signal between our study groups represents actual differences in neuronal activation as compared to other possible underlying mechanisms, like altered vascularization, remains unknown.

Conclusion

Our findings suggest that prenatal opioid exposure affect brain activation patterns during a working memory-selective attention task. Increased prefrontal activation in the exposed group in the most cognitive demanding tasks could represent compensatory mechanisms to less efficient task related networks. However, results should be considered preliminary until replicated in larger samples better suited to explore subtle differences and account for potential confounding factors.

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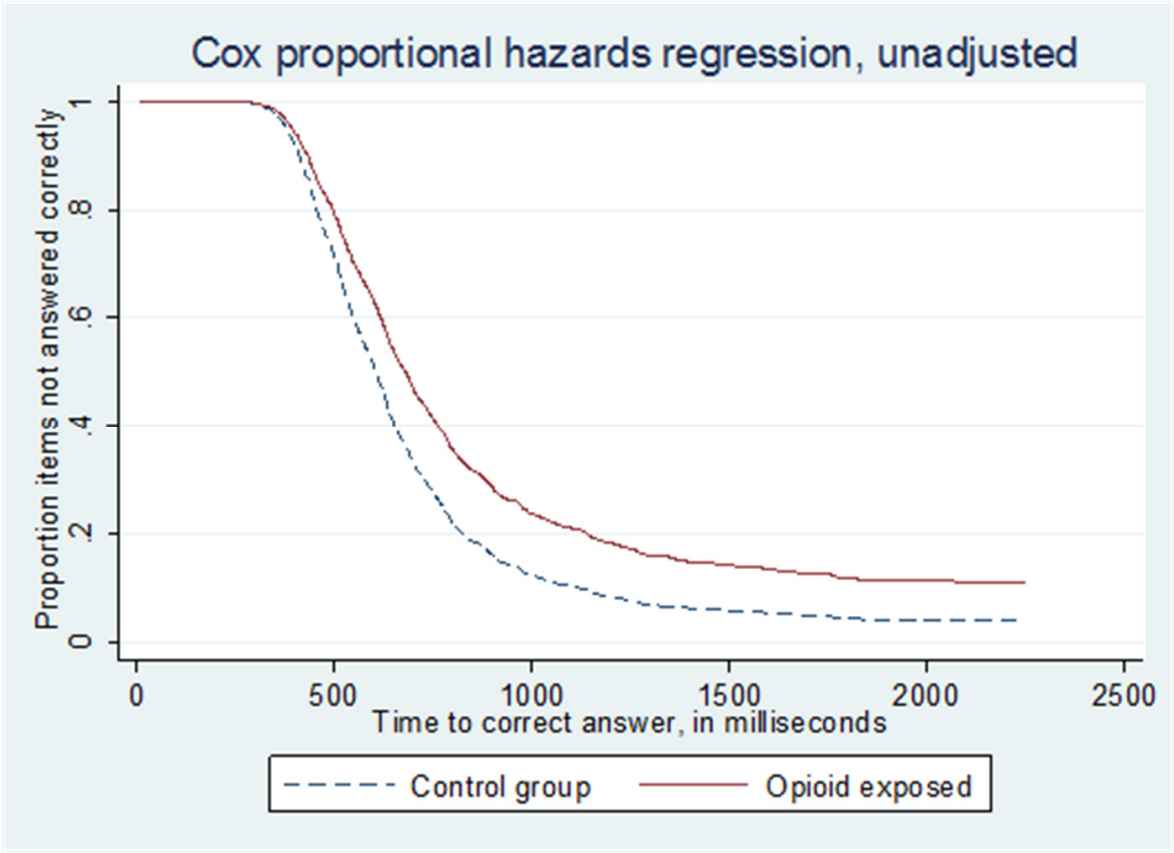
Figure legends

Figure 1 Proportion of items not answered correctly in the working memory-selective attention task for 11 prenatally opioid-exposed children and 11 control children combined for four experimental conditions as estimated by a Cox proportional hazards model with frailty to account for dependency between multiple answers from the same child. Altogether 1430 tasks were analysed showing higher proportion of items not answered correctly in the exposed group ($p = 0.030$).

Figure 2 Within-group BOLD activation patterns for the opioid-exposed group ($n = 11$) and the unexposed control group ($n = 12$) separated by the four experimental conditions. Surface renderings of activated clusters that survived corrections for multiple comparisons with a cluster-extent based threshold at FWE corrected $p < 0.05$. *Abbreviations:* BOLD, blood-oxygen-level-dependent; FWE, family-wise error; L, left; R, right.

