Investigating the role of maternal immune activation on neurodevelopmental trajectories: connecting neuroimaging, behavioural, and molecular phenotypes

Elisa Guma, MSc

Doctor of Philosophy

Integrated Program in Neuroscience
Departments of Psychiatry, Neurology and Neurosurgery
McGill University, Montreal, Canada

June 22, 2021

A thesis submitted to McGill University in partial fulfillment of the requirements for the degree of Doctor of Philosophy

I would like to dedicate this thesis to my parents,

Tullio Guma and Dana Perin Guma.

I would not be where I am today without your love,

unwavering support, and encouragement.

Abstract

Brain development is a remarkable and complex set of processes under the organizational control of genetic, environmental, and immune regulation. The tightly regulated nature and interdependence of these processes make them vulnerable to a variety of risk factors. Converging lines of evidence suggest an association between prenatal exposure to maternal infection and increased risk for a host of neurodevelopmental disorders in offspring, including schizophrenia and autism spectrum disorder. Infection in the mother results in an increased production of proinflammatory cytokines, which can alter the delicate immune balance between the maternal and fetal environments; this may result in abnormal immune profiles in the developing brain, interfering with the regulatory roles of these immune molecules in brain development. Interestingly, neurodevelopmental processes and maternal immune responsiveness vary across gestation, which indicates that the gestational timing of exposure to maternal immune activation *in utero* may influence the nature or severity of offspring disruptions. The majority of studies investigating the effects of maternal immune activation have focused on cross-sectional investigations in the adolescent and adult period. However, it is unclear how proximally to the exposure developmental abnormalities can be detected, and how these evolve throughout the lifespan, potentially resulting in psychiatric disorders.

This thesis involves the integration of whole-brain neuroimaging with behavioural, transcriptional, and cellular phenotyping to investigate the effects of maternal immune activation on offspring development from gestation to adulthood using the mouse as a model system. **Chapters 1** and **2** provide a general introduction and background information on the effects of maternal immune activation across epidemiological and experimental studies. **Chapter 2** also covers the neuroimaging and statistical methodology used in this dissertation. **Chapter 3** presents a systematic review of the literature leveraging whole-brain imaging techniques to study the cross-species effects of prenatal exposure to maternal immune activation on offspring development. This work allowed for the identification of important gaps in our knowledge, which were addressed in the subsequent three chapters. **Chapter 4** presents a published study which characterizes patterns of altered neurodevelopmental trajectory in mice prenatally exposed to maternal immune activation either early or late in gestation. Early exposure transiently altered development in

adolescence, followed by a normalization in adulthood. Using a data-driven approach, regions of interest were selected for transcriptional profiling, wherein genes regulating signaling molecules critical to embryonic brain development were dysregulated due to prenatal maternal immune activation. **Chapter 5** builds upon these findings investigating the effects of maternal immune activation on the embryo and neonatal brain. Striking neuroanatomical alterations were observed in the embryo brain, while limited changes were observed in neonate brain and behaviour. **Chapter 6** examines whether exposure to maternal immune activation increases susceptibility to additional risk factors later in life, namely, adolescent cannabis exposure. Findings from this chapter suggest that exposure to multiple risk factors induces greater neurodevelopmental deviations than a single risk factor, or no risk factor at all. Finally, **Chapter 7** provides a final summary and discussion of these findings. Altogether, this thesis integrates various modalities across differing scales of resolution, from whole-brain neuroimaging, to behaviour, transcription, and electron microscopy to investigate dimensions of altered development throughout the lifespan. It provides evidence for dynamic neurodevelopmental changes from gestation to adulthood associated with maternal immune activation, a known risk factor for a range of psychiatric disorders.

Résumé

Le développement du cerveau est un ensemble de processus remarquables sous le contrôle organisationnel de la régulation génétique, environnementale et immunitaire. La nature réglementée et l'interdépendance de ces processus les rendent vulnérables à divers facteurs de risque. L'exposition prénatale à une infection maternelle peut augmenter le risque de développer des troubles neurodéveloppementaux chez la progéniture, comme la schizophrénie et les troubles du spectre autistique. L'infection chez la mère entraîne une production accrue de cytokines proinflammatoires, qui peuvent altérer l'équilibre immunitaire entre les environnements maternel et fœtal; cela peut entraîner des profils immunitaires anormaux dans le cerveau en développement, interférant avec les rôles régulateurs de ces molécules. Pendant la grossesse, la réactivité immunitaire maternelle et les processus neurodéveloppementaux du fœtus varient. Ainsi, la période de la gestation durant laquelle l'activation immunitaire maternelle est subie peut influencer la gravité des perturbations sur la progéniture. La majorité des études sur l'activation immunitaire maternelle se sont concentrées sur des enquêtes transversales entre l'adolescence et l'âge adulte. Cependant, on ne sait pas à quel point les anomalies du développement peuvent être détectées et comment celles-ci évoluent.

Cette thèse présente l'intégration de la neuroimagerie avec autres modalités pour étudier les effets de l'activation immunitaire maternelle sur la progéniture, de la gestation à l'âge adulte, chez la souris. Les **chapitres 1 et 2** donnent une introduction des connaissances sur les effets de l'activation immunitaire maternelle basées sur des études épidémiologiques et expérimentales. Le **chapitre 2** couvre également les méthodologies utilisées dans cette thèse. Le **chapitre 3** présente une revue systématique des études utilisant l'imagerie cérébral pour étudier les effets de l'activation du système immunitaire maternel sur le développement de la progéniture. Ce travail a permis d'identifier d'importantes lacunes dans nos connaissances, ce qui a motivé les trois chapitres suivants de cette thèse. Le **chapitre 4** présente une étude publiée qui caractérise les altérations des trajectoires neurodéveloppementales chez les souris exposées à une activation immunitaire maternelle, soit au début ou à la fin de la gestation. Une exposition précoce durant l'adolescence a modifié le développement suivi d'une normalisation à l'âge adulte. Guidée par les données, des régions d'intérêt ont été sélectionnées pour le profilage transcriptionnel. Dans ces régions, on a observé une dérégulation des gènes régulant le développement cérébral embryonnaire. Le **chapitre**

5 étudie les altérations neuroanatomiques dans le cerveau de l'embryon et chez les nouveau-nés suite à une activation immunitaire maternelle au début ou à la fin de la gestation. De grandes altérations neuroanatomiques ont été observé chez l'embryon mais les altérations chez les nouveau-nés étaient très limitées. Le **chapitre 6** vise à évaluer si l'activation immunitaire maternelle augmente la sensibilité envers l'exposition des adolescents au cannabis, un autre facteur de risque. Les résultats suggèrent que l'exposition à plusieurs facteurs de risque induit des écarts neurodéveloppementaux plus importants que dans le cas d'un seul facteur de risque. Enfin, le **chapitre 7** fournit un résumé final et une discussion des résultats. Au total, cette thèse intègre diverses modalités sur différentes échelles de résolution, de la neuroimagerie du cerveau au comportement, à la transcription et à la microscopie électronique pour étudier les différents impacts liés à l'altération du développement tout au long de la vie. Il fournit des preuves de changements neurodéveloppementaux dynamiques, de la gestation à l'âge adulte, associés à l'activation du système immunitaire maternel, un facteur de risque connu pour de nombreux troubles psychiatriques.

Table of Content

List of figures

List of Tables

List of abbreviations

ACC: anterior cingulate cortex
ASD: autism spectrum disorder
ASST: attentional set shifting task
CB: cannabinoid
CRP: C-reactive protein
CS: cross-sectional
CT: computed tomography
DBM: deformation based morphometry
DEG: differentially expressed gene
DTI: diffusion tensor imaging
dHIP: dorsal hippocampus
E: early (GD9)
FDR: false discovery rate
FGF: fibroblast growth factor
fMRI: functional magnetic resonance imaging
GD: gestational day
IL: interleukin
L: late (GD17)
LPS: lipopolysaccharide
LV: latent variable
MIA: maternal immune activation
MRI: magnetic resonance imaging
OFT: open field test
PET: positron emission tomography
PFC: prefrontal cortex
PLS: partial least squares
PND: postnatal day

POL: poly I:C

POLY I:C: polyinosinic:polycytidylic acid

PPI: prepulse inhibition

QC: quality control

ROI: region of interest

RRHO: rank rank hypergeometric overlap

SAL: saline

SD: standard deviation

SONT: social novelty test

SOPT: social preference test

T: Tesla

THC: delta-9-tetrahydrocannabinol

TLR: toll-like receptor

TNF: tumor necrosis factor

vHIP: ventral hippocampus

USV: ultrasonic vocalization

Acknowledgements

The content of this thesis reflects my development as a scientist over the course of my doctoral degree. Much of this growth can be attributed to the guidance and mentorship of many incredible and inspiring scientists who deserve recognition. First and foremost, I would like to thank my PhD supervisor and mentor, Dr. Mallar Chakravarty, for his unwavering support and guidance through every step of this process. Mallar, it's been an incredible privilege and pleasure to work with you. Thank you for your enthusiasm, for the many inspiring conversations developing scientific ideas and experiments, and for giving me the space to explore my scientific curiosities while pushing me to grow and learn new skills. Thank you also for always having my back, for creating an environment in which I could thrive, and for providing me with so many opportunities to showcase my work. Your drive, collaborative spirit, passion for science, and ability to lead and mentor many young scientists is admirable. I have learned so much from you over the course of this degree and I sincerely thank you.

I would next like to thank a number of professors with whom I collaborated. First, Dr. Rosemary Bagot, thank you for taking the time to train me in how to perform RNA sequencing experiments and for providing constructive feedback on my research and writing. I admire your scientific thinking and ability to clearly and succinctly distill important information; you are an inspiring scientist and role model. I would also like to thank Dr. Bratislav Mišić for teaching me new statistical methods with patience and clarity. To Drs. Patricia Boksa and Lalit Srivastava, my advisory committee, thank you for sharing your incredible knowledge of the field and for ensuring that my thesis was addressing relevant and important questions for advancing our understanding of risk factors for neurodevelopmental disorders. Thank you to Dr. Joseph Rochford and Dr. Tak Pan Wong for your guidance in implementing and analyzing animal behavioural data.

Importantly, this work would not have been possible without support from various funding agencies. Thank you the Fonds de la recherche en santé du Quebec, the Integrated Program in Neuroscience, the Bruce Sells Award, and the Kappa Kappa Gamma Foundation for investing in my research and scientific development.

The content of this thesis represents a large volume of work, which I could never have accomplished without the support of my colleagues. To my peers at the CoBrA lab, thank you for making coming to work so much fun. I have learned so much from all of you over the course of this degree and feel fortunate to have been part of such a fun and collaborative team. In particular, Dr. Gabriel Devenyi, it has been a pleasure being your officemate, and I could not have completed this degree without your help and support with image processing, statistics, coding, and more. I admire your dedication to ensuring we are using the best methods possible, and your willingness and ability to problem solve and support others. To Lani Cupo, working closely with you over the last few years has been truly wonderful; your passion for science, your curiosity, drive to learn new things, and willingness to help others is admirable. I am grateful for your help in running experiments and for your friendship. To Daniel Gallino, thank you for your flexibility in scanning

evenings, weekends, and long days, and for doing all of this with such a fun and positive attitude; it's been a true pleasure working with you. To Saashi Bedford, thank you for your friendship and support during your time at the CoBrA lab and for making the lab environment fun and lively. To Raihaan Patel, your calm presence and patience, and ability to make complex ideas very clear is admirable, as is your generosity with pastries and chocolate (the latter was definitely critical to my success). I want to thank Emily Snook for making data collection and analysis incredibly fun; your creative spirit and scientific rigor made working with you a real pleasure. To Chloe Anastassiadis, thank you for your support and for helping me with some of that toughest behavioural data acquisition. Thank you to Stephanie Tullo for being a supportive officemate, to Dr. Eric Plitman for your guidance on systematic reviews, and to Gabriel Desrosiers-Gregoire, Aurelie Bussy, and Dr. Jurgen Germann for your help with scripts and statistics. Thank you to my other colleagues at the Douglas; Teresa Joseph, Lourdes Fernandez de Cossio, and Maude Bordeleau for teaching me about maternal immune activation and animal behavioural experiments.

Last, but certainly not least, I am forever grateful to my wonderful family, to whom I dedicate this thesis. Papa` e Mamma, Tullio e Dana Guma, grazie per i vostri sacrifici, il vostro sostegno ed incoraggiamento, per aver sempre creduto in me, e per avermi dato tutto ciò di cui avrei potuto aver bisogno. Thank you for being so understanding and supportive during this long journey; I am incredibly grateful. To my lovely siblings, Linda, Maria Grazia, and Marco Guma, thank you for being my best friends, for being a great source of inspiration, of support, but most importantly, of laughter, jokes, and silly fun. Thank you, Matthias Pries, for being my rock, for being so understanding and supportive, and for keeping me grounded. Thank you for infusing my life with joy and love during a very challenging year, and for helping me celebrate the big and small wins. I am lucky and grateful to have you in my corner and to have shared part of this journey with you. Nonna Arcangela and Zio Roberto Perin, thank you for creating a safe haven for me every Sunday evening. Thank you to Hilda and Gerhard Pries for your warmth, support, and generosity. Thank you to my friends Romane Dufoix, Lea Perret, Erin Yong Ping, Genny Cyr, and Dr. Lauryn Conway for your friendship and encouragement, for always believing in me and for making sure that I maintained a healthy work-life balance. Finally, thank you to the Visual Arts Center Ceramics Studio for helping me foster my creative side and discover my love for ceramics.

Contribution of authors

The original work in this thesis spans **Chapters 3, 4, 5**, and **6**. For each of these manuscripts, I led the experimental design, data collection, data processing, statistical analyses, and interpretation under the incredible guidance and supervision of Dr. M Mallar Chakravarty. I wrote the first draft of these manuscripts and incorporated revisions from co-authors and peer reviewers. Each study features a number of co-authors whose contributions were integral and invaluable to the completion of this work. Their contributions are demarcated below:

Chapter 3: Elisa Guma, Eric Plitman, M Mallar Chakravarty. The role of maternal immune activation in altering the neurodevelopmental trajectories of offspring: a translational review of neuroimaging studies with implications for autism spectrum disorder and schizophrenia. *Neuroscience & Biobehavioral Reviews*, 104:141-157, 2019.

- Eric Plitman: provided guidance on methodology, and the secondary check for article selection and inclusion in the systematic review
- M Mallar Chakravarty: supervised the study

Chapter 4: Elisa Guma, Pedro do Couto Bordignon, Gabriel A. Devenyi PhD, Daniel Gallino, MSc, Chloe Anastassiadis, Vedrana Cvetkovska, Amadou Barry, Emily Snook, Jurgen Germann, Celia M.T. Greenwood, Bratislav Mišić, Rosemary C. Bagot, M. Mallar Chakravarty. Early or late gestational exposure to maternal immune activation alters neurodevelopmental trajectories in mice: an integrated neuroimaging, behavioural, and transcriptional study. *Biological Psychiatry*, In Press, 2021. DOI: https://doi.org/10.1016/j.biopsych.2021.03.017.

- Pedro do Couto Bordignon: performed the RNA sequencing analyses.
- Gabriel A Devenyi: supported image processing, statistical analyses, and interpretation of analyses.
- Daniel Gallino: performed all the *in vivo* scanning and provided technical support for animal experiments.
- Chloe Anastassiadis: contributed to the behavioural data collection, and behavioural video analysis
- Vedrana Cvetkovska: performed the RNA extraction.
- Emily Snook: contributed to the behavioural video analysis
- Amadou Barry: provided Pedro do Couto Bordignon with support for the RNA sequencing analyses.
- Celia M.T. Greenwood: supervisor of Amadou Barry.
- Jurgen Germann: provided support for statistical analyses and data visualization.
- Bratislav Mišić: supported the implementation and results interpretation of the partial least squares analysis.
- Rosemary C. Bagot: provided support for the RNA sequencing analyses as well as with the writing of the paper.

- M Mallar Chakravarty: supervised the study and provided guidance in conceptualization of the study, interpretations of results, determination of follow up analyses, and writing of the manuscript
- All authors: provided critical or conceptual support and revised the manuscript

Chapter 5: Elisa Guma, Maude Bordeleau, Emily Snook, Gabriel Desrosiers-Gregoire, Shoshana Spring, Jason P. Lerch, Brian Neiman, Gabriel A Devenyi, Marie-Eve Tremblay, M Mallar Chakravarty. Early and late exposure to prenatal maternal immune activation alters fetal and neonatal mouse neurodevelopment. In preparation for *Molecular Psychiatry*

- Emily Snook: performed the ultrasonic vocalization data collection and neonate brain collection under my supervision and helped with the embryo collection.
- Maude Bordeleau: performed the tissue processing and electron microscopy.
- Gabriel Desrosiers-Gregoire: provided assistance in the initial embryo harvesting, assessing feasibility, and piloting scanning.
- Shoshana Spring: performed all of the *ex vivo* MRI scanning.
- Jason Lerch: provided assistance for the *ex vivo* MRI scanning, and experimental design.
- Brian Neiman: provided assistance for the *ex vivo* MRI scanning.
- Gabriel A Devenyi: provided support in MRI preprocessing, registration, and analysis. He also supported the implementation of the shift function to analyze behavioural data.
- Marie-Eve Tremblay: provided expertise and guidance for selecting the experimental design for the electron microscopy experiments and is a supervisor of Maude Bordeleau.
- M Mallar Chakravarty: supervised the study and provided guidance in conceptualization of the study, interpretations of results, determination of follow up analyses, and writing of the manuscript
- All authors: provided critical or conceptual support and revised the manuscript

Chapter 6: Elisa Guma, Lani Cupo, Daniel Gallino, Luc Moquin, Alain Gratton, Joe Rochford, M Mallar Chakravarty. Investigating the synergistic effects of prenatal maternal immune activation and adolescent cannabis use. In preparation.

- Lani Cupo: contributed to all data collection, including MRI and behavioural data acquisition, and daily injections
- Daniel Gallino: performed all the *in vivo* longitudinal MRI acquisition.
- Luc Moquin: performed the gas chromatography mass spectrometry data collection to validate the THC concentrations.
- Alain Gratton: supervised work performed by Luc Moquin.
- Joe Rochford: provided support for prepulse inhibition data collection and interpretation.
- M Mallar Chakravarty: supervised the study and provided guidance in conceptualization of the study, interpretations of results, determination of follow up analyses, and writing of the manuscript
- All authors: provided critical or conceptual support and revised the manuscript

Other related lead-author publications

Elisa Guma, Lenka Andrýsková, Milan Brázdil, M. Mallar Chakravarty, Klára Marečková (2021). Perinatal maternal mental health and amygdala morphology in young adulthood*, Under review*.

Elisa Guma, Jill Rocchetti, Gabriel A. Devenyi, Arnaud Tanti, Axel P. Mathieu, Jason P. Lerch, Guillaume Elgbeili, Blandine Courcot, Naguib Mechawar, M Mallar Chakravarty, Bruno Giros (2019). Role of D3 dopamine receptors in modulating neuroanatomical changes in response to antipsychotic administration. *Scientific Reports,* 24;9(1):7850.

Elisa Guma, Jill Rocchetti, Gabriel A Devenyi, Arnaud Tanti, Axel P Mathieu, Jason P Lerch, Guillaume Elgbeili, Blandine Courcot, Naguib Mechawar, M Mallar Chakravarty, Bruno Giros (2018). Regional brain volume changes following chronic antipsychotic administration are mediated by the dopamine D2 receptor. *Neuroimage,* 176:226-238.