Figure 3 Surface renderings of group differences in BOLD activation for the two most cognitive demanding experimental conditions with cluster-wise corrections for multiple comparisons (FWE corrected; $p < 0.05$). (a) color 2-back, (b) word 2-back. In both these conditions the opioid-exposed group ($n = 11$) showed increased BOLD activation relative to unexposed controls ($n = 12$) *Abbreviations:* BOLD, blood-oxygen-level-dependent; FWE, family-wise error; L, left; R, right.

Figure 1


Cox proportional hazards regression, unadjusted
Proportion items not answered correctly
0
.2
.4
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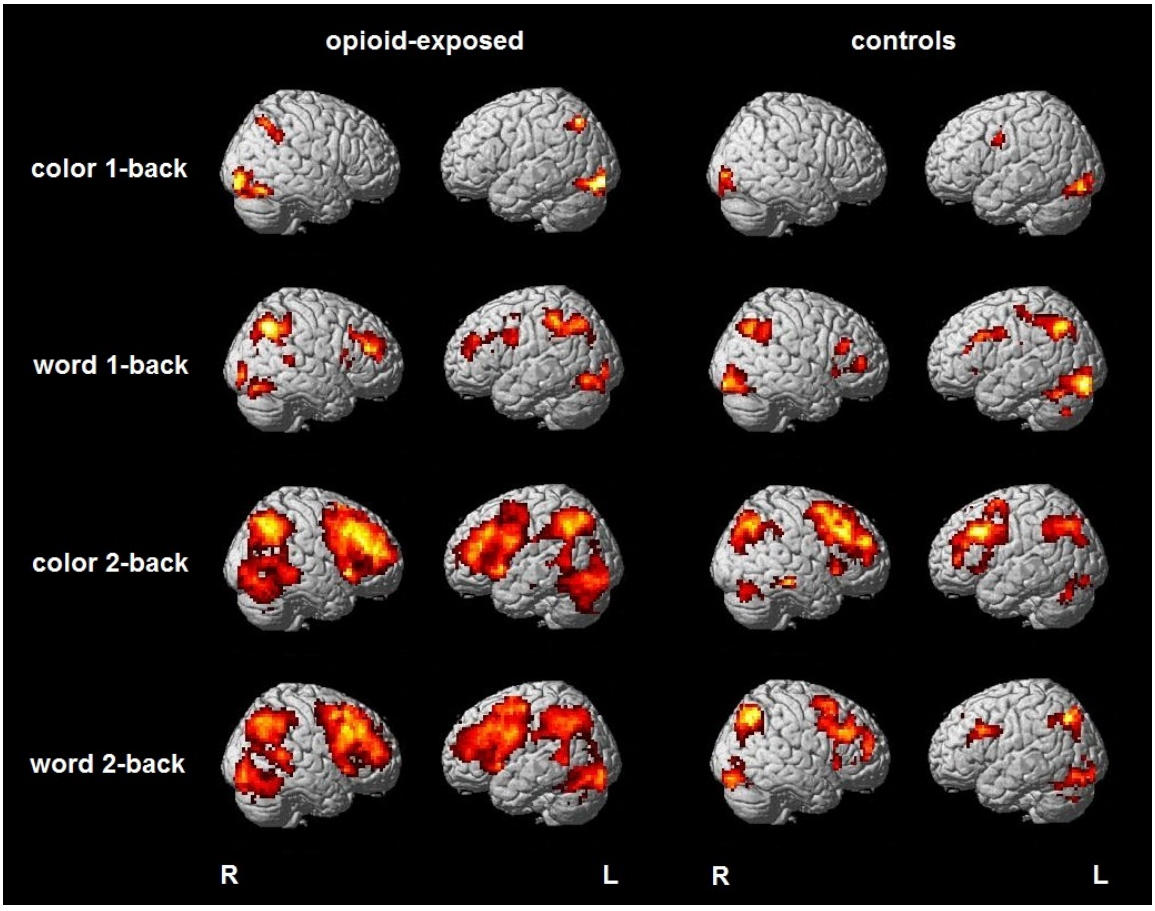
.8
1
0
500
1000
1500
2000
2500
Time to correct answer, in milliseconds
Control group
Opioid exposed

Figure 2


opioid-exposed
controls
color 1-back
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color 2-back
word 2-back
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Figure 3