Sina Hafizi*, **Elisa Guma***, Alex Koppel, Tania Da Silva, Michael Kiang, Sylvain Houlse, Alan A. Wilson, Pablo M. Rusjan, M. Mallar Chakravarty, Romina Mizrahi. TSPO expression and brain structure in the psychosis spectrum. *Brain, Behaviour, and Immunity*, 74:79-85.
*authors contributed equally

Elisa Guma, Gabriel A Devenyi, Jurgen Germann, M Mallar Chakravarty, Marita Pruessner (2017). Neuroanatomical and clinical sex differences in individuals at clinical high risk for psychosis. *Frontiers in Psychiatry*, 8:291.

Other related co-authored publications

Lani Cupo, Eric Plitman, **Elisa Guma**, M Mallar Chakravarty (2021). The Straight Dope: A Systematic Review of Neuroimaging and Acute Cannabis Exposure in Age-of-Risk for Psychosis. *In press Neuropsychopharmacology*.

Eric Plitman, **Elisa Guma,** Martin Lepage, Jamie Near, M. Mallar Chakravarty (2019). Using proton magnetic resonance spectroscopic imaging to study glutamatergic alterations in patients with schizophrenia: A systematic review. *Schizophrenia Research*, 210:13-20.

Daniel Gallino, Gabriel A Devenyi, Jurgen Germann**, Elisa Guma,** Chloe Anastassiadis, M Mallar Chakravarty (2019). Longitudinal assessment of the neuroanatomical consequences of deep brain stimulation: Application of fornical DBS in an Alzheimer's mouse model. *Brain Research*, 1715: 2132-223

Golia Shafiei, Ross D. Markello, Carolina Makowski, Alexandra Talpalaru, Matthias Kirschner, Gabriel A. Devenyi, **Elisa Guma,** Patric Hagmann, Neil R. Cashman, Martin Lepage, M. Mallar Chakravarty, Alain Dagher, Bratislav Mišić, Spatial patterning of tissue volume deformation in schizophrenia reflects brain network architecture (2020), *Biological Psychiatry,* **87:** 727-735.

Elsa Isingrini, Lea Perret, Quentin Rainer, Benedicte Amilhon, **Elisa Guma**, Aranud Tanti, Garence Martin, Jennifer Robinson, Luc Moquin, Fabio Marti, Naguib Mechawar, Sylvain Williams, Alain Gratton, Bruno Giros (2016). Resilience to chronic stress is mediated by noradrenergic regulation of dopamine neurons. *Nature Neuroscience,* **19**: 560-563.

Original contributions of thesis

Chapter 3

- Comprehensive literature review of all neuroimaging studies investigating the effects of MIA in offspring across 5 species: human, rabbit, macaque, rat, mouse
- By focusing on neuroimaging, a translational assay, we bridge the gap between clinical and preclinical findings on the effects of MIA-exposure
- Links gestational timing of MIA exposure and age of investigation in the offspring to different neuroanatomical deficits, providing a framework through which to interpret the disparate findings in the literature
- Identified areas for future investigation; specifically:
    - better longitudinal mapping,
    - the need for comparison of effects across gestational timings,
    - the need for preclinical studies focusing on offspring early life, and
    - the need for inclusion of males and females

Chapter 4

- Longitudinal *in vivo* characterization of the effects of MIA-exposure at early or late gestation on offspring neurodevelopmental trajectories from childhood to adulthood shows that early exposure induces greater alterations to brain structure and behaviour
- The alterations observed due to early MIA-exposure are most pronounced in the adolescent/early-adult period, but normalize in later adulthood
- Integration of neuroanatomical and behavioural data from deeply phenotyped mice at the time of greatest deviation (adolescence) revealed brain-behaviour patterns used to inform region of interest selection for transcriptional phenotyping
- Transcriptional phenotyping of adolescent brains in the more affected group (early MIA-exposure) revealed transcriptional regulation in a number of genes, including those involved in the fibroblast growth factor signaling, particularly in the dorsal hippocampus
- Applying a human neuroimaging-based experimental design for the study of neurodevelopment to rodent research to allow for better inter-species translation
- Novel framework for investigating neurodevelopment across disorders in rodents: integrating longitudinal neuroimaging, behaviour, transcriptomics

Chapter 5

- Novel investigation of the effects of early and late MIA-exposure on embryo and neonatal brain anatomy using high-resolution *ex vivo* MRI
- Building on Chapter 4, this extends the investigation of the effects of MIA-exposure on offspring development, from gestation to adulthood
- The observation that early and late MIA-exposure have differential effects on embryo brain anatomy, with late exposure causing greater neuroanatomical alterations

- Evidence of sex-dependent alteration in the density of dark neurons and dark glia in the late exposed MIA-exposed embryo brains, with an increase in females and a decrease in males
- The use of MRI to guide selection of regions of interest for *ex vivo* anatomical investigation in hopes of gaining a better understanding of the neuroanatomical underpinnings associated with MRI signal
- The observation that neuroanatomy is somewhat unaffected in the neonate brains and may represent a period of normalization
- Identification of subtle impairments in communicative abilities in neonates exposed to early MIA
- The integration of neuroanatomical and behavioural data

Chapter 6

- Investigation of the effects of early MIA-exposure and adolescent THC exposure on neurodevelopmental trajectories
- The identification of subtle effects on neuroanatomy due to MIA, which recapitulate those identified in Chapter 4, although at a subthreshold effect
- The identification of significant alteration in neuroanatomy associated with THC exposure, which normalize in later adulthood
- The observation that exposure to both risk factors, prenatal-MIA and adolescent THC induces deviations in neuroanatomical trajectory, most pronounced post-treatment, with some sustained alterations in adulthood
- Behavioural phenotypes are largely similar between groups, apart from some subthreshold alterations in anxiety-like behaviour in mice exposed to both risk factors
- Integration of neuroanatomical and behavioural phenotypes reveals the need to further explore sex-differences

CHAPTER 1: Introduction

In any given year, one in five Canadians experiences a mental illness, which has lifelong implications ("Mental Health Commission of Canada Commends Federal Investment in Child and Youth Mental Health, 2011). Many of these disorders, such as schizophrenia and autism spectrum disorder, are thought to have a developmental origin, and typically emerge in the childhood or adolescent period (Rapoport, Giedd, and Gogtay 2012; Inui, Kumagaya, and Myowa-Yamakoshi 2017). Their onset may interfere with important milestones, such as attainment of higher education, employment, marriage, and parenthood (Keshavan and Paus 2015; Keshavan et al. 2014; Paus, Keshavan, and Giedd 2008). The burden of mental illness extends beyond the affected individual, impacting family, society, and the healthcare system; as such, it is the leading cause of disability in Canada (Lim et al. 2008).

Fetal brain development consists of finely tuned spatially and temporally constrained events, which may be negatively influenced by exposure to a suboptimal intrauterine environment, significantly impacting the health of offspring throughout their postnatal life (Gluckman et al. 2008; Rees and Inder 2005). Epidemiological studies have documented numerous environmental exposures in gestation as risk factors for the onset of neurodevelopmental and psychiatric disorders later in the lifespan, such as autism spectrum disorder and schizophrenia (van Os, Kenis, and Rutten 2010; Gardener, Spiegelman, and Buka 2009). These include maternal infection during pregnancy, maternal stress or malnutrition, and obstetric complications (Knuesel et al. 2014; Brown and Derkits 2010; Guma, Plitman, and Chakravarty 2019). Converging evidence from several research disciplines has identified maternal infection during pregnancy, such as rubella, herpes simplex virus, cytomegalovirus, toxoplasmosis, chorioamnionitis, and others, as potent disruptors of fetal neurodevelopment, leading to congenital brain abnormalities, mental retardation, learning disabilities, and more (Wormser and Tolan 2006; Knuesel et al. 2014; Estes and McAllister 2016; Brown and Meyer 2018). Further, there is a wealth of epidemiological and experimental evidence associating exposure to prenatal maternal infection with increased risk for autism spectrum disorder, schizophrenia, bipolar disorder, and cerebral palsy (Knuesel et al. 2014). Given the breadth of pathogens associated with abnormalities in offspring neurodevelopment, it has been proposed that the maternal inflammatory response to the pathogen, rather than the pathogen itself, is interfering with normal brain development. Importantly, maternal immune

activation may be induced by other more common lifestyle factors such as chronic stress, poor nutrition, obesity, and more (Hantsoo et al. 2019). Given the range of stimuli that can increase maternal inflammation, there is an urgent need to understand its effects on offspring outcomes.

The mechanisms by which maternal immune activation (MIA) during pregnancy disrupts fetal brain development leading to psychopathology remain to be elucidated. Exposure to increased inflammation *in utero* may interfere with normal fetal brain development, particularly if it occurs during sensitive developmental windows. Determining which developmental windows, or gestational timepoints, are most sensitive to this risk factor may be critical in our understanding of how it may increase risk for psychopathology later in life. Another important gap in our understanding is the identification of the age at which deviations may be detected in the offspring, and the evolution of neurodevelopmental alterations throughout their lifespan. This level of characterization may not only inform our understanding of how this risk factor affects brain development but may aid in the identification of sensitive windows for intervention. A final consideration is that not all maternal infections lead to the development of neuropsychiatric disorders suggesting that this risk factor may act as a disease primer, making the brain more sensitive to exposures later in life (Estes and McAllister 2016). There are multiple postnatal risk factors whose exposure may further perturb neurodevelopment, including urban birth and upbringing, migrant status, child abuse or neglect, adolescent use of recreational drugs of abuse, amongst others (van Os, Kenis, and Rutten 2010).

One such risk factor is adolescent cannabis exposure, the most widely used recreational drug. The main psychoactive compound of cannabis, delta-9-tetrahydrocannabinol (THC), is responsible for the "high" felt during cannabis use and is associated with negatively affecting brain maturation and behaviour. Furthermore, both heavy use of cannabis and use of high potency cannabis (i.e., with very high THC concentration) has been associated with the emergence of psychosis in adolescence (Stefanis et al. 2004; Di Forti et al. 2014). Importantly, the THC content of cannabis has been increasing over the last few decades, as has the use of synthetic THC analogues (termed 'spice') (Freeman et al. 2019; El Sohly et al. 2016). Given the link between THC exposure and adverse outcomes, it is imperative to understand the neurobiological effects of THC exposure. Understanding how it may disrupt brain maturation is of the utmost importance to Canadians given the recent legalization of cannabis (Bloomfield et al. 2016; Volkow et al. 2016). Significant consideration should be given to understanding the impact of exposure on mental

health outcomes, especially as access to cannabis increases, and the perceived risk of consumption is low (Nashed, Hardy, and Laviolette 2020). Currently, there are limited studies that examine this association directly at the neurobiological level (Cupo et al. 2021). As is the case with MIA-exposure, not all individuals exposed to chronic cannabis use in adolescence develop psychosis, suggesting that pre-existing vulnerabilities may exist. Some evidence points to genetic predispositions (Caspi et al. 2005; Estrada et al. 2011b), however other early life risk factors may also play a significant role. To this end, we use animals exposed to MIA *in utero* as well as chronic adolescent THC exposure to model the potential additive effects of exposure to multiple risk factors. To our knowledge, no rodent work has been done to investigate longitudinal brain wide and behavioural changes following exposure to MIA and adolescent THC.

Specific Objectives of the Thesis

The overarching goal of this thesis is to investigate the effect of either early or late gestational exposure to maternal immune activation (MIA) on offspring neurodevelopmental trajectories. Additionally, we assessed how exposure to a second risk factor in adolescence, a developmentally sensitive period in which many disorders emerge, to further characterize if the risk of MIA exposure is sufficient on its own or can be exasperated by exposure to a second risk factor. In an established MIA mouse model, we acquired whole-brain longitudinal *in vivo* and *ex vivo* neuroimaging data across the lifespan of offspring (childhood to adulthood), performed multi-behavioural and transcriptional phenotyping, as well as post-mortem electron microscopy to gain a better understanding of the effects of MIA on offspring development to span varying scales of brain architecture. This comprehensive investigation allowed us to specifically address the following questions:

1) How prenatal exposure to maternal immune activation alters brain development and behaviours from childhood to adulthood;
2) How prenatal exposure to maternal immune activation alters embryonic and neonatal brain development; and
3) how these variations are further modulated by a second hit, namely adolescent exposure to the main psychoactive component of cannabis, THC.

CHAPTER 1

This thesis comprises an introduction **(Chapter 1**), a detailed background and neuroimaging methods sections (**Chapter 2**), followed by a comprehensive systematic review on the effects of MIA on offspring brain development across species as measured by neuroimaging methods (**Chapter 3**). Additionally, it includes three separate chapters which address the research questions above (**Chapters 4, 5, 6**). The background section introduces the reader to the epidemiological and experimental evidence, and potential neurobiological mechanisms associating both prenatal MIA and adolescent THC exposures as risk factors for neurodevelopmental and neuropsychiatric disorders. Further, the neuroimaging and transcriptional background covers important empirical foundations for the MRI-derived metrics and statistical analysis used throughout the thesis.

CHAPTER 2: Background

2.1 Evidence from epidemiology on maternal immune activation

2.1.1 Evidence for elevated risk for schizophrenia due to maternal infection

Schizophrenia is a debilitating neuropsychiatric disorder affecting 1% of the population worldwide. The complexity of the disease is profound, presenting with broad neuroanatomical and neurochemical alterations (Kahn et al. 2015; Selemon and Zecevic 2015). Symptoms usually begin in adolescence and early adulthood include cognitive impairments to memory and attention (which may present even earlier in childhood), positive symptoms such as hallucinations and delusions, and negative symptoms such as social and motivational deficits (Stachowiak et al. 2013). In recent decades, it has been increasingly accepted that schizophrenia is a disorder of neurodevelopment and that both genetic and environmental risk factors increase the likelihood of developing the disorder. Maternal infection has been identified as a risk factor that may alter neurodevelopment potentially leading to schizophrenia later in life (Selemon and Zecevic 2015; Rapoport et al. 2005).

The earliest evidence for the association between *in utero* exposure to maternal infection and increased risk for schizophrenia comes from ecological epidemiological studies that use naturally occurring epidemics, typically influenza epidemics, to define exposure status in the mother. One of the first observation dates back to the Spanish influenza (influenza A) epidemic of 1918-1919, which killed 50-100 million people in 1 year (more people than World War I), with the preponderance of deaths amongst 20–40-year-olds rather than children and elderly, common to other epidemics (Kendell and Kemp 1989; Yudofsky 2009). One of the few advantages of such a devastating event is that it allowed for the observation, recorded first by Karl A. Menninger, of the association between the increased prevalence of psychotic disorders in those exposed to maternal influenza.

Further, studies conducted in Finnish, Danish, and English populations identified higher prevalence of schizophrenia among individuals *in utero* during the severe 1957 A2 influenza epidemic in comparison to those whose *in utero* periods coincided with comparable non-epidemic periods. Mednick and colleagues found that individuals who were in their second trimester of fetal development during this epidemic were at higher risk for being hospitalized for schizophrenia as

adults. This was true when compared to individuals born in the same month and same city for the 6 previous years, in which there was not an influenza epidemic. Similar observations were found in Danish populations (Mednick et al. 1988; Barr, Mednick, and Munk-Jorgensen 1990). Similarly, studies conducted in England and Wales reported an 88% increased risk of developing schizophrenia for individuals born 5 months after the peak of the A2 influenza pandemic compared to the previous 2 and subsequent 2 years (O'Callaghan et al. 1991). Periconceptual exposure to genital and reproductive infections has also been associated with a fivefold increase in schizophrenia risk (Babulas et al. 2006); further, higher rates of schizophrenia in offspring of women receiving treatment for urinary tract infections during pregnancy have also been reported, especially in those with a family history of the disease (Clarke et al. 2009).

Even though, based on these studies, there was convincing evidence for second trimester exposure to maternal influenza and increased risk for developing schizophrenia, other reports did not replicate the association in Australian, Croatian, and Dutch cohorts (Morgan et al. 1997; Erlenmeyer-Kimling et al. 1994; Susser et al. 1994). This is possibly due to significant limitations in those studies; in nearly all of these studies the presence of exposure to influenza was solely based on whether an individual was *in utero* at the time of the influenza epidemic. There was no confirmation of maternal influenza during pregnancy. By relying on these criteria, approximately 70% of individuals who were *in utero* during the 1957 influenza epidemic but were not exposed would have been misclassified as exposed (Kilbourne 2006; Brown and Derkits 2010).

In order to overcome some of the limitations inherent to ecological studies relying on reports of epidemics, subsequent epidemiological studies have leveraged population-based birth cohort studies in which infection status was recorded for individual pregnancies. In some cases, infection status was determined by medical records, and in other cases it was prospectively acquired using serologic biomarkers of infection or inflammation in pregnant mothers, such as maternal serum samples, in combination with offspring outcomes later in life. These have further established the link between maternal infection and inflammation with psychotic disorders such as schizophrenia, and bipolar disorder. Below I will focus on the major birth cohort studies of prenatal infection and schizophrenia; most of the serologic studies come from three cohorts. These include the Child Health and Development Studies (CHDS) cohort composed of all the births from 1959-1967 in Alameda County, California (van den Berg, Christianson, and Oechsli 1988), the Collaborative Perinatal Project (CPP) cohort comprised of multiple birth cohorts born between

1959-1967 in different regions of the United States (Klebanoff 2009), and a Danish national birth cohort comprised of all pregnancies in that country since 1981 (Olsen et al. 2001). In the first two cohorts, maternal serum samples were archived during pregnancy, whereas the Danish cohort used filter paper blood spots taken from neonates to infer maternal status. These samples were used to confirm seropositivity for specific infections as measured by immunoglobulin G (IgG) antibody titer against specific pathogens. In confirmation of the previous ecological investigations, studies from all three birth cohorts found an increased risk for psychotic disorders or schizophrenia in offspring whose mothers experienced infection during pregnancy, confirmed by seropositivity for different pathogens. A nested case-control study from the CHDS cohort found a 3-fold increase in risk for developing schizophrenia for serologically confirmed exposure to influenza in the first (but not second) half of pregnancy, and a 7-fold increase for exposure occurring during the first (but not the second or third) trimester (Brown, Begg, et al. 2004)**.**

Further evidence comes for exposure to *Toxoplasma gondii (T. gondii),* which causes toxoplasmosis, and can live in the host for a long period of time without displaying overt symptoms. Infants who have been exposed *in utero* have been found, in some cases, to develop serious eye and brain damage. In the same (CHDS) cohort, exposure to *T. gondii* IgG antibody (from maternal sera) *in utero* was associated with increased risk for developing schizophrenia; specifically, women with the highest antibody levels (upper decile) had 2.6-fold increased risk of having offspring with schizophrenia (Brown et al. 2005). A similar observation was made in the Danish birth cohort based on neonatal levels of *T. gondii* IgG antibody measured on filter paper blood spots. They found that infants with the highest IgG levels (upper quartile) had a 1.78-fold increased risk for developing schizophrenia; the risk was further elevated when focusing specifically on early onset cases (Mortensen et al. 2007).

Neonatal exposure to the Herpes Simplex Virus (HSV) Type 2 (HSV-2), a sexually transmitted virus, has also been associated with adverse neonatal outcomes such as congenital abnormalities and psychiatric illness, similar to those following exposure to *T. gondii* (Fa et al. 2019). Within the multi-site CPP, a small case-control study coming from the Rhode Island cohort found that individuals with psychosis had mothers with increased levels of serum IgG antibody for HSV-2 at delivery (Stephen L. Buka et al. 2002). A larger follow-up study of 200 individuals with psychosis and 500 matched controls from multiple CPP sites (Boston, Providence, Philadelphia) confirmed this association. They found a 1.6-fold increase in risk for psychosis, and a 1.8-fold

increase in risk for schizophrenia in offspring whose mothers were seropositive for HSV-2 (Buka et al. 2008). Interestingly, this finding was not replicated in the CHDS birth cohort, however they had a smaller sample, and thus may have been underpowered (Brown et al. 2006).

Exposure to rubella has also been associated with increased risk for developing schizophrenia, as documented by medical records and maternal sera (A. S. Brown et al. 2000). Studies from the Rubella Defects Evaluation Project birth cohort, based on the 1964 rubella pandemic, have identified that 20% of individuals whose mothers who had rubella during pregnancy (confirmed clinically and serologically), went on to develop schizophrenia spectrum disorders (Brown et al. 2001; Patterson 2009). This 20% increase is a 15- to 20-fold increase in prevalence relative to the typical ~1%. Further, maternal bacterial infection during pregnancy has been strongly associated with psychosis in offspring. A study of births in Copenhagen in 1959-1961 found increased risk for schizophrenia in offspring whose mothers had a documented infection in the first (odds ratio [OR]=2.53) or second (OR=1.17) trimesters (Sorensen et al. 2009). A recent study from the CPP cohort has replicated this finding. They report maternal bacterial infection during pregnancy was strongly associated with psychosis in offspring (OR=1.8). Further, they identified that multisystemic bacterial infections, which cause a more severe inflammatory response in the mother, were associated with an even higher risk (OR 2.9), and that male offspring were more likely than females to develop psychosis following *in utero* exposure to maternal bacterial infection (Lee et al. 2020). Importantly, there is extensive evidence linking exposure to maternal infection with increased risk for neuropsychiatric illness, however some important confounds need to be considered, including maternal psychiatric conditions, socio-economic status, and other factors contributing to maternal health such as stress, nutrition, and more. Some of these studies do account for many of these confounds, however, not all cohorts may have access to all of this information. These factors are discussed in more detail in **section 2.3.**

2.1.2 Evidence for elevated risk for autism spectrum due to maternal infection

Autism spectrum disorder (ASD) is a heterogeneous neurodevelopmental disorder characterized by impaired social behaviours and communications, as well as restricted and repetitive interests (Joseph, Tager-Flusberg, and Lord 2002; Wetherby et al. 2007; Joshi et al. 2017). Like schizophrenia, there is a significant genetic component (Geschwind 2011; Geschwind

and State 2015), however it is well accepted that certain environmental exposure increases the risk for developing this disorder (Noriega and Savelkoul 2014; Dietert, Dietert, and Dewitt 2011; L. Liu et al. 2016; Lyall, Schmidt, and Hertz-Picciotto 2014). Although epidemiological studies investigating exposure to maternal infection *in utero* as a risk factor for aberrant neurodevelopment has focused on schizophrenia, mounting evidence indicates that maternal infection may also increase the risk for ASD in the offspring (Atladóttir et al. 2010; Jiang et al. 2016).

A study using data from the Danish Medical Birth Register, which identified all children born in Denmark from January 1, 1980 to December 31, 2005, and has all records of hospital admissions, found that hospitalization for viral infection in the first trimester was associated with a higher rate of ASD diagnosis (hazard ratio=2.98). They also found that hospitalization for any infection (mainly driven by bacterial infections) in the second trimester was associated with the development of ASD in the offspring (hazard ratio=1.3) (Atladóttir et al. 2010). This study only focused on infections requiring hospitalization, suggesting that they were quite severe, and thus excluded the majority of infections were likely less severe and treated as outpatients. Similar work using self-reported data on infection and febrile episodes from the Danish National Birth Cohort (women recruited from 1996 to 2002) found little association between mild common infectious diseases or febrile episodes during pregnancy and ASD in offspring. However, they did find that influenza exposure during gestation was associated with a twofold increased risk for infantile ASD, and that long episodes of fever caused a threefold increase in risk for both infantile ASD and ASD (Atladottir et al. 2012). Conversely, another study investigating newborn screening filter paper blood specimens from children born in 1994 to mothers residing in San Francisco, California, surprisingly found that lower IgG, HSV IgG, and *T. Gondii* IgG antibody levels in neonates was associated with increased risk for ASD (Grether et al. 2010). This indicates that ASD risk may be associated with an imbalance in inflammatory markers. Another group investigating mothers pregnant in the Kaiser Permanente Northern California region reported no association between maternal infection and risk for developing ASD in offspring; they only found associations for severe infections requiring hospitalization, specifically bacterial infection, or exposure to multiple infections during pregnancy (Zerbo et al. 2015).

2.1.3 Evidence for maternal infection as a risk factor for other disorders

The bulk of epidemiological findings for *in utero* exposure to maternal infection and subsequent risk for disorders in the offspring has focused on schizophrenia and ASD, however there is some evidence for other disorders. A few epidemiological studies have evaluated maternal immune activation associated with bipolar disorder in offspring, but findings are mixed. A study using the CHDS cohort found that offspring exposed to maternal influenza at any time during pregnancy were almost 4 times more likely to develop bipolar disorder than those who were not exposed (OR=3.82), based on prospective physician diagnoses of maternal influenza (Parboosing et al. 2013). Further, when restricting analysis to only include bipolar disorder with psychotic features there was a significant 5-fold increase for third trimester exposure, and a not statistically significant 6-fold increase for second trimester exposure (Parboosing et al. 2013). This may provide evidence for exposure to maternal infection *in* utero as a risk factor for a range of psychotic disorders and not just schizophrenia. However, based on maternal serum samples, others have only found an association for bipolar disorders with psychotic features (5-fold increase; (OR=5.03, 95% confidence interval [CI]=1.38–18.38), but not for all bipolar cases (OR=1.26, 95% [CI]=0.65–2.44) (Canetta et al. 2014). Finally, some groups have found no association between exposure to maternal infection and bipolar disorder (Alan S. Brown 2015). For example, seropositivity for HSV-2, HSV-1, or cytomegalovirus collected by neonatal dried blood spots were found not to be related to bipolar disorder in a Danish birth cohort (Mortensen et al. 2011).

Another study from the CHDS cohort found that maternal exposure to *T. gondii* was related to an increased risk for affective psychosis, which include bipolar disorder, however they did not directly test for bipolar disorder (Xiao et al. 2009). A Finnish study investigating the effects of the 1957 A1/Singapore influenza virus epidemic found that children born between mid-November 1957 to mid-August 1958 had an increase in hospital diagnoses for major affective disorders, especially if their mothers were in the second trimester of pregnancy during the influenza epidemic. These effects were stronger in men, particularly for unipolar depression, but also for bipolar disorder and other forms of affective disorders (Machon, Mednick, and Huttunen 1997).

2.2 Inflammatory milieu and development

2.2.1 Innate and adaptive immunity

The mammalian immune system has evolved a complex series of immune responses to defend against several different types of pathogens including bacteria, viruses and parasites, as well as injury, to ensure health and survival (Loubser 2012). This is typically divided into two lines of defense. The first line is phylogenetically ancient, and present in most vertebrates and invertebrates and is very rapid. It involves elicitation of the innate immune response, a stereotyped set of defense mechanisms that are not pathogen specific (Ellis, Mouihate, and Pittman 2005). It primarily consists of fever, which is a highly conserved response to infection; it is metabolically costly, involving an increase in core body temperature, which leads to a reduction in viral and bacterial loads in the host (Kluger et al. 1998). Additionally, macrophage cells found in tissue, and neutrophil granulocytes, found in blood and tissue are recruited to enclose the pathogen and phagocytose it. The innate immune response is also associated with increased inflammation, activation of the hypothalamic-pituitary-adrenal (HPA) axis, and various sickness-induced behaviours (lethargy, hypersomnia, anhedonia) (Wolman 1988).

The second line of defense is termed adaptive or humoral immune response. This response is pathogen specific and has evolved in higher vertebrates. The main effectors of the adaptive immune response are lymphocytes and antibodies, allowing the organism to have "immunological memory" (Goronzy and Weyand 2012). It requires more time to be effective and is developed throughout the lifespan. It is less relevant to the work presented in this thesis, as we are focused on the role of the innate immune response.

2.2.2 Cytokines in normal development

Cytokines also contribute to normal central nervous system development and are expressed during fetal brain development (Mehler and Kessler 1997). The presence of cytokines and chemokines have been detected in fetal forebrain cells as early as 5 weeks of gestation, suggesting they play a role in normal brain development (Mousa et al. 1999). They play an important role in the regulation of neuronal migration and synaptic plasticity (Rostène, Kitabgi, and Parsadaniantz 2007; Bauer, Kerr, and Patterson 2007). These processes are tightly regulated, thus, even subtle

increases in cytokine concentration due to maternal immune response may disrupt the maternal-fetal cytokine balance and have deleterious effects on the developing fetus.

Interleukin (IL)-6, in particular, has been identified as a key contributor to the pathological effects observed in disorders such as schizophrenia and ASD (Meyer, Feldon, and Dammann 2011; Prata et al. 2017; Smith et al. 2007). It plays important roles in inducing differentiation, regulating axonal guidance and synapse formation (Parker-Athill, Carla Parker-Athill, and Tan 2010). It is still unclear how these effects are mediated, and whether or not certain maternal cytokines cross the placental barrier (Aaltonen et al. 2005; Zaretsky et al. 2004) or simply increase placental inflammation (Ashdown et al. 2006; Zaretsky et al. 2004; Girard et al. 2010), however it is believed that maternal immune activation somehow triggers fetal immune activation.

Although cytokines have been found to support various aspects of brain development *in utero*, the role and function of specific cytokines may change depending on the developmental phase in the prenatal and postnatal brain. For example, IL-1β, a pro-inflammatory cytokine, functions as an astroglial growth factor in the fetal (rat) brain. Further, *in vitro* evidence suggests that it is capable of converting rat mesencephalic progenitor cells into dopamine cells in combination with IL-6 (Ling et al. 1998; Potter, Ling, and Carvey 1999). If concentrations of IL-1β and IL-6 are too low, they no longer promote the differentiation of dopamine neurons (Jarskog et al. 1997). Furthermore, elevated IL-6 levels have been shown to decrease survival of fetal serotonin neurons (Jarskog et al. 1997). Similarly, low or high concentrations of TNF- α, and high concentrations of IL-1β and IL-6 have been shown to disrupt cortical neuron dendrite development in culture (Gilmore et al. 2004). Importantly, there is an age-dependency to the roles of these cytokines, as they are expressed at low levels in the adult brain, and only upregulated in response to host defence of injury (Ratnayake et al. 2013).

2.2.3 The role of the placenta: maternal-fetal immune regulation

The placenta plays a central role in regulating the rate and timing of fetal growth and development via nutrient transport, endocrine regulation, and serves as an important interface for maternal-fetal immune regulation (Hsiao and Patterson 2012). It is also intimately associated with the mechanisms underlying various obstetric complications such as growth restriction, hypoxia, chorioamnionitis, and related neurodevelopmental complications (Nugent and Bale 2015). This

transient organ is unique as it has both fetally and maternally derived cells (Hsiao and Patterson 2012). The maternal compartment, or decidua, is the most superficial layer and is densely packed with maternal immune cells (Hsiao and Patterson 2012). Beneath the decidua is a layer of fetally derived trophoblast cells, which release hormones and endocrine factors to support maternal and fetal health. Lastly, beneath the trophoblast layer, there is the villous space (chorionic villi in humans or labyrinth layer in mice). Here, both maternal and fetal blood circulate separated by two layers of fetal trophoblast cells (syncytiotrophoblasts), which filter the entrance of nutrients, oxygen, etc. from the maternal to fetal bloodstream (**Figure 2.1**) (Hsiao and Patterson 2012).

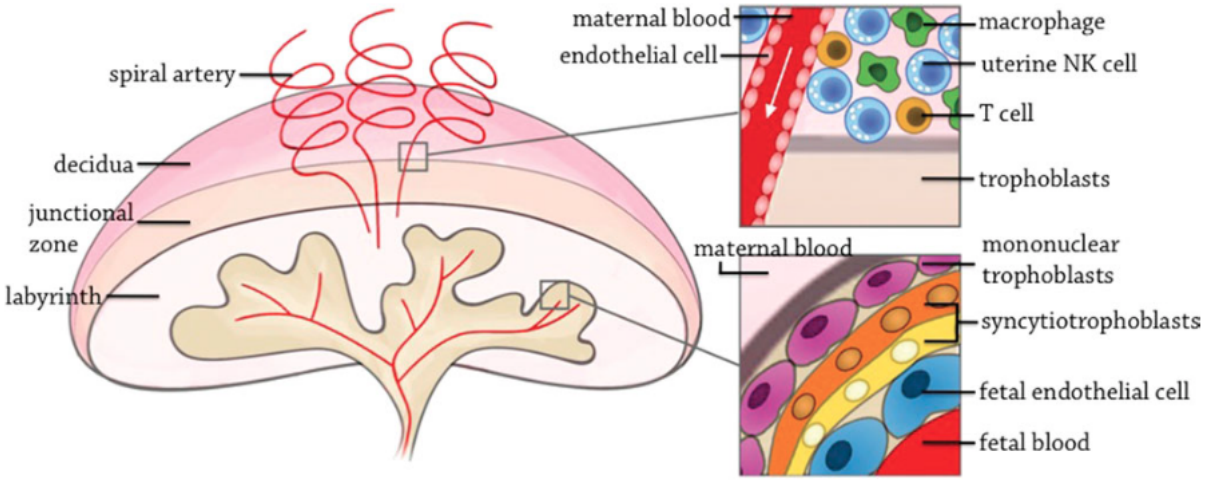


Figure 2.1 The maternal–fetal interface in the mouse placenta. Maternal and fetal cells interact directly in the placenta, during gestation. The placenta has dual origin: the outer layer (decidual) is composed of maternal immune cells, while the underlying layers (junctional zone and labyrinthine layers - chorionic villous layer in humans) are composed of fetally derived cells (trophoblasts and leukocytes). Spiral arteries (maternal immune and endothelial cells) cross the maternal cells in the decidua, and the fetal cells in the junctional zone (upper right). In the labyrinth layer, intervillous spaces are lined by fetal syncytiotrophoblast cells, mononuclear trophoblast cells, and fetal endothelial cells that separate maternal from fetal blood (lower right). Figure from (Hsiao and Patterson 2012).

As discussed below (**2.2.4**), normal pregnancy involves several changes to the immune system (including pro-inflammatory phases and immunosuppressed ones). The placenta still retains its ability to respond to and detect infection and inflammation (Heerema-McKenney 2018). MIA upregulates the production of soluble immune factors, such as pro-inflammatory cytokines, chemokines, and reactive oxygen species, which travel to the labyrinth layer via maternal blood

and are detected by placental receptors (Hsiao and Patterson 2012; Zhang et al. 2007). The degree to which maternal pro-inflammatory cytokines can cross the placental barrier to access the fetal compartment is somewhat unclear. The placenta is known to be more permeable to cytokines if damaged, however less is known regarding permeability in the healthy placenta, and whether this differs by gestational stage (Chandorkar et al. 1999). Under normal conditions, there is an increase in the number of immunosuppressive regulatory T cells around implantation, which prevents the proliferation and activation of pro-inflammatory T helper type (Th)17 cells (Robertson, Care, and Moldenhauer 2018). Certain cytokines such as IL-6 have been found to play a critical role in maintaining the balance between regulatory T cells and Th17 cells; an increase in IL-6 would shift the balance towards increased Th17 cells, and thus a more pro-inflammatory state (Kimura and Kishimoto 2010). Some have studied the human placenta at term and found that IL-6 as well as low amounts of IL-1, and TNF- α cross the placental barrier (Zaretsky et al. 2004; Kent, Sullivan, and Elder 1994; Aaltonen et al. 2005), but that IL-8 does not (Reisenberger et al. 1996). One caveat is that these studies assessed perfusion rates in placentas after delivery; it is possible that delivery may change placental functions in comparison to how it behaves *in utero*. Therefore, more evidence is necessary to demonstrate that cytokines can actually cross the placental barrier. In order to maintain normal functioning and structural integrity, the placenta produces and secretes cytokines (Meyer, Feldon, and Yee 2009; Jonakait 2007). It is possible that elevated cytokine levels from the maternal blood in response to infection do not actually cross the placenta, but rather, induce elevated production of cytokines from the placenta itself. This may explain the presence of elevated cytokines in the amniotic fluid and fetal circulation (Ratnayake et al. 2013).

An *in vitro* study performed on trophoblast cells from the human first trimester placentas (obtained following elective termination) found that stimulation of the Toll Like Receptor (TLR)-3 receptors with polyinosinic:polycytidylic acid (poly I:C) (but not TLR-4 via LPS) induced an inflammatory response; these cells release pro-inflammatory mediators such as interferon beta, secretory leukocyte protease inhibitor, amongst other intracellular factors (Abrahams et al. 2006). Interestingly, the permeability of the placenta may change throughout gestation; for example IL-6 has been found to cross the placenta at mid-gestation, but not in late gestation, in rats (Dahlgren et al. 2006). Others have shown that MIA in early/middle (GD 9) gestation in the mouse elevates protein IL-6 levels in the brain without increasing endogenous fetal IL-6 production, suggesting some transplacental passage of maternal IL-6 to the fetal system (Meyer et al. 2006). TGF-β1 has

also been shown to cross the placenta in mice (Letterio et al. 1994). Conversely, immune stimulation late in mouse gestation with bacterial endotoxin mimetic, lipopolysaccharide (LPS), has been shown to induce increases in TNF-α, IL-1β and IL-6 in maternal plasma and placenta, but not in the fetal liver or brain, but to increase IL-1β in fetal plasma, suggesting transplacental transfer (Ashdown et al. 2006) It is unclear whether other pro- or anti-inflammatory cytokines or chemokines (or other immune molecules) can efficiently cross the placenta. Further, there are some structural and functional differences between human and rodent placentas. Both have hemochorial placentas, which means that the trophoblast layer is in direct contact with maternal blood, not separated by endothelium (Schmidt et al. 2015). However, an important difference lies in the fact that mice have an inverted yolk sac placenta, which becomes active early in pregnancy and persists to term; the yolk sac is completely absent from humans (Schmidt et al. 2015). This structure plays important functions in rodent development, as malformations to the yolk sac are related to offspring abnormalities (Beckman et al. 1990). Further, it may affect the way in which chemicals and or/pharmaceutical cross the placenta (Beckman et al. 1990). Additionally, rodents are multiparous, with one placenta per fetus; it is possible that not all placentas function in the exact same manner, potentially influencing each fetus differently.

2.2.4 Maternal immune system during pregnancy

During pregnancy the maternal endocrine and immune systems must undergo numerous changes to create a hospitable environment for the growing fetus. Typically, there are three distinct immunological phases that are characterized by distinct biological processes. The first and early second trimester of pregnancy represent a first, strong pro-inflammatory phase. During this time implantation and placentation occur, the blastocyst is required to break through the epithelial lining of the uterus in order to successfully implant causing damage to the endometrial tissues. Additionally, maternal blood vessels must be recruited to secure adequate placental-fetal blood supply. These processes require significant cycling between cell death and cell repair; further in order to induce repair of the uterine epithelium and removal of cellular debris, a pro-inflammatory environment is required (Dekel et al. 2010; Mor and Cardenas 2010), which further contributes to the feeling of morning sickness women may experience in the first trimester.

During the second immunological phase an anti-inflammatory state is induced, and the mother, placenta, and fetus achieve a symbiotic relationship. The fetus is undergoing rapid growth

and development, and the mother tends to feel better, no longer suffering from nausea and fever (Mor and Cardenas 2010; Morelli et al. 2015). This corresponds mainly to the second trimester and part of the third.