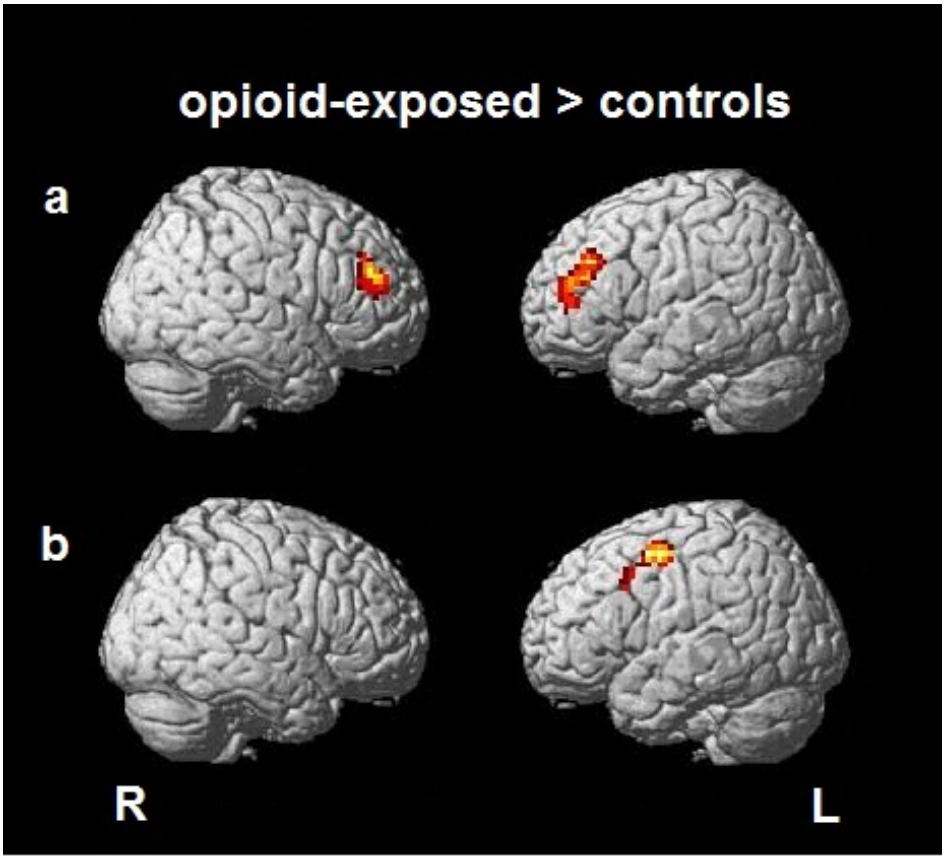

Table 1 Sample characteristics for 11 prenatally opioid-exposed children and 12 unexposed controls

Variable, *statistic*	**Exposed group (*n* = 11)**	**Range**	**Control group (*n* = 12)**	**Range**	***p***
Males, *n (%)*	6 (55)	n/a	6 (50)	n/a	0.842
Age at scan (months), *mean (SD)*	146.1 (13.3)	116–160	146.0 (10.6)	123–160	0.986
Head circumference (cm), *mean (SD)*	54.9 (1.4)	52.5–57.0	54.5 (1.7)	51.5–58.2	0.547
Height (cm), *mean (SD)*	153 (11.8)	127–169	150 (9.3)	130–167	0.560
Weight (kg), *mean (SD*)	43.9 (11.7)	22.8–64.5	43.4 (10.0)	29.3–68.8	0.906
Left handedness, *n (%)*	0 (0)	n/a	1 (8.3)	n/a	0.522
ADHD, *n (%)*	7 (64)	n/a	1 (8.3)	n/a	0.009
Birth weight (g), *mean (SD)*	2956 (520)	2300–4010	3545 (431)	3040–4200	0.007

Abbreviations: ADHD, attention-deficit/hyperactivity disorder; n/a, not applicable; *p* = *p* values for group difference (controls vs. exposed) from independent *t*-test (continuous variables) and Fisher's exact test with mid-p correction (dichotomous variables).

Table 2 Results from Cox regression of time to correct response on a working memory-selective attention task in 11 prenatally opioid-exposed children and 11 unexposed controls

		Unadjusted models			Adjusted for birth weight[b)]		
Variable	***Category***	**HR[a)]**	**95% CI**	***p***	**HR[a)]**	**95% CI**	***p***
Group	Opioid-exposed	1.00	Reference	0.030	1.00	Reference	0.164
	Controls	1.46	(1.04, 2.06)		1.29	(0.90, 1.83)	
Birth weight group	2000 – 2500g	1.00	Reference	0.002	1.00	Reference	0.010
	2500 – 3000g	1.50	(0.81, 2.77)		1.50	(0.83, 2.70)	
	3000 – 3500g	1.87	(1.20, 2.90)		1.62	(1.02, 2.57)	
	3500 – 4000g	2.43	(1.40, 4.21)		1.89	(1.00, 3.56)	
	4000 – 4500g	1.04	(0.60, 1.80)		0.87	(0.48, 1.55)	
Difficulty level	Color 1-back	1.00	Reference	< 0.001	n/i		
	Word 1-back	0.81	(0.70, 0.94)		n/i		
	Color 2-back	0.34	(0.28, 0.40)		n/i		
	Word 2-back	0.39	(0.33, 0.46)		n/i		

Abbreviations: HR, hazard ratio; CI, confidence interval; *p* = *p*-value for the variable from likelihood ratio test; *n/i*, not included; a) Estimated HR (instant probability of a correct answer) from Cox regression using a frailty model to account for dependency between multiple answers from the same participant; b) All children performed the same tasks, so by the design difficulty level was independent of exposure group and was not adjusted for in the model.

Table 3 Within-group analyses: Peak voxel descriptions for BOLD activation in significant clusters from whole brain analyses in 11 prenatally opioid-exposed children and 12 unexposed controls

Task	*Group*	*A*natomical area[a]	Cluster size[b]	Peak T[c]	Peak coordinates (MNI)		
					x	*y*	*z*
Color 1-back	*Opioid-exposed*	R Inferior occipital gyrus	329	4.80	33	-88	-7
		L Superior parietal gyrus	182	4.64	-27	-64	47
		L Inferior occipital gyrus	230	4.38	-30	-88	-7
		L Supplementary motor area	146	4.09	-3	14	50
		R Angular gyrus	133	3.73	39	-64	50
	Controls	L Fusiform gyrus	163	4.43	-42	-82	-16
		L Precentral gyrus	99	3.89	-39	2	32
		R Middle occipital gyrus	106	3.74	39	-88	-1
Word 1-back	*Opioid-exposed*	R Middle frontal gyrus	1503	5.40	45	44	20
		L Parahippocampal gyrus	2985	4.99	-33	-43	-1
		R Inferior occipital gyrus	84	4.82	33	-88	-4
		R Cerebellum	162	4.30	33	-79	-25
		L Precentral gyrus	98	3.73	-48	11	32
	Controls	R Lingual gyrus	311	5.35	24	-91	-16
		L Fusiform gyrus	583	4.67	-36	-85	-16
		L Middle frontal gyrus	1388	4.60	-48	17	35
		R Middle frontal gyrus	919	4.22	39	44	8
		R Inferior parietal gyrus	312	4.14	51	-49	41
Color 2-back	*Opioid-exposed*	R Middle frontal gyrus	19057	5.93	36	56	17
	Controls	L Middle frontal gyrus	4939	5.21	-48	17	38
		L Superior occipital gyrus	778	5.17	-18	-64	38
		R Fusiform gyrus	137	4.59	48	-31	-16
		R Precuneus	848	4.50	30	-52	29
		R Cerebellum	123	4.12	39	-76	-28
		L Thalamus	81	3.71	-12	-13	8
		L Cerebellum	149	3.71	-45	-70	-28
		R Thalamus	119	3.70	12	-10	11
Word 2-back	*Opioid-exposed*	R Superior frontal gyrus, dorsolateral	17891	5.47	27	17	50
	Controls	R Angular gyrus	788	4.87	33	-61	47
		R Lingual gyrus	273	4.74	24	-88	-16
		R Inferior frontal gyrus, triangular part	1082	4.62	39	29	23
		L Inferior parietal gyrus	454	4.61	-30	-70	41
		L Fusiform gyrus	331	4.23	-39	-73	-16
		L Precentral gyrus	228	4.02	-45	5	29
		L Supplementary motor area	218	4.00	3	20	50

Abbreviations: BOLD, blood-oxygen-level-dependent; L, left; MNI, Montreal Neurological Institute; R, right; a) Local maxima labeling from Anatomical Automatic Labeling (AAL); b) Cluster size in voxels (3 × 3 × 3 mm), only clusters that survived correction for multiple comparisons with a cluster-extent based threshold at family wise error (FEW) corrected $p < 0.05$ are shown; c) t-values from one-sample *t*-tests.

Table 4 Between-group analyses: Peak voxel descriptions for BOLD activation in clusters with significant group differences between 11 prenatally opioid-exposed children and 12 unexposed controls

Task	*Contrast*	*A*natomical area[a]	Cluster size[b]	Peak T[c]	Peak coordinates (MNI)		
					x	*y*	*z*
Color 2-back	*Opioid-exposed* > *Controls*	L Middle frontal gyrus	277	5.78	-33	38	23
		R Middle frontal gyrus	186	5.17	33	41	17
Word 2-back	*Opioid-exposed* > *Controls*	L Precentral gyrus	148	5.00	-39	-4	53

Abbreviations: BOLD, blood-oxygen-level-dependent; L, left; MNI, Montreal Neurological Institute; R, right; a) Local maxima labeling from Anatomical Automatic Labeling (AAL); b) Cluster size in voxels (3 × 3 × 3 mm), only clusters that survived correction for multiple comparisons with a cluster-extent based threshold at family wise error (FEW) corrected $p < 0.05$ are shown; c) t-values from independent *t*-tests.