The final immunological phase occurs close to delivery when the fetus is developed, and its organs are functional. This final phase is characterized by increased inflammation; during parturition there is an influx of immune cells which promotes uterine contractions to birth the fetus and expel the placenta (Romero et al. 2006). Differences in pro- and anti-inflammatory states and corresponding cytokine profile may affect maternal sensitivity to infectious diseases. Women tend to be more susceptible to illness during the first half of pregnancy, with a decline in risk during the second half of pregnancy (Jamieson, Theiler, and Rasmussen 2006; Mor and Cardenas 2010). This timing corresponds with epidemiological evidence of increased risk for adverse outcome in offspring for women who do fall ill in the first half of pregnancy (Barr, Mednick, and Munk-Jorgensen 1990).

2.3 Possible mediators between prenatal infection and altered fetal development

2.3.1 Cytokines as possible mediators

Mounting evidence from epidemiological studies detailed above, as well as preclinical research, support the link between exposure to maternal infection and heightened risk for neuropsychiatric disorders. However, how is it possible that such a diverse group of pathogens all increase risk for neurodevelopmental disorders? Several models have attempted to explain how an infection experienced by the mother could increase the risk of neurodevelopmental anomalies in the offspring. The common thread to the diverse group of pathogens is the maternal immune response. This involves an increase in the production of pro-inflammatory cytokines, which are a family of low molecular weight soluble proteins that play a critical role in the host response to infection, adaptive, and innate immunity (Curfs, Meis, and Hoogkamp-Korstanje 1997).

Cytokines are typically classified according to their functions in either inducing (pro-inflammatory) or suppressing (anti-inflammatory) inflammation. Some pro-inflammatory

cytokines include IL-1β, IL-6, and tumor necrosis factor (TNF)-α. These play a role in host defence against a variety of infectious agents or diseases by promoting fever and recruitment of other immune cells such as monocytes, macrophages, and T and B lymphocytes (Ratnayake et al. 2013; Dinarello 2000). They may also control immune cell differentiation and homeostasis, as well as cell apoptosis and inhibition of protein synthesis (Meyer, Feldon, and Yee 2009). Anti-inflammatory cytokines such as IL-10 and Transforming Growth Factor-Beta (TGF-β) are immunomodulators important for limiting excess inflammatory reactions (Opal and DePalo 2000). They act in concert with specific cytokine inhibitors and soluble cytokine receptors to repress the expression of inflammatory cytokines by activated macrophages. IL-10, specifically, can act on inflammation at multiple levels by upregulating endogenous anti-cytokines and downregulating pro-inflammatory cytokine receptors (J.-M. Zhang and An 2007).

Cytokines are released by a variety of immune cells (and other cell types), particularly helper T cells and macrophages, in response to a range of environmental stimuli (Curfs, Meis, and Hoogkamp-Korstanje 1997; Borish and Steinke 2003). They bind to specific cell-surface receptors which are often expressed on glial cells, such as microglia (resident immune cells of the brain) and astrocytes, as well as neuronal cells. When they bind to a cell, they trigger a signaling cascade that leads to an increase in the production of immune signaling molecules, as well as changes in gene expression in that target cell (Meyer, Feldon, and Yee 2009).

While the increase in pro-inflammatory cytokines has been shown to disrupt neurodevelopment, this may not be the full picture. It has been hypothesized that the disruption is due to a shift in balance of pro- and anti-inflammatory cytokines, and that an excess increase in either could disrupt normal brain development (Meyer, Feldon, and Yee 2009). Interestingly, overexpression of either the pro-inflammatory cytokine IL-6 (Smith et al. 2007) or the anti-inflammatory cytokine IL-10 (Meyer et al. 2008) have both been shown to induce cognitive and behavioural abnormalities in offspring. Interestingly, when viral mimetics are administered to IL-6 knockout mice, or when they are administered with IL-6 blocking antibodies, offspring deficits are no longer detected, whether at the behavioural or transcriptional level (Smith et al. 2007).

Autoimmune disorders, characterized by increased inflammation, have also been associated with increased risk for neurodevelopmental disorders and can be thought of as a pathogen free model of MIA. Maternal Type I diabetes (Xiang et al. 2018), rheumatoid arthritis (Wojcik et al. 2017), systemic lupus (Vinet et al. 2015), and thyroid disease (Brown et al. 2015)

have been identified as risk factors for neurodevelopmental disorders including ASD, schizophrenia, attention hyperactivity deficit disorder, and mood disorders.

2.3.2 Evidence for cytokines as mediators in schizophrenia

In order to further investigate the role of immune markers such as pro-inflammatory cytokines and chemokines in neurodevelopmental disruption following maternal immune activation, some have investigated whether or not elevation of specific maternal cytokines was associated with increased risk for schizophrenia in offspring. One study based on the Prenatal Determinants of Schizophrenia Study (Susser et al. 2000) found a significant association between mean second-trimester serum levels of IL-8 in mothers of offspring who developed schizophrenia spectrum disorders (n=59; n=105 controls) (Brown et al. 2004); no associations were observed with other pro-inflammatory cytokines such as IL-1β, IL-6, and TNF-α. Increased exposure to maternal IL-8 (also known as chemokine [C-X-C motif] ligand 8) was also associated with more severe neuroanatomical abnormalities in the offspring experiencing schizophrenia spectrum disorders, discussed in more detail in **Chapter 3**, including reduced volume of the posterior cingulum, left entorhinal cortex, and larger ventricular volume (Ellman et al. 2010). This chemokine may be stimulated by both bacterial and viral infections and is associated with chorioamnionitis (Shimoya et al. 1997; Brauner et al. 2001; Adachi et al. 1997). It plays a role in the immune response by activating T lymphocytes and participating in a cascade of events that results in an increase in oxygen free radicals (Baggiolini 1998).

Another study leveraging the Providence cohort of the CPP found that for 27 subjects with psychosis and 54 matched controls, elevated maternal TNF-α levels measured at term were associated with schizophrenia and psychotic disorders in the offspring (Buka et al. 2001). This was further associated with a history of infection during the third trimester of pregnancy. This peptide plays a central role in human immune response to infection. No associations were found in IL-1, IL-2, IL-6, and IL-8 in this cohort. In both of these studies, however, neither maternal obesity, nor other lifestyle factors known to increase systemic levels of inflammation were controlled for, which may influence the findings (Ellulu et al. 2017). A more recent study used maternal serum samples from Philadelphia Cohort of the National Collaborative Perinatal Project collected between 1959 to 1965 at several sites across the USA. They observed increased concentrations of

pro-inflammatory cytokines TNF-α, IL-1β, and IL-6 in maternal serum of offspring who later developed psychosis; interestingly, they analyzed data based on week of gestation and found that the greatest differences were present for the first half of pregnancy (weeks 7-20), with no difference in the second half of pregnancy (Allswede et al. 2020). While the evidence provided above heavily suggests that there is reasonable evidence associating elevated cytokine levels to schizophrenia risk, there are conflicting reports. For example, in two Scandinavian birth cohorts (Danish and Swedish), neonatal cytokine profiles were found not to differ between individuals with schizophrenia and controls, indicating that further research is required to better identify the link between MIA-exposure and offspring risk (Nielsen et al. 2015; Gardner et al. 2013).

2.3.3 Evidence for cytokines as mediators in autism spectrum disorder

Building on epidemiological evidence, the Early Markers for Autism (EMA) study is a population-based, nested case-control study in which maternal blood was collected mid-pregnancy, and infant blood collected at birth in hopes of examining biological markers associated with risk for developing ASD. This cohort includes mother-baby pairs born in California between July 2000 and September 2001 (Brown 2012). In this cohort of 84 individuals with ASD and 159 controls, 17 cytokines were assayed from the maternal serum samples in relation to later ASD diagnosis. IL-4, IL-5, and interferon-gamma were found to be significantly elevated in women whose children developed ASD. Although not specifically associated with maternal infection, the pro-inflammatory cytokine profile observed was consistent with the phenotype of allergic asthma, a chronic inflammatory disorder (Goines et al. 2011).

Additional work from the Danish Historic Birth Cohort examined cytokine profiles in samples of amniotic fluid for ASD cases (331) and controls (698). Higher levels of TNF-α and TNF-β were found in the amniotic fluid of children who went on to develop ASD. For subjects born after 1993, an ASD diagnosis was also associated with increased IL-4 and -5 levels (Abdallah et al. 2013). A limitation of this work is that amniotic fluid is thought to be more reflective of the fetal rather than maternal cytokines levels. However, the EMA study reported increases in the same pro-inflammatory cytokines, IL-4 and IL-5, in maternal serum to be associated with increased ASD risk, indicating that maternal increases in pro-inflammatory cytokines could lead to fetal increases in the same cytokines.

2.3.4 Fever as a possible mediator

Fever often accompanies an increase in pro-inflammatory cytokines during infection and is considered a possible mediator of prenatal infection and abnormal fetal neurodevelopment. Fever during pregnancy has been associated with increased risk for developing schizophrenia, particularly in the first half of pregnancy, based on a retrospective self-report questionnaire of mothers whose children developed schizophrenia or schizoaffective disorders (Torrey, Rawlings, and Yolken 2000). Fever during pregnancy has also been identified as a risk factor for ASD in various large birth cohort studies including prolonged fever (Atladottir et al. 2012), untreated fevers in the first and second trimesters (Hornig et al. 2018; Zerbo et al. 2013; Croen et al. 2019), or third trimesters (Brucato et al. 2017). However, many of these studies could not separate the effects of the fever from the infection, as they tend to occur in tandem. Other studies have reported associations between fever during pregnancy and other structural and functional deficits in offspring including growth retardations, malformations, and fetal death in very extreme cases. In line with some of the observations made with MIA-exposure, fever exposure in the first trimester has been associated with more severe structural malformations including neural tube defects, congenital heart defects, and oral clefts (Sass et al. 2017; Dreier, Andersen, and Berg-Beckhoff 2014). Further, there is preclinical evidence for cell death, membrane disruptions, vascular disruptions, placental infarction following high fever exposure (Edwards 2006; Edwards, Saunders, and Shiota 2003).

2.3.5 Stress and the Hypothalamic-Pituitary-Adrenal (HPA) axis

Elevated cytokine levels and increased inflammation have been associated with numerous types of stress (Hantsoo et al. 2019). The relationship between stress and inflammation is mediated via the crosstalk between the hypothalamic-pituitary-adrenal (HPA) axis and the immune system. Glucocorticoids, a type of corticosteroid hormone released by the adrenal glands (part of the HPA axis) in response to stressors, play an important role in the crosstalk between the body's stress and immune systems. These molecules have been found to impact innate and adaptive immunity by modulating activity of numerous immune cells (e.g., monocytes and macrophages) and immune mediators (e.g., cytokines and chemokines), often exerting anti-inflammatory action (Hantsoo et al. 2019). For example, glucocorticoids have been shown to exhibit anti-inflammatory action by

decreasing febrile response and cytokine release in response to infection (Barnes 1998). They have also been found to inhibit both the vasodilation and increased vascular permeability that typically occurs following an increase in inflammation. Corticotropin-releasing factor (CRF), an upstream regulator of stress pathway activation has also been shown to interact with the immune system, exerting anti-inflammatory action peripherally in various injury models (Karalis et al. 1995; Wei et al. 1986).

The timing at which immune molecules and stress hormones are upregulated seems to be critical in determining the downstream effects. Although glucocorticoids seem to have anti-inflammatory roles, if glucocorticoids are circulating in elevated levels prior to an immune challenge, they actually enhance the immune response rather than dampen it. Thus, elevated stress may synergize with inflammation to create an unfavourable inflammatory state (Sapolsky, Romero, and Munck 2000). CRF, may also act as an immune stimulant rather than suppressant, enhancing B and T cells proliferation in response to various antigens (Wei et al. 1986). As discussed in section **2.2.4**, the inflammatory milieu undergoes large changes during gestation, to which the glucocorticoid system adapts. For example, inflammatory responses to acute stresses are typically dampened during pregnancy. If the regulation between cytokines and glucocorticoids is hampered, due to factors such as chronic stress, a maladaptive stress response, or an inability to dampen cytokine release in response to stress, there may be negative effects on offspring outcomes (Cohen et al. 2012).

Associations between prenatal maternal stress and offspring outcomes have been heavily studied; most often, negative outcomes are reported for offspring, with higher incidence of neuropsychiatric disorders and problematic behaviour (MacKinnon et al. 2018). Few studies have directly investigated the relationship between maternal stress, MIA, and offspring outcomes in conjunction. Increased levels of prenatal maternal stress have been correlated with higher circulating levels of pro-inflammatory cytokines IL-6 and TNF-α, particularly in the first trimester, which tends to be more "pro-inflammatory" in nature (Coussons-Read et al. 2005); this increase in first trimester IL-6 and TNF-α levels has been associated with poor pregnancy outcomes and a greater chance of premature birth, both unfavourable for the offspring (Miller et al. 2017; Coussons-Read et al. 2012). Previous work has found that IL-8 levels during pregnancy mediated the relationship between chronic maternal stress due to socioeconomic status and risk of neurological abnormalities in offspring (Gilman et al. 2017). More recently, increased IL-6 levels

across pregnancy due to various lifestyle factors has been associated with increased amygdala volume and connectivity in infants, a structure sensitive to the effects of maternal stress (Buss et al. 2012), as well as poor impulse control and working memory in toddlers (further discussed **Chapter 3**) (Graham et al. 2018; Rudolph et al. 2018). In rodents, prenatal stress has been associated with increased inflammation markers in the fetal brain, including activated microglia, and elevated levels of pro-inflammatory cytokines (IL-1β, IL-18, TNF-α, and IL-6) and chemokines (CCL1, CXCL12) (Ślusarczyk et al. 2015). Others have observed that offspring exposed to prenatal stress, and increased MIA, display alterations in anxiety, social, and locomotor behaviour often in a sex-dependent manner, potentially mediated by increased inflammation in the placenta (Babri, Doosti, and Salari 2014; Bronson and Bale 2014). This evidence suggests there is an interesting reciprocal interaction between these two systems in programming fetal development, and that the stress response may be partially responsible for some of the downstream alterations observed following MIA-exposure (Howerton and Bale 2012).

2.3.6 Oxidative stress

Oxidative stress is a term used to describe an excess production of reactive oxygen species due to an imbalance between the generation of reactive oxygen species and the availability of endogenous antioxidants to sequester the reactive oxygen species (Chatterjee 2016). Reactive oxygen species can react with lipids, proteins, and DNA leading to damage, cell injury and death. Many factors can induce an imbalance between reactive oxygen species and their sequestration throughout the lifespan, however, of interest to this thesis is what occurs during pregnancy. Pregnancy introduces a metabolic challenge that must be met by both the mother and developing fetus; it is also associated with elevated levels of oxidative stress relative to the nonpregnant state (Marseglia et al. 2014). Abnormally high levels of oxidative stress have been linked to pregnancy complications such as abnormal placentation, preeclampsia, fetal growth restrictions, and premature birth or pregnancy loss (Marseglia et al. 2014). However, the contributions of oxidative stress to the pathogenesis and progression of neonatal disease are only partially understood.

Oxidative stress and inflammation may be highly interrelated processes. An excess in reactive oxygen species can trigger an upregulation in pro-inflammatory genes and transcription factors leading to the onset of inflammation. Interestingly, inflammation can also lead to an

increase in oxidative stress and reactive oxygen species overproduction (Chatterjee 2016). Therefore, inflammation and oxidative stress are inexorably interrelated processes that can amplify each other, potentially increasing the impact each has on altering neurodevelopmental processes.

Several groups have investigated the role of oxidative stress on the central nervous system (CNS) of offspring prenatally exposed to MIA. Increases in markers of oxidative stress have been observed in the fetal rat brain both several hours following maternal immune activation (via LPS injection) (Oskvig et al. 2012; Lanté et al. 2007), but also in early postnatal life and adulthood (Paintlia et al. 2004, 2008; Lanté et al. 2007, 2008). Interestingly, maternal treatment with the antioxidant N-acetylcysteine (a drug safe for pregnant women) was observed to prevent development of oxidative stress in the fetus due to MIA (Paintlia et al. 2008; Lanté et al. 2008). Cotreatment with this antioxidant was also shown to prevent white matter injury in offspring exposed to LPS induced MIA (Paintlia et al. 2008).

2.3.7 Maternal nutrient deficiency and obesity

Inflammation has also been associated with nutrient deficiency. Increased cytokine production due to infection has been shown to increase production of various proteins, including the zinc-binding protein metallothionein. An increase in production of metallothionein in the body will subsequently cause maternal and fetal zinc levels to drop, which may have detrimental effects on pregnancy and fetal development (Boksa 2010). This increase in metallothionein and drop in zinc has been observed following LPS treatment in wild-type mice, but not metallothionein knock-out mice, indicating the central role of metallothionein in modulating some of the secondary metabolic effects due to inflammation (Carey et al. 2003). Furthermore, IL-6, a pro-inflammatory cytokine consistently increased in maternal blood following MIA, has been shown to increase the production of zinc transporters, further contributing to zinc depletion (Liuzzi et al. 2005). Some have observed that supplementation of the maternal diet with increased zinc during pregnancy was able to prevent prenatal MIA-induced (via LPS) behavioural abnormalities in recognition memory, indicating a putative therapeutic role for zinc supplementation for pregnancies affected by elevated inflammation (Coyle et al. 2009). Maternal iron status may also play an important role in modulating immune responses during pregnancy, wherein iron deficiency often occurs simultaneously with infection in many pregnancies (Harvey and Boksa 2014a). Additionally, iron

deficiency has been identified as a risk factor for neurodevelopmental disorders such as schizophrenia or ASD in offspring (Atladóttir et al. 2010; Brown and Derkits 2010), and to exacerbate MIA induced inflammatory response in rodent models (Harvey and Boksa 2014b). Omega-3 polyunsaturated fatty acid and vitamin D deficiency have also been shown to further exacerbate MIA induced deficits in offspring; both of these compounds have been shown to strengthen the immune system and produce anti-inflammatory effects; thus, insufficient levels may lead to poorer control over inflammatory challenges (Labrousse et al. 2018; Luan et al. 2018). Deficits in many of these nutrients seem to exacerbate inflammatory responses, thus their supplementation may have anti-inflammatory effects, providing potential treatment avenues, or tools for mitigating negative outcomes.

In addition to increased systemic inflammation, individuals with obesity often suffer from malnutrition, with reports of inadequate iron, zinc, calcium, magnesium, copper, folate, and vitamin (A, B12, D) intake as a result of poor diet (Astrup and Bügel 2019). Further, obesity is associated with low-grade inflammation from chronic activation of the innate immune system (H. Lee, Lee, and Choue 2013; Ellulu et al. 2017). During pregnancy, a time in which inflammation is already increased, obese women tend to have even higher levels of circulating pro-inflammatory cytokines than women who are not obese (van der Burg et al. 2016). Further, both human epidemiologic studies and animal research have identified that prenatal and lactational exposure to maternal obesity and high-fat diet are associated with increased risk for neurodevelopmental and psychiatric disorders in offspring (Edlow 2017). These include ASD, schizophrenia, attention deficit hyperactivity disorder, cerebral palsy, anxiety, depression, and eating disorders (Edlow 2017). Further, evidence from rodent studies has shown that maternal high-fat diet leads to social and cognitive deficits as well as increased anxiety in offspring, but that these deficits are attenuated following reversal to normal diet during lactation (Kang et al. 2014). Thus, obesity and diet induced inflammation may play a role in MIA-associated developmental disruption and risk for neurodevelopmental disorders in offspring.

2.3.8 Maternal care

Maternal care is a powerful programmer of fetal outcomes, and may be affected by MIA, possibly propagating the effects of MIA to following generations. In some rodent studies,

investigators cross-fostered prenatally MIA-exposed and control pups to control for possible differences in maternal care. Some have measured maternal behaviour in both MIA-exposed and control dams and found no differences (Baharnoori, Bhardwaj, and Srivastava 2010). Similarly, others have also reported no effect of maternal care on offspring behavioural outcomes (Meyer et al. 2006). However, findings are mixed; some have observed aberrant maternal care due to MIA-exposure, which enhanced depression-like behaviour in female rodent offspring specifically (Ronovsky et al. 2017). Thus, maternal care is an important modulator of fetal outcomes, however its role in influencing MIA-induced deficits remains unclear.

2.4 Animal models as investigative tools

Epidemiological studies linking maternal immune activation to neurodevelopmental disorders are associative, therefore, they cannot provide causal evidence associating the two events, nor can they provide much insight into the mechanisms underlying this causal link. Motivated by the epidemiologic evidence, animal models of maternal infection or immune activation using non-human primates and rodents have been created, allowing scientists to study the impact of this risk factor on offspring neurodevelopment and behaviour on much shorter timescales, and with the potential to probe underlying biological mechanisms (Reisinger et al. 2015; Brown and Meyer 2018; Meyer 2014). In fact, there is a large body of evidence describing the behavioural, neurochemical, neuroanatomical, and neurophysiological disruptions observed in these offspring (Brown and Derkits 2010; Boksa 2010; Meyer et al. 2008). One cannot expect animal models to fully recapitulate the complexity of human brain function and behaviour. However, they still remain a useful tool, as specific symptoms and neuroanatomical features observed in human neurodevelopmental disorders can be captured in animal models (Meyer and Feldon 2012).

There is a class of animal models based on prenatal exposure to live pathogens, such as the influenza virus, *Toxoplasma gondii*, or *Streptococcus B*; these are useful for verifying the epidemiologic studies in a casual manner. Administering live pathogens is most ecologically valid and reflective of the human situation, as it elicits the full spectrum of immune response, however their use in the laboratory requires stringent biosafety levels, which may not be accessible to all research laboratories (Brown and Meyer 2018). Therefore, there exists another class of animal model that make use of immune activating agents that stimulate the innate immune system.

Stimulation in response to pathogens is initiated when components of the pathogen bind to pattern-recognition receptors (Zou et al. 2013). This sets off a signaling cascade, including the upregulation of cytokines, which are important for clearing the pathogen and developing adaptive immunity (Y.-G. Li et al. 2012).

One commonly used compound is the bacterial endotoxin lipopolysaccharide (LPS), a gram-negative bacterial cell wall component that mimics a bacterial infection by binding to toll like receptor (TLR)-4. Of interest for this thesis is the other most commonly used mimetic, the synthetic analogue of double-stranded RNA polyinosinic:polycytidylic acid (poly I:C) (Reisinger et al. 2015; Brown and Meyer 2018). Double-stranded RNA is a by-product of viral replication and is recognized by pattern-recognition receptors including the TLR-3 receptors and retinoic acid-inducible gene-I-like receptor I (RIG-I) (Zou et al. 2013). RIG-I receptors are localized in the cytoplasm and recognize RNA derived from different viruses; they initiate the recruitment of signaling molecules which stimulate the immune system, causing an upregulation in pro-inflammatory cytokines and transcription factors, such as interferons, inflammatory kinases, and nuclear factor kappa B (NF-kβ), amongst others (Zou et al. 2013). TLR-3 is expressed in the endoplasmic reticulum and delivered to endosomes where it recognizes the double stranded RNA, and further engages a series of adaptor proteins and pro-inflammatory molecules (interferons and NF-kβ) (Zou et al. 2013). These compounds were initially used to test whether the increase in maternal cytokines were mediating many of the neurodevelopmental deficits rather than specific pathogens. Finally, a third class of models exists based on specific immunopathological processes implicated in the etiology of neuropsychiatric disorders, such as the administration of specific pro-inflammatory cytokines associated with allergic disorders such as asthma (Schwartzer et al. 2015; Schwartzer et al. 2017), or autism-related maternal autoantibodies (Braunschweig, Golub, et al. 2012; Braunschweig, Duncanson, et al. 2012; Martínez-Cerdeño et al. 2016).

The most commonly used animal models are those that make use of viral and bacterial mimetics (Brown and Meyer 2018; Estes and McAllister 2016). Although this model does not recapitulate the full spectrum of immune reactions typically elicited by exposure to live infectious pathogens, they do exhibit many relevant behavioural abnormalities (as discussed in section **2 .4.3**), and there are many advantages (Reisinger et al. 2015). First, they allow researchers to control the duration and intensity of the maternal immune response by adjusting the dose. This affords researchers the possibility to distinguish between effects due to subtle or potent immune activation

on offspring outcomes. Furthermore, it allows researchers to investigate how the specific gestational timing of these insults impacts outcomes. Finally, it has provided researchers with the understanding that many of neurodevelopmental abnormalities observed in the offspring are not necessarily due to exposure to a pathogen, but rather to the immune response of the mother, which is a significant advancement in our understanding of the interplay between the immune system and development (Weber-Stadlbauer and Meyer 2019).

2.4.1 Critical gestational windows for association between prenatal MIA and psychiatric disorders in the offspring

As outlined in section (**2.1**), epidemiological findings provide a strong case for the role of MIA-exposure in increasing risk for aberrant neurodevelopment in offspring, however, there is some controversy over the degree to which gestational timing plays a role in determining outcomes in the offspring. Not all studies are powered to investigate the differences due to gestational timing of MIA-exposure, however, those that have specifically investigated it report interesting associations. Many of these studies have identified the second trimester of gestation, or mid-gestation, as a critical period for exposure to influenza or other types of infections, leading to increased risk for schizophrenia (Torrey, Rawlings, and Waldman 1988; O'Callaghan et al. 1995; Brown et al. 2000; Mednick, Huttunen, and Machón 1994). Others have identified exposure in the first half of pregnancy as conferring greater risk (Brown and Susser 2002). More recently, first trimester exposure to both viral and bacterial infections has been associated with increased rates of psychosis in offspring, with no effects associated with exposure in later stages of pregnancy; these studies made use of maternal serum samples to confirm infection, rather than making temporal associations or using self-report information (Brown et al. 2004; Allswede et al. 2020; Sorensen et al. 2009). These epidemiological findings provide motivation for wanting to investigate the effects of MIA in the first and second trimesters of human pregnancy. To this end, in this thesis the effects of prenatal MIA-exposure were investigated at two different gestational windows in the mouse, GD 9 and GD 17, which correspond to the end of the first, and end of the second trimester in humans, respectively. We hope to disentangle whether there is a difference in the downstream effects based on the gestational timing of the immune challenge. Below, we

discuss important preclinical behavioural (**2.5**) and neurobiological (**2.6**) findings. All MRI-based findings are discussed in **Chapter 3**.

2.4.2 Comparative neurodevelopment: humans vs. rodents

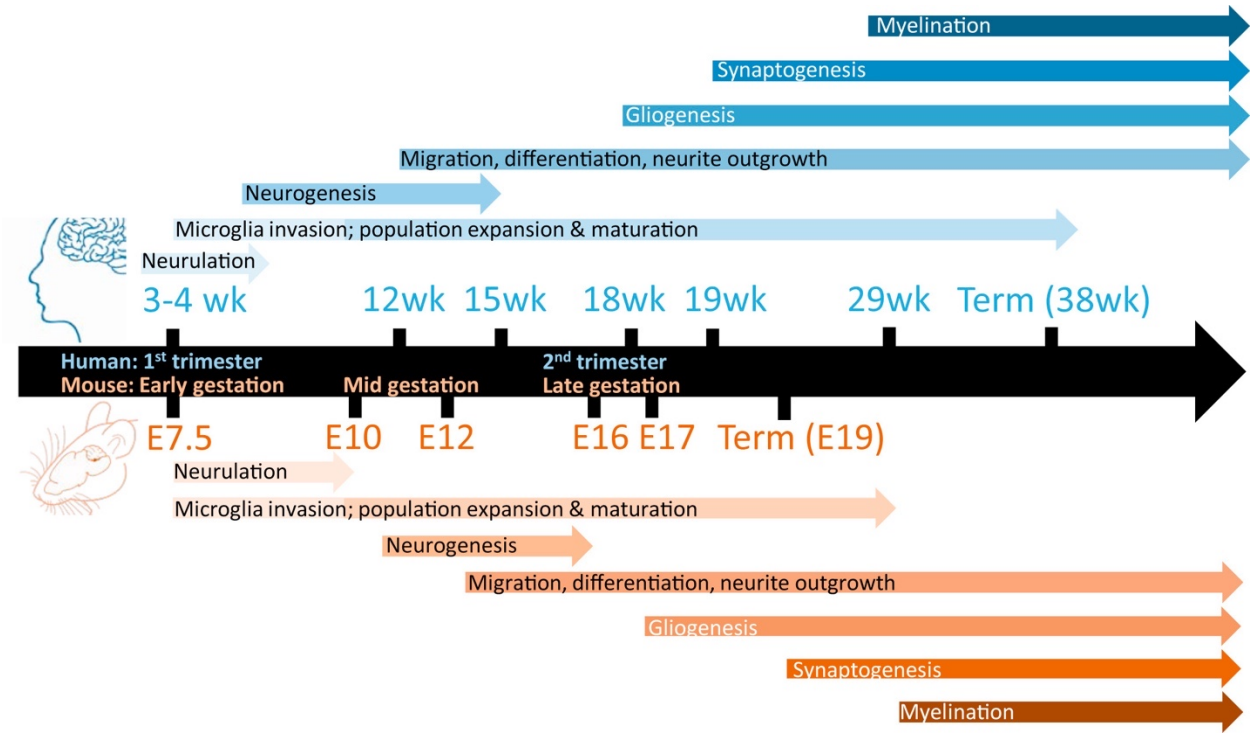


Figure 2.2. Timeline comparing important neurodevelopmental milestones in human and rodent gestation, from (Gumusoglu and Stevens 2019).

Rodents are the most widely used animal models to study human disease, however there are some questions of comparability across species that continue to create controversy. No model is likely to fully mimic human disease or development, however with appropriate understanding of equivalent developmental benchmarks, we can make progress (**Figure 2.2**).

Much of the work approximating cross-species homology is decades old, with the most detailed work based on human to rat comparisons (Clancy et al. 2007). However, we can still use this information as a starting point. Based on gross morphology, gestational day (GD) 10.5 in mice is thought to be equivalent to 28 days post-conception in humans. Further, the rat brain at PND 1-10 is thought to be equivalent to the human third trimester with PND 7 corresponding roughly to the human brain at birth (Clancy et al. 2007; Dobbing and Sands 1979). However, if one focuses

on GABAergic neurotransmission development (Romijn, Hofman, and Gramsbergen 1991), for example, or cortical development (Ohmura and Kuniyoshi 2017), rat PND 12-13 corresponds more closely to the human brain at birth, suggesting that not all developmental events may align properly, and that it may be more useful to draw comparisons across more specific developmental events.

With respect to cortical development, the first trimester is a critical period for cell proliferation and neurogenesis; in humans, corticogenesis begins at ~4.5 weeks of gestation, followed by emergence of neuronal precursors shortly thereafter (Selemon and Zecevic 2015). At ~7 weeks of gestation, thalamo-cortical and dopaminergic fibers begin to develop, followed by the formation of the cortical plate at ~8 weeks of gestation (Selemon and Zecevic 2015). With respect to neurogenesis, in humans and nonhuman primates, hippocampal neurogenesis peaks at GD60, and they are born with 80% of the dentate gyrus granule cells (Semple et al. 2013; Rakic and Nowakowski 1981). In the mouse, the neural tube forms, and neurogenesis begins at GD 9 and ends at GD 15. Hippocampal neurogenesis peaks around GD 14-16 in rodents, and they are born with only 15% of granule cells present in the dentate gyrus, indicating that much of the *in utero* development occurring in humans and nonhuman primates occurs postnatally in rodents (Semple et al. 2013; Rakic and Nowakowski 1981).

Myelination and synapse formation both begin postnatally in rodents but prenatally in humans, so they may be better studied in other species (Gumusoglu and Stevens 2019). Some comparative immunofluorescent studies have suggested that white matter development and axonal outgrowth in the rodent central nervous system at PND 1-3 corresponds to 23-32 weeks of gestation in the human neonate, while PND 7 is closer to 32-36 weeks of gestation (Craig et al. 2003). Based on these timelines, PND 10 would correspond roughly to a term human infant with respect to white matter development, which is slightly later than the estimation based on gross morphology highlighted above (Semple et al. 2013). By PND 10-14 the process of myelination is actively progressing; mature oligodendrocyte markers such as myelin basic protein are detectable by PND 20, when this process is thought to peak (Semple et al. 2013).

Synapse formation in the developing human brain begins around 20 weeks of gestation with density increasing rapidly right after birth, reaching 50% of adult levels by 2 years of age (McAllister 2007). This is a region-dependent process, with earlier peaks in primary cortical areas (8-12 months of age), followed by higher order associative areas (2-4 years of age) (Lenroot and

Giedd 2006). In rodents, the critical period of synaptogenesis occurs during the first three postnatal weeks of life, peaking around week 2.

Microglia colonize the human brain between the 4th and 24th week of gestation (Menassa and Gomez-Nicola 2018), and the mouse brain at GD 9, making this developmental period sensitive to potential inflammatory insults (and one of the gestational timepoints at which we induce MIA in this thesis). By GD 17 (the second gestational timepoint at which we induce MIA) in the mouse, many neurodevelopmental processes are complete, however cell migration to form cortical layers, and myelination are both ongoing (Semple et al. 2013). Therefore, it seems that insult during earlier gestational periods has the ability to cause more damage than one incurred later in gestation given the more active nature of earlier brain development, whereas insults occurring later in gestation may induce more subtle organizational changes.

2.4.3 Behavioural findings in MIA-exposed offspring

Prenatal exposure to MIA results in a wide variety of behavioural phenotypes in offspring. Below we outline the most relevant findings to neurodevelopmental and psychiatric disorders.

2.4.3.1 Sensorimotor deficits

MIA has been associated with a range of sensorimotor deficits in offspring including impaired prepulse inhibition (PPI) to an acoustic startle, sensory and motor behaviours, exploratory behaviour, and latent inhibition (Boksa 2010; Gumusoglu and Stevens 2019). PPI, which measures the ability to filter sensory information, is the most frequently reported deficit in rodent literature, possibly due to the feasibility, reproducibility, and automatization of the test (Boksa 2010). Further, it has also been shown to be impaired in individuals with schizophrenia (Casa et al. 2016), as well as children, but not adults, suffering from ASD (Cheng et al. 2018). In mice, poly I:C exposure in early (GD 9) and mid-gestation (GD 12.5-15) has been associated with PPI deficits (Smith et al. 2007; Meyer et al. 2008; Meyer, Nyffeler, Yee, et al. 2008; Meyer et al. 2006; Zhang and van Praag 2015), however these deficits have not been observed for exposure in late gestation (GD 17) (Meyer et al. 2006). PPI deficits have also been observed in mouse offspring whose pregnant mothers were exposed to IL-6 (Smith et al. 2007) or to a live influenza virus (Shi et al. 2003), and in rats following mid-gestation exposure to poly I:C (Zuckerman et al. 2003) or

LPS administered every other day during pregnancy (Borrell et al. 2002). Thus, deficits develop in both mice and rats, irrespective of immunostimulant. Further, it appears that exposure in the early- and mid-gestation period is more likely to induce to sensorimotor perturbations. Adult locomotor deficits have also been observed by others in rats exposed to poly I:C at GD 13-15 (Aavani et al. 2015).

2.4.3.2 Anxiety-like and depression-like behaviours

Anxiety- and depression-like behaviours have often been examined in MIA exposed offspring, including exploratory behaviour, elevated plus maze, sucrose preference, and the forced swim test (Ronovsky et al. 2016). Exposure to poly I:C at GD 9 (but not GD 17) in mice has been associated with increased anxiety-like behaviour in adult offspring, as measured by decreased time in the center zone of the open field test (Meyer et al. 2006)). Some have reported adolescent but not adult deficits in the open field test following poly I:C induced MIA-exposure (Ozawa et al. 2006). Interestingly, LPS exposure at GD 17 has been associated with increased anxiety as measured by the elevated plus maze (Hava et al. 2006).

Poly I:C exposure at GD 12.5 in C57BL6/N mice has been associated with decreased sucrose preference and increased despair in the forced swim test in adult offspring (Khan et al. 2014). The same exposure in C57BL/6J mice is associated with increased anxiety (as measured by the marble burying task (Schwartzer et al. 2013)). Poly I:C exposure at GD 15 has also been associated with increased despair behaviour in adulthood (Zhang and van Praag 2015).

Interestingly, adult mice exposed to LPS on GD 9.5 also exhibit increased anxiety- and depression- like behaviours including avoidance of open arms in the elevated plus maze and the center of an open field, as well as the lit (anxiogenic) side of a light/dark box; increased despair has also been observed in the forced swim test and tail suspension test (Depino 2015). Similar deficits have been observed in rats prenatally exposed to LPS at GD 10.5 (Y.-L. Lin and Wang 2014). Exposure in both early and mid-, but also late gestation seems to induce a range of offspring deficits in this behavioural domain.

2.4.3.3 Social and communication deficits and repetitive behaviours

Social and communication deficits are a core feature of ASD pathology and are often observed in individuals with schizophrenia (Mier and Kirsch 2015; Baron-Cohen 1988).

Repetitive, restrictive behaviours are another core feature of ASD, sometimes observed in individuals with schizophrenia, although less consistently than social deficits (Tracy et al. 1996).

The ultrasonic vocalizations (USV) induced by maternal separation is the earliest complex behavioural assay that can be measured in rodents (apart from assessing basic motor skills); they are typically used to assay impairments in communication, but alteration can also be an indication of stress or altered bonding with the mother (Scattoni, Crawley, and Ricceri 2009). Abnormalities in USVs typically arise in the first two postnatal weeks, with a peak in vocalization occurring at PND 8-10 (Scattoni, Crawley, and Ricceri 2009). MIA-exposed mouse offspring have shown decreased vocalization number and complexity at PND 10 following poly I:C exposure at GD 12.5, with deficits persisting in adulthood only in male offspring evident during social interaction (Malkova et al. 2012). Decreased vocalizations at PND 8 have also been observed following GD 12.5 LPS exposure in mice at PND 8 (Cossío et al. 2017), and in rats at PND 4 and 5 following GD 15 and 16 LPS exposure (Baharnoori, Bhardwaj, and Srivastava 2010). In contrast, poly I:C exposure at GD 12.5 in mice has also been found to decrease USVs at PND 6 and 8 but increase USVs at PND 10 and 12 (Pendyala et al. 2017). Increased USVs at PND 9 have been reported by other groups who also use poly I:C exposure at GD 12.5 (Choi et al. 2016; Shin Yim et al. 2017). The gestational timing and immunogen used may influence the directionality of the changes in USVs (increases or decreases), however, this behaviour seems to be consistently affected.

Rodent models of neurodevelopmental disorders using MIA have often observed deficits in social interaction and social approach. Using the three-chambered social approach task, various groups have exhibited deficits in adult offspring with maternal poly I:C exposure at GD 12.5 (Malkova et al. 2012; Shin Yim et al. 2017; Pendyala et al. 2017; Choi et al. 2016), and maternal LPS exposure at the same GD (Cossío et al. 2017), wherein they spend less time exploring a novel mouse than a non-social object relative to control mice. Others, however, have found no deficits following the same poly I:C treatment (Schwartzer et al. 2013). Poly I:C exposure at GD 15 (D. Mattei et al. 2017) and GD 17 (Bitanihirwe et al. 2010) has also been associated with impaired social behaviour in adult mouse offspring. MIA-exposed rhesus monkeys have not displayed deficits in social interaction, however some abnormal social behaviours have been observed when interacting specifically with novel monkeys, such as cooing (Bauman et al. 2014), or abnormal eye fixation (Machado et al. 2015).

Repetitive behaviours in rodents are typically exhibited as compulsive digging (in the marble burying task) or compulsive grooming. Adult mice previously exposed to poly I:C at GD 12.5 have been shown to have higher levels of compulsive and repetitive behaviours as measured by the marble burying task and self-grooming (Malkova et al. 2012), with some observing greater deficits in males (Xuan and Hampson 2014). Others have observed social deficits in adolescent, but not adult rats exposed to poly I:C at GD 13-15 (Aavani et al. 2015). Similar results in social and repetitive behaviours have been observed with LPS exposure at the same gestational day, with more pronounced deficits in male offspring (Cossío et al. 2017), whereas others have observed greater social deficits in females and increased repetitive behaviours in males (following LPS injection at both GD 11.5 and 12.5) (Xuan and Hampson 2014). Interestingly, altered inappropriate social behaviour, increased repetitive and stereotypic behaviours, and decreased vocalizations have been observed in macaque offspring exposed to either maternal treatment with poly I:C (Bauman et al. 2014; Machado et al. 2015) or immunoglobulin G isolated from mothers whose children developed ASD (Martin et al. 2008). Maternal immune stimulation with IL-6 (Parker-Athill and Tan 2010; Smith et al. 2007) or IL17-a (Choi et al. 2016) has also been associated with abnormal social and repetitive behaviours in offspring. Thus, social deficits are often observed across a variety of species following prenatal MIA-exposure, although a number of negative reports do exist.

2.4.3.4 Cognitive deficits and memory impairments

Impairments in memory, cognition, and cognitive flexibility have been consistently observed in individuals with schizophrenia and ASD, as well as in animals exposed to MIA prenatally. Deficits on recognition memory, as measured through the novel object or novel place recognition tests, have identified deficits in adult mouse MIA-exposed offspring following poly I:C exposure at GD 9 (Li et al. 2014), from GD 12 to 17 (Ozawa et al. 2006), as well as LPS exposure at GD 8 (Coyle et al. 2009) and at GD 15 and 16 (Wischhof et al. 2015). Spatial and working memory disruptions have also been observed in various maze tests (Morris water maze, Y maze, T maze, radial arm maze) for mouse offspring exposed to poly I:C at GD9 (MacDowell et al. 2017), GD 15 (Zhang and van Praag 2015), and GD 17 (Giovanoli et al. 2015). However, others have found deficits to emerge only when tasks reach their most challenging trials (such as longer trials in the water maze) following poly I:C exposure at GD 9 or 17 (Meyer, Nyffeler, Yee,

et al. 2008; Meyer et al. 2006), or only in adult male rats following LPS exposure at GD 15 and 16 (Batinić et al. 2016), while others have observed no differences at all (Abazyan et al. 2010).

Cognition has also been measured via latent inhibition, which is the process of inhibiting the learning of associations when first presented with a neutral stimulus representing non-association (Feifel and Shilling 2013). Impaired latent inhibition has been reported in adult mice following poly I:C exposure at GD 9 (Giovanoli et al. 2013; Meyer, Nyffeler, Schwendener, et al. 2008), GD 12.5 (Garay et al. 2013) late (GD 17) exposure to poly I:C (Bitanihirwe et al. 2010). Adult rats exposed to poly I:C at GD 15 have also exhibited deficits in latent inhibition (Zuckerman et al. 2003; Piontkewitz, Arad, and Weiner 2011), but not in younger rats (Piontkewitz, Arad, and Weiner 2011). Attentional set shifting, or cognitive flexibility impairments have also been observed in MIA-exposed offspring. One study observed these deficits to be apparent in adult mice exposed to poly I:C at GD 9, with a dependence on prefrontal parvalbumin positive GABAergic interneurons (Canetta et al. 2016). Deficits have also been observed in mice exposed to poly I:C at GD 12.5 (Amodeo et al. 2019) and in male, but not female, adult rats exposed to poly I:C at GD 15 (Y. Zhang et al. 2012; Kleinmans and Bilkey 2018). Again, there is some heterogeneity in these findings, which may be associated with a number of experimental design choices.

2.4.3.5 Behavioural findings with a lens on GD 9 and 17 exposure

Animal models provide useful tools for examining critical windows of exposure to MIA. In this thesis, we focus on exposure to prenatal poly I:C treatment (5mg/kg) on gestational day (GD) 9 (corresponding to the end of the first trimester in humans), which has been associated with suppression in exploratory behaviour, abnormalities in selective association learning, impairments in sensorimotor gating (reduced PPI), enhanced sensitivity to amphetamine (a dopamine receptor agonist), and deficiency in spatial working memory (Meyer, Nyffeler, Yee, et al. 2008; Meyer et al. 2006). We also focus on identical treatment at GD 17 (corresponding to the end of the second trimester in humans), which is shown to lead only partially overlapping behavioural impairments. These include perseverative behaviours, deficits in spatial working memory and recognition memory, and a potentiated response to the NMDA-receptor antagonist dizocilpine (MK-801) (Meyer, Nyffeler, Yee, et al. 2008; Meyer et al. 2006; Bitanihirwe et al. 2010).

2.4.3.6. Limitations of the MIA animal model: inconsistencies in reporting

Importantly, there are several important limitations to the use of the MIA animal models. Behavioural, neuroanatomical, both morphological and cellular, and transcriptional changes in animal models of MIA are highly susceptible to the dose or immunostimulant used, the gestational timing, the individual immune and thermogenic response of the mother (Mueller et al. 2019). Additionally, housing conditions, strain, sex, age, amongst many other factors may influence outcomes (Kentner et al. 2019; Weber-Stadlbauer and Meyer 2019). These are further discussed in **Chapter 3**, and section **7.3** and **7.4**. Increasing the detail and transparency in reporting is one way to improve the reproducibility of these findings (Kentner et al. 2019; Weber-Stadlbauer and Meyer 2019).

2.5 Neurobiological outcomes of MIA exposure

2.5.1 Neurogenesis, neural progenitors, and migration

MIA-exposed offspring exhibit a range of neuropathologies that align with human neurodevelopmental disorders, including reduced cortical thickness and hippocampal volume, increased ventricular size, and cerebellar alteration (see **Chapter 3** for more detail). Further, patches of disorganized cortex have also been observed (Shin Yim et al. 2017). The cellular mechanisms underlying many of these changes are likely quite complex, however it is possible that alterations in neurogenesis and proliferation may impact gross morphology later in life. Reductions in the number of proliferative neural stem cells in the cortex, paired with a reduction in cortical thickness have been observed in embryos at GD 18 who were exposed to MIA at GD 12.5 with poly I:C (Tsukada et al. 2015). It is possible that microglial dysfunction plays a role here, as they have been shown to phagocytose neural precursor cells in the cortex, particularly when they are in a more activated state (Cunningham, Martínez-Cerdeño, and Noctor 2013).

Alterations in neurogenesis have also been observed in MIA models. Reduced proliferative cells (labeled with Bromodeoxyuridine/5-bromo-2'-deoxyuridine [BRdU]) have been observed in the dentate gyrus of the rat fetus (GD 18-19) and juvenile (PND 14) following MIA (via LPS) on GD 15 and 16 (Cui et al. 2009). Similar decreases in proliferating cells which are fated to give rise to hippocampal neurons have also been observed by others (Piontkewitz et al. 2012; Mattei et

al. 2014; Khan et al. 2014). Some have observed regional dependence on the decreased neurogenesis, with decreases occurring in the hippocampus but not the subventricular zone or rostral migratory stream, two other neurogenic niches (Graciarena et al. 2013). However, the findings are mixed, as others have found decreased neurogenesis in the olfactory bulb as well as the subventricular zone, resulting in fewer adult born neurons and olfactory deficits (Liu et al. 2013). Finally, animal models show that curtailing cell proliferation in early gestation produces schizophrenia-like pathology in the prefrontal cortex of adolescent and adult as well as key behaviors and symptoms of the disease (Reisinger et al. 2015).

In addition to being a neurogenic niche, the ventricular zone is the site of initial neuronal migration for neurons destined to the neocortex; this process begins at the end of the first trimester into the second trimester of pregnancy (Minakova and Warner 2018). There is evidence that MIA-exposure may interfere with or damage cells involved in neurogenesis during critical developmental windows, which may contribute to some of the impairments observed in exposed offspring. Transcriptional alterations to genes involved in cell migration, laminar fate, and cortical organization have been observed in fetal brains of mice exposed to MIA in mid-gestation (poly I:C GD 15) (Lombardo et al. 2018). Additionally, a reduction in number of reelin-positive cells in the hippocampus has been reported in mice exposed to LPS early in gestation (Depino 2015; Meyer et al. 2006); similar findings have been reported in the cortex (Fatemi et al. 1999). Interestingly, IL-6 exposure in mice has been associated with delayed migration of cortical interneurons (Gumusoglu et al. 2017). Thus, there is evidence to suggest that exposure to elevated cytokines *in utero* due to MIA-exposure (or direct exposure to pro-inflammatory cytokines) may disrupt both neurogenesis and neuronal migration in the offspring brain.

2.5.2 Microglia and immune molecules

Microglia are thought to play a key role in mediating neurodevelopmental disturbances due to MIA, as they are important regulators of neuronal differentiation and maturation, as well as synaptic pruning and stabilization; they are regulated by cytokines and chemokines, as well as various neurotrophic factors and neurotransmitters (Ginhoux et al. 2010; Hutchins et al. 1990).

Microglia are the resident immune cells of the brain, so it is no surprise that they have been investigated in the context of MIA, and often found to be altered. These cells migrate from the

yolk sac and infiltrate the brain in early gestation prior to the closure of the blood-brain barrier, (GD 9 in the mouse, 4 weeks gestation in humans) and form a resident pool throughout life (Thion, Ginhoux, and Garel 2018; Thion and Garel 2017). They support innate and adaptive immunity in the brain, but also play critical roles in neurodevelopment, such as in circuit and synapse refinement (Thion, Ginhoux, and Garel 2018). Early in development, they are the only glial cell in the brain and are a major source of cytokines in the CNS. When microglia are stimulated by inflammatory cytokines and TLR ligands, they increase production of reactive oxygen species, and produce inflammatory chemokines and cytokines that can be toxic to mature neurons and oligodendrocytes (Pratt et al. 2013). Inflammatory perturbations to the brain in critical developmental stages can drive these cells to assume a more pathological role, rather than support neurodevelopment. These changes can be short term, or persistent. Interestingly, abnormal microglial function has been observed in a multitude of neurodevelopmental/psychiatric disorders such as schizophrenia, ASD, but also depression (Monji et al. 2013; Sellgren et al. 2019; Koyama and Ikegaya 2015; Suzuki et al. 2013; Réus et al. 2015).

Alterations in microglial cell number, density, and activation state (characterized by change in morphology and expression of certain markers such as CD11b and Iba-1) have been observed in preclinical MIA models, mirroring those observed in human ASD and schizophrenia post-mortem studies. These changes have been observed as early as in embryonic stages; in a sheep model of mid- or late-gestation LPS administration increased microglial number and reactivity was observed a few days following exposure in the offspring brain (Dean et al. 2011; Hutton et al. 2008). Further, embryonic alterations to microglia following MIA-exposure have also been observed in rodents; increased numbers of activated microglia in the forebrain have been detected following LPS exposure at GD 9, as have brain-wide increases in density and activation following LPS at GD 17.5 (Le Belle et al. 2014; Tronnes et al. 2016). Similarly, microglia isolated from the fetal mouse brain 3 hours post LPS challenge (GD 17) expressed greater levels of pro-inflammatory genes such as IL-1β, TNF-α, and IL-6 (Schaafsma et al. 2017). Conversely, others have observed no significant changes to microglial density in the fetal mouse brain when focusing specifically on the hippocampus (Smolders et al. 2015). Thus, it is possible that the changes are regionally dependent, or that differences in methodology or species could affect outcomes. Increased microglial number or activation have been found to deplete neural progenitor cells in the fetal brain, to disrupt synaptic development, which would both lead to abnormal neurocircuit

development later in life (Paolicelli and Gross 2011; Cunningham, Martinez-Cerdeno, and Noctor 2013).

Thus, MIA-exposure seems to alter microglial activity and morphology at a time proximal to exposure, however, it is also thought to prime microglia to respond differently to stimuli later in life. For example, microglia may be either more (Schaafsma et al. 2017; Giovanoli et al. 2013) or less (Cao et al. 2015; Schaafsma et al. 2017) reactive to immune challenges experienced later in the lifespan following priming. In support of this hypothesis, MIA-exposure both early (Ling et al. 2006) or late (Ling et al. 2006) in gestation has been shown to elicit larger than expected responses to an immune challenge in adulthood, with prolonged pro-inflammatory cytokine release and microglial activation.

Additionally, alterations to microglial activation, number, density, and function have also been observed postnatally in MIA models. Both early (Juckel et al. 2011) and mid-gestation (Van den Eynde et al. 2014) poly I:C exposure has been shown to increase active microglia densities, as has early (Ling et al. 2006) and late (Girard et al. 2010) LPS exposure. Further, adult alterations to microglia as well as other immune molecules have been shown to influence synaptic plasticity (Welberg 2014; Tremblay and Majewska 2011; Levin and Godukhin 2017).

Finally, there is significant evidence that microglia follow temporarily different trajectories in development in males and females, particularly in early development (Hanamsagar and Bilbo 2016; Yanguas-Casás et al. 2018; Nelson, Saulsbery, and Lenz 2019). A greater number of microglia (as much as twice the number), particularly those in an activated state, have been observed in male compared to female neonatal mouse brains (Lenz et al. 2013; Schwarz, Sholar, and Bilbo 2012). Furthermore, greater numbers of microglia have been observed in the female brain than the male in the adolescent to adult period (Schwarz, Sholar, and Bilbo 2012). These developmental sex differences may underlie some of the disparity in prevalence of neuropsychiatric disorders between sexes. Early (GD 9) MIA-exposure has been associated with male-specific changes in microglial activation in adulthood (Hui et al. 2018); Manitz and colleagues report similar findings in the adolescent male brain following GD 9 MIA as well (Manitz et al. 2016). These data suggest that microglial signaling may be differentially impacted by an early immune challenge in males and females, and that the male brain may be more susceptible to microglial activation following an immune challenge, as they tend to have higher

levels of activated microglia in the early postnatal period (Lenz et al. 2013; Schwarz, Sholar, and Bilbo 2012).

2.5.3 Synaptic structure and function

Alterations in dendritic development and synaptic structure and function has been observed in many neurodevelopmental and psychiatric disorders (Gumusoglu and Stevens 2019). Given the role of microglia in synaptic pruning during normal development (Paolicelli and Gross 2011), and abnormal activation of these cells following MIA-exposure (Mattei et al. 2017; Pratt et al. 2013), it is plausible that MIA-mediated dysregulation of microglia causes deficits in synapse formation and pruning (Paolicelli and Gross 2011). Further, this may mechanistically link prenatal immune disruption to psychiatric risk (Gumusoglu and Stevens 2019). One mechanism for over-pruning may be direct phagocytosis of synapses by overactive microglia (Galloway et al. 2019; Weinhard et al. 2018). Specific cytokines and chemokines which would be present at higher levels following MIA, such as IL-1β, IL-6, and TNF-α have direct roles in establishing synaptic function and dendritic morphology (Beattie et al. 2002; Gilmore et al. 2004). Therefore, there are various potential avenues by which an elevation in inflammation may affect the synaptic landscape.

MIA exposure in mid-gestation (poly I:C on GD 12.5) has been associated with reduced cortical dendritic spine density, as well as decreased turnover, altered connectivity, and an imbalance in excitatory and inhibitory synaptic signaling (Pendyala et al. 2017). Decreased spine density and function have been reported in layer 5 pyramidal neurons of the somatosensory cortex in both juvenile (PND 17) and adult (PND 90) offspring, also following poly I:C exposure at GD 12.5 (Coiro et al. 2015), as well as in adult offspring exposed to LPS exposure at GD 15-16 (Baharnoori, Brake, and Srivastava 2009). Conversely, others have observed either increased spine density of hippocampal granule cells in male but not female offspring exposed to mid-gestational LPS (GD 15), while others report no differences in layer 3 specifically (following LPS at GD 15 and 16) (Baharnoori, Brake, and Srivastava 2009). The disparity in these findings may be due to an age-dependency on the effects; increased spine density on hippocampal neurons have been reported in the juvenile period (PND 21), but decreased density in adulthood (PND 90) (Lin and Wang 2014). Finally, there is evidence for abnormal expression of presynaptic (synaptophysin) and postsynaptic (PSD-95) proteins in MIA models (Meyer 2013; Giovanoli et al. 2016; Hao et al.

2010), both of which have been observed in post-mortem studies of individuals with schizophrenia and ASD (Penzes et al. 2011; Estes and McAllister 2016; Solek et al. 2018; Varghese et al. 2017). The synaptic disruptions observed in the hippocampus and prefrontal cortex may underlie many of the learning and memory deficits observed in MIA models, as well as many psychiatric disorders (Khan et al. 2014; Kim et al. 2016; Oh-Nishi et al. 2010). Thus, there is clear evidence for MIA-induced disruption in normal synaptic development, however more work is required to identify the trajectory of these changes throughout the lifespan, as age dependent differences have been reported. Further, the regional specificity of these changes remains to be elucidated, as previous work seems to be limited to discrete cortical and hippocampal regions.

2.5.4 Specific neuronal subpopulations

As cells migrate in development, they continue to differentiate. MIA-exposure may interfere with the successful completion of this process. GABAergic interneurons may be more vulnerable to these insults than pyramidal neurons as their migration to the cortex, originating from the medial ganglionic eminence, is much longer than that of pyramidal cells, which originate from the cortical subventricular zone (Lavdas et al. 1999; Pleasure et al. 2000; Anderson et al. 2001). In rodent models, exposure to MIA has been shown to reduce functional GABAergic transmission in a specific interneuron subpopulation expressing parvalbumin (PV). Additionally, aberrant signaling has been observed in the prefrontal cortex and as well as the dysgranular zone of the somatosensory cortex (Canetta et al. 2016; Shin Yim et al. 2017). These interneurons play important roles in plasticity and maturation of neocortical circuitry in development, acting as neuromodulators and balancing excitatory input from glutamatergic pyramidal neurons (Butt et al. 2017; Morgane Sonia Thion et al. 2019; Keshavan et al. 2014). Given their important roles in modulating the development and function of cortical circuits, PV interneurons dysfunction is thought to play a central role in the neural abnormalities observed in neurodevelopmental and psychiatric disorders (Keshavan et al. 2014). Promising evidence from rodent work has shown that manipulating their activity in adult offspring exposed to MIA may rescue some behavioural deficits triggered by MIA exposure (Canetta et al. 2016; Shin Yim et al. 2017).

In addition to alterations in GABA-ergic cell function, the excitatory-to-inhibitory switch of GABA signaling has been shown to be delayed in offspring exposed to poly I:C early in

gestation indicative of aberrant neurodevelopment (Corradini et al. 2018). Furthermore, altered expression of glutamic acid decarboxylase-67, an enzyme critical for GABA production has been observed due to MIA-exposure in the hippocampus, thalamus, and prefrontal cortex (Cassella et al. 2016; Nouel et al. 2012).

Later-developing structures such as the cerebellum are also often disrupted in MIA-exposed offspring both at the gross anatomical level as discussed in **Chapter 3**, but also at the cellular level. Decreases in Purkinje cell volume have been reported in juvenile but not adult rat offspring exposed to MIA late in gestation (Wallace et al. 2010). Early-mid gestational MIA has been associated with increased Purkinje cell number in juvenile and adult mice (Aavani et al. 2015), but decreased Purkinje cell density in the neonatal period (Pendyala et al. 2017).

2.5.5 Transcriptional and epigenetic changes due to MIA

It is possible that exposure to MIA *in utero* leads to a dysregulation in gene expression, which may in turn have detrimental effects on brain development. As discussed in section **1.5.1**, transcriptional alterations to genes involved in cell migration, laminar fate, and cortical organization have been observed in fetal brains of mice exposed to MIA in mid-gestation (poly I:C GD 15) (Lombardo et al. 2018). Others have found alterations to the GABA transcriptome in the prefrontal cortex of adult, and, to a lesser extent, adolescent mice exposed to MIA late in gestation (poly I:C GD 17). Previous work has observed altered expression of both microRNAs and messenger RNAs in the brain of young (PND 21) mice exposed to MIA in mid-gestation (GD12.5) (Sunwoo et al. 2018) and in early postnatal life (Hicks and Middleton 2016).

Transcriptional analysis of mouse frontal cortices at PND 189, following MIA at GD 12.5 (20mg/kg, intraperitoneal [i.p.]) identified alterations in genes critical to fetal brain development, including genes involved in glutamatergic neurotransmission, potassium ion channel activity, and mTOR signaling (Amodeo et al. 2019). Comparison of GD 12.5 and GD 17.5 MIA-exposure (poly I:C 5mg/kg, intravenous [iv]) on the adult brain transcriptome was found to induce very subtle changes in transcription (Connor et al. 2012). However, investigating the effects of MIA-exposure at GD 12.5 on the fetal brain identified an acute upregulation in genes associated with hypoxia, immune signaling, and angiogenesis 6 hours following MIA-challenge, associated with changes in cortical lamination emerging in the embryo brain at GD 14.5 and peaking at GD 17.5 (Canales

et al. 2021). Recent work leveraging single-cell RNA sequencing to profile transcriptional changes in the mouse fetal brain following MIA (GD 12.5) have observed perturbations in cellular pathways associated with mRNA translation, ribosome biogenesis, and stress signaling. Moreover, they found activation of the integrated stress response in male and not female offspring, dependent on the activation of IL-17a (Kalish et al. 2020). Finally, activation of the integrated stress response was associated with a reduction in mRNA translation and altered proteome synthesis (Kalish et al. 2020). These data provide exciting insight into MIA-associated neurodevelopmental impairments, identifying potent transcriptional regulation occurring as early as in the fetal brain.

2.6 Sex differences in MIA: focus on neurodevelopmental disorders

Sex differences in the prevalence and presentation of neurodevelopmental and neuropsychiatric disorders are well documented. There is a male bias for early-emerging disorders with a neurodevelopmental basis, affecting attention, motor control, language and social function. The diagnosis of ASD, for example, shows a marked male preponderance, as do other early onset antisocial disorders such as developmental language disorder and attention-deficit hyperactivity disorder (Xuan and Hampson 2014). In contrast, there is a female bias for adolescent-emerging eating and mood disorders (Bao and Swaab 2010). Sex differences have also been reported in the age of onset of psychotic disorders, which tends to be earlier in males, and later in females (with a second peak of incidence later in life) (Gogos et al. 2020), as well as in the symptomatology, wherein males tend to suffer from more negative symptoms and women from more affective symptoms (Canuso and Pandina 2007). Thus, sex differences should be considered when investigating neurodevelopmental models.

Epidemiological reports on potential sex-dependent effects of MIA are mixed. A greater rate of adult schizophrenia in females rather than males whose mothers were exposed to influenza epidemics in England and Wales between 1938 and 1965 (Takei et al. 1994). However, as discussed in **2.1**, there is no confirmation of infection in these mothers by hospital records or serology. A recent study analyzing data from 15,421 pregnancies form the Collaborative Perinatal Project, which included 116 offspring with a confirmed psychotic disorder did find that mothers who experience severe bacterial infection during gestation, with ascertained infection status, were more likely to have offspring with psychosis, and that this effect was more pronounced in male

offspring than females (Lee et al. 2020). Finally, others have reported no sex differences in outcome due to prenatal exposure to maternal infection, again based on medical records from the National Health Insurance Research database (Fang et al. 2015). Thus, further investigation may be required to better understand if MIA-exposure may be a contributing factor to the sex differences observed in prevalence and outcomes in neurodevelopmental and neuropsychiatric disorders.

Not until recently, most studies on the effect of MIA used male rats or mice exclusively (as discussed in **Chapter 3**). Sex differences have been observed in the development of neuroanatomical changes due to MIA-exposure in rats (poly I:C GD 15), wherein males exhibited an earlier deviation in both brain volume and behaviour than females rats did (Piontkewitz, Arad, and Weiner 2011). A male bias in behavioural abnormalities has also been observed in PPI and attentional set-shifting behaviours, while others only observe greater male impairment in spatial working memory tasks, but not in PPI or locomotor activity (Gogos et al. 2020). Greater social and communicative impairments and increased stereotypic behaviours have also been reported in male relative to female mice prenatally exposed to MIA (LPS, GD 11.5 and 12) (Xuan and Hampson 2014) consistent with the ASD phenotype. Decreased cerebellar Purkinje cells, synapse formation in the hippocampus, and abnormal microglial activation have been reported to affect males more than females (Haida et al. 2019; Hui et al. 2020). Thus, although the evidence is mixed, there seems to be an increased susceptibility in male MIA-exposed offspring in both human and animal research.

2.7 Adolescent cannabis use as a risk factor for psychosis

The landscape of cannabis use is undergoing unprecedented change. Cannabis consumption is on the rise, as is the THC content in both the cannabis plant, and synthetic products. Prospective, longitudinal, epidemiological studies consistently report an association between pre-psychosis cannabis use and the onset of a psychotic episode (Arseneault et al. 2002) after controlling for other drugs (Volkow et al. 2016; Zammit et al. 2002). This is particularly evident if use begins before the age of 16 years (Keshavan et al. 2014; French et al. 2015). The age of sensitivity corresponds to the developmental period of the endocannabinoid system (specifically the cannabinoid [CB]-1 receptor), which plays a fundamental role in brain development.

2.7.1 Epidemiological evidence

The association between cannabis consumption and induction of psychotic-like symptoms was first reported over 60 years ago; a study was first published in 1958 describing psychotic-like symptoms in healthy volunteers following ingestion of cannabis (Ames 1958). A 15 year follow up of over 45000 Swedish conscripts observed a higher relative risk for schizophrenia among high cannabis consumers (defined as having used cannabis more that 50 times; 6.0 compared with non-users) (Andréasson et al. 1987). A similar analysis of the same cohort with an additional 5000 individuals revealed a 6.7 adjusted odds ratio in heavy cannabis users (Zammit et al. 2002) A longitudinal population-based study on 4000 Dutch subjects also identified a threefold increase in psychotic symptoms three years following reports of heavy cannabis use (Van Os et al. 2002). Furthermore, others have reported a dose-dependent response in which increased use is associated with increased risk (Fergusson, Horwood, and Ridder 2005; Moore et al. 2007). A longitudinal study of regular cannabis users between 15-18 years of age reported higher levels of schizophrenia-like symptoms at age 25 relative to controls who had never used cannabis or had only used it a handful of times (Arseneault et al. 2002). Others have found no association between cannabis use in adolescence and psychosocial deficits in adulthood (Macleod et al. 2004). A study of 3500 19-year-olds in Greece found that adolescent cannabis use was positively associated with both subclinical positive and negative symptoms as measured by a 40-item Community Assessment of Psychic Experiences questionnaire; this association was strongest for those that initiated use prior to 16 years of age (Stefanis et al. 2004). These findings provide evidence for adolescent cannabis exposure as a risk factor for later onset of psychosis symptoms. Finally, a number of studies have identified cannabis use to be significantly associated with decreasing the age of onset of schizophrenia (Sugranyes et al. 2009).

However, results are mixed; studies from Australia and the UK have identified rapid increases in cannabis use with no clear evidence of increased psychosis in their general populations (Degenhardt, Hall, and Lynskey 2003; Hickman et al. 2007). Thus, the association between cannabis use and development of psychosis-spectrum disorders is a matter of debate. Multiple scenarios exist in which individuals abuse cannabis for a number of years before symptom onset, individuals develop symptoms immediately after a single use, or individuals initiate cannabis consumption after symptoms have already been established (Malone, Hill, and Rubino 2010). Furthermore, individuals with pre-existing vulnerabilities due to genetic background, or previous

exposure to other environmental risk factors may be at greater risk for developing a cannabis induced psychosis-spectrum disorder or experience a worsening of symptoms associated with cannabis use (Hall, Degenhardt, and Teesson 2004; Van Os et al. 2002; Henquet et al. 2004). One possible genetic risk factor is the Val158Met polymorphism of the catechol-O-methyltransferase (COMT) gene, involved in synaptic reuptake and recycling of dopamine (and other catecholamines) (Montag, Jurkiewicz, and Reuter 2012), and cannabis use such that those with the mutation who abuse cannabis tend to had a significantly earlier age of onset for psychosis (Pelayo-Terán et al. 2010). This polymorphism results in a ~40% decrease in functional enzyme activity, resulting in higher synaptic dopamine levels following neurotransmitter release, increasing dopaminergic stimulation of the postsynaptic neuron (Chen et al. 2004). Finally, there is evidence to suggest that the timing and frequency of exposure may also play a significant role in predicting individual outcomes.

The association between cannabis use and chronic psychosis is stronger in individuals who are heavy or frequent users during adolescence or who use cannabis with high THC potency (Di Forti et al. 2014; Galvez-Buccollini et al. 2012). In fact, frequent use of cannabis with high THC potency has been associated with a 6-fold increase in risk for schizophrenia (Di Forti et al. 2015). Therefore, there is a rationale to investigate THC specifically in the context of schizophrenia. To date, there are limited studies that have investigated how adolescent THC exposure in adolescent impairs brain development, potentially leading to a psychotic episode.

In addition to increasing risk for psychosis, chronic cannabis consumption in adolescence has been associated with depression and anxiety. An epidemiological study following 1920 individuals from 1994-1996 in the Baltimore area found that individuals with depressive symptoms were four times more likely to also abuse cannabis. Data from four Australian cohort studies (6900 individuals) also lends support to this association, as they found a modest increase in depressive symptoms to be associated with heavy cannabis use; this effect was strong in adolescence but declined in adulthood (Horwood et al. 2012). Others have observed an association between cannabis use in adolescence and increased anxiety in adulthood (Degenhardt et al. 2013). Again, it remains unclear whether cannabis consumption is responsible for the emergence of these symptoms, or if individuals are consuming cannabis as a way to ease their pre-existing symptoms.

2.7.2 Mechanism of action

The main psychoactive component of cannabis (or marijuana) is delta-9-tetrahydrocannabinol (THC), a compound that activates the endocannabinoid system via CB-1 (and to some extent CB-2) receptors in the brain. The discovery of THC as the psychoactive component in cannabis led to the identification of the endogenous CB-1 G protein-coupled receptor to which THC could exert specific and saturable binding (Devane et al. 1988). CB-1 receptors are in high concentration in the hippocampus, amygdala, basal ganglia, many regions of the cerebral cortex and cerebellum (DeLisi 2008; Herkenham et al. 1991). This receptor was found to exist presynaptically, and to inhibit neurotransmitter release from the axon terminal (Malone, Hill, and Rubino 2010). More specifically, activation of CB-1 receptors suppresses GABA release, which can disrupt development of pyramidal cell and parvalbumin containing basket cell circuitry creating an imbalance in excitatory-inhibitory signaling in the brain, which may ultimately lead to psychotic symptoms (Bloomfield et al. 2016). Similar imbalances have been identified in MIA-exposed offspring as discussed in section **2.5.4**. Additionally, given the ability of the CB-1 receptors to function as retrograde signals and traverse back across the synapse, they must play a critical role in the maintenance and determination of synaptic plasticity (Shrivastava et al. 2014). Cannabis has also been shown to increase dopamine release acutely, which may underlie positive psychotic symptoms (hallucinations, delusions) (Keshavan et al. 2014). THC-mediated increase in dopamine signaling may affect the maturation of the dopamine system, which regulates motor function, cognition, motivation, and emotional processing; these behaviours are typically impaired in psychosis (Shrivastava et al. 2014). Finally, CB-1 receptors have also been observed on glutamate, serotonin, and acetylcholine releasing neurons, in addition to the GABA-ergic and dopaminergic ones already mentioned (Freund, Katona, and Piomelli 2003). Interestingly, CB-1 receptors have also been identified at low levels within microglial populations (Cabral et al. 2008), and have been observed to affect glial cell function in response to cellular injury (Walter et al. 2003). These may be interesting avenues for further investigation, as microglial activation has been implicated in many of the downstream neurodevelopmental aberrations due to MIA-exposure.

2.7.3 Pharmacokinetics of THC

2.7.4.1 Smoking

Smoking is the principal route of cannabis administration. It provides rapid and efficient absorption from the lungs to the brain (Huestis 2007), making it the preferred route of dosing for most individuals. THC absorption by inhalation has a bioavailability of 2-56% from cigarettes (McGilveray 2005; Huestis 2007). The source of plant material, composition of the cigarette, and the inter- and intra- subjects' smoking efficiency and variability may all affect the amount of THC absorbed. Canadian marijuana is thought to have approximately 15% average THC content but can be as high as 30% (Health Canada. https://www.canada.ca/en/health-canada/services/drugs-medication/cannabis/about.html). Due to these factors, it is sometimes difficult to establish a clear relationship between THC content in cigarettes and peak THC plasma concentrations. Work from Huestis and colleagues implemented a strict smoking protocol and performed extremely rapid blood sampling in 6 volunteers using two THC dose levels (1.75 and 3.55%). They found that 2 minutes after the first puff, THC concentrations were detectable in blood, peaking at 9 minutes (Huestis et al. 1992). Interestingly, THC and its metabolites were found in plasma for up to 7 days after smoking (Huestis, Henningfield, and Cone 1992). Further, bioavailability seems to increase with use, as heavy smokers have been found to obtain higher bioavailability (23% to 27%) of THC than light marijuana smokers (10% to 14%) (Toennes et al. 2008).

2.7.4.2 Other routes of administration

Fewer studies have examined the disposition of THC and its metabolites following oral cannabis administration via consumption of edible cannabis products, or "edibles". It is readily absorbed, and may be the preferred route of administration for therapeutic purposes such as in the management of pain, nausea, anxiety, or glaucoma, amongst other applications (Nashed, Hardy, and Laviolette 2020) (to avoid negative impact of smoking (Huestis 2007)). This route of administration leads to slower absorption, as well as a lower and more delayed peak concentration (Law et al. 1984); bioavailability has been found be 6% 1-5 hours post ingestion, and to reach 10-20% 4-6 hours post ingestion, comparable to the bioavailability achieved by light smokers (section **2.7.4.1**).

Although THC is not typically used intravenously, the pharmacokinetics have been investigated for this method to evaluate the association between cannabinoids and psychosis in experimental studies. In a double-blind, randomized, and placebo-controlled study, 0, 2.5, and 5mg of THC were administered intravenously to healthy individuals. They found plasma concentrations to peak after 10 minutes and found increases in psychotic symptoms (D'Souza et al. 2004).

2.7.4.3 Distribution and metabolism

THC plasma concentrations decrease rapidly following the end of smoking as it is lipophilic and begins to distribute rapidly through well vascularized organs and tissues (Lucas, Galettis, and Schneider 2018), including the lung, liver, heart, and brain (McGilveray 2005). Interestingly, however, only 1% of an administered dose was found to reach the brain in animal studies, suggesting that much of the THC is sequestered to fat tissues (Agurell et al. 1986; Adams and Martin 1996). Using radioactively labeled THC, previous animal work has observed the highest concentration of THC in the lungs (Lemberger et al. 1970). Distribution may be influenced by body size, body composition, and other factors affecting permeability of blood-tissue barriers (Lucas, Galettis, and Schneider 2018). It has been shown that smoking leads to significantly higher blood THC concentrations than both oral and vaporized administration, with oral achieving the lowest concentrations (Farokhnia et al. 2020).

THC is primarily metabolized in the liver (as well as the intestines and brain (Huestis 2007)) into 11-hydroxy-THC (11-OH-THC), shown to have psychotomimetic properties, and 11-carboxy-THC (11-COOH-THC), which can be excreted in the feces and urine (Gaston and Friedman 2017). However, up to 80 other metabolites of THC have been identified. 11-hydroxy-THC tends to appear rapidly and peak shortly after THC intake (~15 minutes) when the drug is smoked, however, with oral administration, THC and 11-hydroxy-THC seem to be present in equal concentrations in plasma, and to peak later, highlighting some additional differences in metabolism due to administration route (Martin et al. 1976).

2.7.4 Endocannabinoid signaling and adolescent development

Endocannabinoid signaling is critical to brain development during gestation as it supports the development of neuronal processes, the proliferation and differentiation of progenitor cells, neuronal migration, axonal guidance, neurite outgrowth, and morphogenesis (Harkany et al. 2007,

2008). Previous studies have found that blockade of the CB-1 receptor in mid- to late gestation impairs progenitor proliferation in the subventricular zone, axonal migration, and cortical organization (Mulder et al. 2008), as well as hippocampal and cortical interneuron development (Berghuis et al. 2005). These studies provide robust evidence for the critical role that the endocannabinoid system plays in driving normative brain maturation and establishing neuronal circuits.

Adolescence represents a critical period for brain development defined by changes in synaptic pruning and density, receptor distribution, brain volume, myelination, and neurotrophic levels (Giedd et al. 1999; Bartzokis et al. 2001; Andersen and Teicher 2008). Many of these changes are particularly notable in higher order brain regions such as the prefrontal cortex, and other association cortices, as well as limbic structures and the hippocampus (Malone, Hill, and Rubino 2010). While the effects of endocannabinoid signaling have not been as clearly mapped out as they have in prenatal brain development, there is evidence to suggest that endocannabinoids do play a critical role in guiding these changes. Additionally, as their brain is still developing, adolescents may be more prone to the psychoactive effects of THC exposure (Iede et al. 2017). Further, cannabis is typically perceived as safe by many, and is consumed on a regular basis by 4.5% of world inhabitants aged 15-64 (Nordentoft 2010), and by up to 10% of young adults in developed countries; (Rapp et al. 2012) its pervasive use warrants a deeper understanding of its effects on brain development and behavioural outcomes in users.

2.7.5 Behavioural outcomes due to cannabis exposure

2.7.5.1 Acute administration

A number of studies have investigated the acute effects of cannabinoids in human subjects. Intravenous THC administration has been associated with an increase in both positive and negative symptoms in healthy individuals (as measured by the Positive and Negative Symptom Scale questionnaire), peaking 80 minutes following administration, and normalizing within 4 hours (D'Souza et al. 2004). A recent meta-analysis by Hindley and colleagues investigated the effects of THC alone and in combination with CBD on psychiatric symptoms in healthy people (compared with placebo); based on 12 eligible studies they found that THC increased both positive and

negative symptom severity with a large effect size (Hindley et al. 2020). Conversely, of the 4 studies investigating THC with CBD, only one found a reduction in symptoms, suggesting that THC administration may acutely induce psychotic-like symptoms, but that there is less consistent evidence for the role of CBD, and its interaction with THC (Hindley et al. 2020). Acute THC exposure has also been associated with increased anxiety (Atakan et al. 2013; Colizzi et al. 2018), feelings of anger, and aggression (Mathew et al. 1992), and decreased motor coordination and attention (Weinstein et al. 2008). This evidence indicates that acute THC exposure can transiently induce a spectrum of symptoms associated with psychotic disorders in healthy individuals, or temporarily worsen symptoms in individuals with a psychotic disorder.

2.7.2.2 Chronic use

Impairment in cognitive functions is often observed following either chronic or acute cannabis use; this is another core feature of psychotic disorders. Heavy cannabis use has been associated with blunted executive function in humans (Keshavan et al. 2014), and blunted spatial working memory in non-human primates (Verrico et al. 2014). Impairments in processing speed, verbal learning and memory, attention, executive function, and object recognition memory have also been observed in chronic cannabis users (Battisti et al. 2010). Further, early onset of cannabis use, which is thought to have more deleterious long-term effects, has also been found to alter cognitive impairment, a process reliant on the dorsolateral prefrontal cortex (Solowij and Michie 2007). Similar cognitive dysfunction has been observed in both chronic cannabis users and individuals with schizophrenia (Solowij and Michie 2007); this may provide further evidence for the central role of the cannabinoid system in the etiology of the disease.

2.7.6 Brain imaging studies of acute and chronic cannabis use

2.7.6.1 Effects of acute THC administration

Acute cannabis exposure has been associated with acute onset of psychotic symptoms, as well as increased risk for neuropsychiatric illness and cognitive alterations. The mechanisms by which acute exposure induces these changes are unclear. Neuroimaging studies provide a non-invasive approach to probe how the brain responds to acute cannabis consumption. This may allow us to better understand the link between cannabis and long term structural and functional changes

associated with onset of disease, however there is great heterogeneity in the findings, as reviewed by Cupo and colleagues (Cupo et al. 2021). Decreases in connectivity in reward and fronto-limbic pathways have been reported following acute THC administration (Rabinak et al. 2018); decreases in activity in regions related with cognitive functions, as well as global reductions in blood flow have also been reported (Jockers-Scherübl et al. 2006). When comparing acute administration in chronic users relative to controls, global resting state activity and prefrontal blood flow was found to be lower in cannabis users than in controls (Jockers-Scherübl et al. 2006). Further, reduced metabolism, as measured by positron emission tomography (PET) imaging, in frontal and anterior cingulate cortices have been observed in chronic THC users relative to controls following an acute dose of THC (Weinstein et al. 2008). Thus, although there is heterogeneity in the methods used and regions investigated it seems as though THC exposure decreases brain activity in regions associated with higher level processing and may decrease overall brain metabolism.

2.7.6.2 Effects of chronic cannabis use

As discussed above, multiple studies demonstrate that acute THC exposure impacts brain activity (and behaviour). The next critical step in understanding how this risk factor impacts brain development at the structural and functional level is to investigate the effects of chronic use. Neuroimaging studies have been performed in patients consuming cannabis with and without psychosis in order to understand if there are associated brain-wide structural and functional changes. As with the literature on acute effects of cannabis exposure, there is a great deal of heterogeneity across studies. As discussed in section (**2.6.2**), CB-1 receptors are in high concentration in the hippocampus, amygdala, basal ganglia, many regions of the cerebral cortex and cerebellum (DeLisi 2008; Herkenham et al. 1991). As such, these regions may be vulnerable to the effects of chronic cannabis use and are also regions known to be affected in schizophrenia (Fusar-Poli et al. 2013). Interestingly, some longitudinal MRI studies of chronic cannabis users have found abnormalities in the CB-1 receptor rich regions, including reduced hippocampal, amygdala (Yücel et al. 2008), and cortical gray matter volume (Koenders et al. 2016). In fact, atrophy of regions associated with memory and affective processing such as the hippocampus and parahippocampal cortex, as well as the amygdala, have been consistently reported (Demirakca et al. 2011; Matochik et al. 2005; Yücel et al. 2008), as has decreased axonal connectivity in the fimbria of the hippocampus and corpus callosum (Zalesky et al. 2012). Heavy cannabis use is associated with a decrease in grey and white matter volume in frontal and parietal lobes and

reduction in cerebral blood flow (Pierre 2010; Battistella et al. 2014). Reports of decreases in grey matter volume in the temporal cortex, parahippocampal gyrus, insula, and orbitofrontal cortex have also been made in heavy relative to recreational users (Battistella et al. 2014). However, while many have reported brain volume decrease, others have reported either increases or no observable differences, indicating that this requires further investigation (Tzilos et al. 2005; Cousijn et al. 2012).

Much of the work discussed above has investigated the effects of heavy cannabis use on brain volume. Interestingly, a recent study investigating the effects of recreational use in a group of 14 year old males and females found that relative to matched controls who were THC-naive, recreational users had decreased grey matter volume in the bilateral cingulate cortex, lingual gyri, and cerebellum, which map onto regions in which the CB-1 receptor is expressed (Orr et al. 2019); these were associated with decreased performance in a perceptual reasoning task, as well as higher symptoms of anxiety (Orr et al. 2019). Therefore, it seems as though chronic cannabis use has pervasive effects on brain anatomy. However, even lower exposure, at recreational doses, may affect both brain structure and function.

2.7.7 Neurobiological outcomes associated with cannabis use

Given the regional expression of CB-1 receptors, and their ability to modulate a number of other neurotransmitter systems, it is plausible that they may be implicated in psychotic disorders. Some post-mortem studies have investigated the density of CB-1 receptors in the brains of individuals with schizophrenia; in those who had not consumed cannabis, increased density of the receptors was observed in the prefrontal cortex, whereas in those who had consumed cannabis, increased density was observed in the caudate-putamen (Dean et al. 2001). Similarly, elevated levels of anandamide, an endogenous cannabinoid agonist, have been found in the cerebrospinal fluid of individuals with schizophrenia who had not consumed cannabis (Dean et al. 2001). This provides some interesting evidence to suggest that alterations to the endocannabinoid system may be implicated in the pathology of schizophrenia, and that these changes may be different from those associated with cannabis consumption independent of schizophrenia.

2.7.8 Animal studies on adolescent cannabinoid exposure

2.7.8.1 Behavioural findings

Preclinical rodent and non-human primate research is critical in our understanding of the association between cannabis or THC exposure and behavioural outcomes (Rubino and Parolaro 2016). Rodent work has shown that adolescent administration of THC or synthetic cannabinoids disrupts behaviours associated with core features of schizophrenia in adulthood. Along the negative symptoms axis, previous studies have found decreased social behaviour in adulthood (O'Shea, McGregor, and Mallet 2006); with regards to anxiety, there are mixed findings from experiments using the open field test or by the elevated plus maze (Mateos et al. 2011; Llorente-Berzal et al. 2013). Along the cognitive symptom axis, THC or synthetic cannabinoids have been shown to disrupt working memory and cognitive flexibility (Verrico et al. 2014; Gomes, Guimarães, and Grace 2014). Finally, disrupted sensorimotor gating and locomotor hyperactivity have also been observed, pointing to dopamine dysregulation, and most related to the positive symptom axis (Gleason et al. 2012; Schneider and Koch 2003). Similar but more severe behavioural alterations were observed in COMT knockout mice, with greater deficits in male offspring (O'Tuathaigh et al. 2010). It is clear that adolescent cannabis/THC exposure induces behavioural changes, however, a wide range of compounds have been investigated, including different types of synthetic cannabinoids, or THC. There is inconsistency in the dose, duration, and administration route of these compounds (Rubino and Parolaro 2016), therefore further investigation is required.

2.7.8.2 Brain imaging findings

There are limited preclinical studies using brain imaging modalities to investigate the effects of THC exposure on the brain. However, a few PET imaging studies provide further evidence of brain-wide alterations. For example, increased global uptake of [F]-FDG has been observed in young adult rats following acute administration of a THC homology (HU-210), indicative of a potential increase in brain metabolism (Nguyen et al. 2012). Another PET imaging study reported an increase in D2 and D3 receptor availability (based on [F]fallypride binding) in the dorsal striatum following three weeks of daily THC injections (in male mice only) (Ginovart et al. 2012). These findings are in line with PET studies reporting increased D2-like receptor availability in individuals with schizophrenia (Seeman 1990).

2.7.8.3 Neurobiological findings

Chronic adolescent administration of THC has been associated with the development of traits associated with psychiatric disorders. Epigenetic changes associated with genes involved in the endocannabinoid system have been reported in the frontal cortex of female rats following chronic adolescent THC exposure (Prini et al. 2018). Altered synaptic pruning has been observed in pyramidal neurons of the rat prefrontal cortex following adolescent THC administration (Miller et al. 2019). Decreased pre- and postsynaptic protein markers have also been observed in the rat hippocampus following chronic THC exposure in adolescence, as well as lower dendritic length and reduced spine density, as measured by Golgi-Cox staining (Rubino et al. 2009). Decreases in glial cell and NDMA receptor density were also observed in these rats, who displayed deficits in spatial working memory (Rubino et al. 2009). Thus, it seems as though chronic cannabis administration, particularly in the adolescent period, influences the structural integrity of dendrites and synapses, potentially leading to circuit dysfunction and remodeling. Further, synaptic alterations have been consistently reported in post-mortem studies of individuals with schizophrenia and in preclinical animal models associated with the disease (Selemon and Zecevic 2015; Oh-Nishi et al. 2010).

2.7.9 Sex-dependent effects of cannabis

A better understanding of sex differences in the context of neurodevelopment and psychiatric disorders is critical. There have been observations of sex differences in the age of onset, symptom profile, neurobiology and prevalence of schizophrenia and autism spectrum disorder (Bedford et al. 2020; Maric et al. 2003), sex differences in inflammatory systems (Schwarz 2016) and in response to MIA-exposure (as highlighted in **2.6**) (Gogos et al. 2020; Estes and McAllister 2016), as well as sex differences in response to THC exposure (Cooper and Craft 2018). Some evidence suggests that males are more likely to initiate drug use earlier than females and are more likely to be heavy users (Kohn, Kittel, and Piette 2004), however this is not a consistent finding. Further, cannabis smoking may be associated with different mental states in males versus females (Kohn, Kittel, and Piette 2004). Sex differences exist in the endocannabinoid system, wherein greater CB-1 receptor density has been observed in male hippocampus, prefrontal cortex, and mesencephalon, and female amygdala (Calakos et al. 2017). There have been some reports that

women tend to feel more anxious, drowsier, and more fatigued than males following THC exposure, although not all studies adjust dose to body weight (Fattore and Fratta 2010; Calakos et al. 2017).

Some reports of sex differences have been observed in brain imaging studies; chronic female users were found to have larger amygdala volume and decreased anterior cingulate and orbitofrontal cortex volume than non-users, with no effects in males (Boulos et al. 2016; McQueeny et al. 2011). In contrast, greater reductions in cerebellar volume and frontal and parietal grey matter volume have been observed in males (Dalwani et al. 2015). Work by French and colleagues found that males who used cannabis and had a high polygenic risk score for schizophrenia had greater decreases in cortical thickness in adolescence (14.5-18.5 years of age) (French et al. 2015). This finding may help parse some of the heterogeneous brain imaging findings, suggesting that alterations in cortical maturation may be sensitive to cannabis consumption interaction with genetic risk for schizophrenia in a sex-specific way, and that there may be additive negative effects across multiple risk factors.

Previous animal work has found that chronic adolescent THC administration decreased CB-1 receptor levels in the amygdala, ventral tegmental area, and nucleus accumbens of female rats, paired with greater behavioural despair and anhedonia in female, but not male rats (Rubino et al. 2008). Greater catalepsy, and locomotor effects have also been observed in female rats (Biscaia et al. 2003). Chronic adolescent THC exposure has also been associated with greater reductions in CB1-receptor expression in female rat hippocampus and striatum than males (Silva et al. 2015). Evidence from animal studies suggests that there may be sex differences in the metabolism of THC, which could explain some of the behavioural and neurochemical differences observed. After acute THC injection, female rats were found to produce more 11-OH-THC than males, particularly in the adolescent period (Tseng and Craft 2004; Wiley et al. 2007); this difference is thought to be modulated by gonadal hormones (Craft et al. 2017). Overall, there is data from human and animal studies to suggest that there may be sex differences in response to chronic THC exposure (Cooper and Craft 2018), however more research is required to elucidate the implications of these differences.

2.7.10 Investigating the combined effects of prenatal MIA and adolescent THC exposure on offspring outcomes

As mentioned previously not all individuals exposed to cannabis in adolescence develop psychosis, suggesting that pre-existing vulnerabilities may exist. Some evidence points to genetic predispositions (Caspi et al. 2005; Estrada et al. 2011a), however other early life risk factors may also play a significant role, such as prenatal MIA-exposure. This is in line with the commonly accepted two-hit hypothesis of schizophrenia, which suggests that a prenatal genetic or environmental exposure disrupts some aspect of brain development, increasing vulnerability to a second hit that may occur later in life (Rapoport et al. 2005). MIA has been investigated in conjunction with other "hits" such as genetic risk factors (DISC1 mutation (Lipina et al. 2013)) or environmental risk factors such as adolescent stress (Giovanoli et al. 2013). Both prenatal MIA and adolescent cannabinoid exposure have been identified as major environmental risk factors for mental illness.

To our knowledge, no rodent work has been done to investigate longitudinal brain wide and behavioural changes following exposure to MIA and adolescent THC, as are presented in this thesis. Interestingly, previous work using PET imaging to longitudinally asses CB1 receptor expression in the offspring of MIA-exposed offspring (GD 15) found that MIA-exposed rats had lower CB1 receptor standard uptake values in the globus pallidus in adolescence, and lower values in the sensory cortex and hypothalamus in adulthood, relative to controls; these findings indicate that MIA may alter the cannabinoid system in adolescence and adulthood, potentially leading to greater sensitivity to drug use in this period (Verdurand et al. 2014).

Additionally, previous work has investigated the effects of these two combined risk factors on gene expression in late adolescence. Pregnant rats were exposed to poly I:C at GD 16, and offspring received daily injections of a synthetic cannabinoid (HU210) from PND 35-49 (Sharon L. Hollins et al. 2016). In line with other investigations of two-hit models (such as MIA and adolescent stress (Giovanoli et al. 2013)), each risk factor alone led to subtle changes in gene expression; however, dramatic and persistent alterations of small non-coding microRNA in the entorhinal cortex associated with transcriptional networks enriched with genes associated with neurotransmission, cellular signaling, and schizophrenia were observed in rats exposed to both risk factors (Hollins et al. 2016; Hollins et al. 2014). Furthermore, work from the same group found a synergistic effect between exposure to MIA and adolescent THC on serotonin receptor binding in

the hippocampus, such that binding was only increased in the group exposed to both risk factors (Dalton et al. 2012). Conversely, another group investigating the impact of these two risk factors found that THC exposure in adolescent rats (PND 45-55) previously exposed to MIA (GD 15) attenuated several MIA-induced deficits in male offspring, such as normalizing the MIA-induced hyperactivity of VTA dopamine cells (Lecca et al. 2019).

Adolescent cannabinoid exposure (synthetic cannabinoid WIN 55,212-2) has also been investigated in conjunction with a different prenatal risk factor, maternal exposure to methylazoxymethanol (MAM) on GD 17; this compound is a DNA methylating agent shown to disrupt neurodevelopment in offspring consistent with those observed in schizophrenia (Gomes, Guimarães, and Grace 2014). This group found that pubertal WIN treatment in normal animals, and prenatal MAM exposure led to schizophrenia-like deficits in adulthood, including deficits in behavioral flexibility, augmented locomotor response to amphetamine, and increased spontaneous activity in VTA dopamine neurons. Contrary to expectations, however, pubertal WIN in conjunction with prenatal MAM did not exacerbate deficits, but rather normalized amphetamine-induced hyperlocomotion (Gomes, Guimarães, and Grace 2014). Clearly, there are interacting effects between exposure to these two risk factors, however the long-term effects remain to be elucidated (Gomes, Rincón-Cortés, and Grace 2016).

2.8 Magnetic Resonance Imaging

Magnetic resonance imaging (MRI) is a safe and non-invasive medical imaging technique used for imaging soft tissue. MRI is a commonly used tool for imaging the human and rodent brain as it offers high spatial resolution and allows for the generation of 3 dimensional images. Importantly, it can be used in applications and studies of the brain including structural, functional, and quantitative imaging.

An MRI scanner consists of a magnet, which maintains a strong magnetic field (B_0, measured in Tesla (T)) which is used to magnetize and polarize protons (hydrogen atoms) in the tissue (Nishimura 1996; Plewes and Kucharczyk 2012). Further, there is a radiofrequency (RF) coil, which can transmit and receive radiofrequency energy to and from the tissue of interest, and a shim coil which is used to correct local inhomogeneities of the main magnetic field (Berger 2002; Elmaoğlu and Çelik 2011). Finally, gradient coils are used to localize the MR signal and exist in

the x, y, and z directions, with B_0 applied in the z direction, through the center of the bore (**Figure 2.3**).

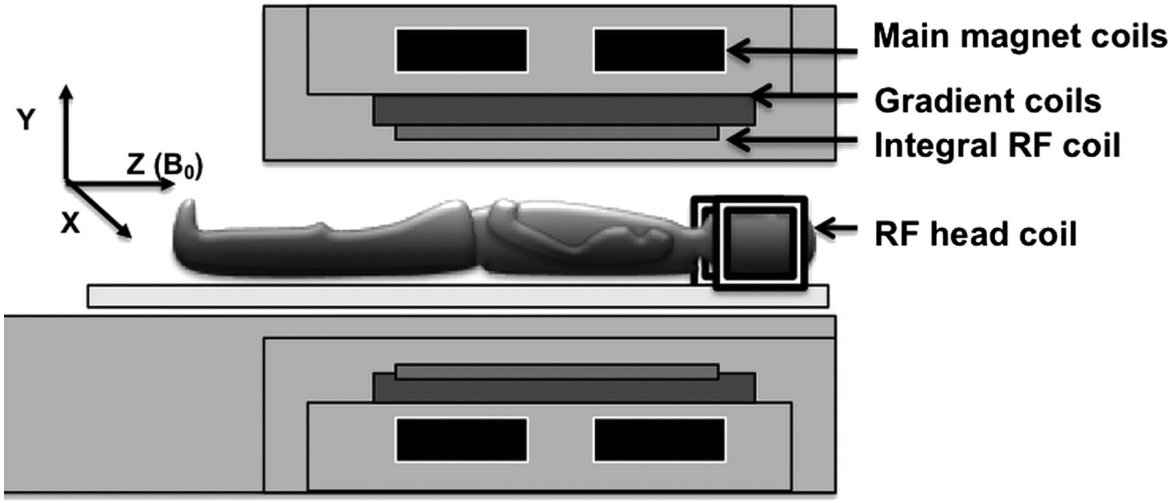


Figure 2.3. Schematic demonstrating the different components of the MR machine relative to each other. The patient lies within the bore of the machine. Their head is surrounded by coils, including the radiofrequency coil, gradient coils, and main magnet coils. From (Currie et al. 2013).

For the majority of MRI-based image contrasts, the signal which we are interested in measuring is derived from protons in tissues, specifically from hydrogen nuclei. These consist of a single proton that carries a positive electrical charge, and spins about its axis, creating its own magnetic field (known as magnetic moment). The procession of these hydrogen atoms creates a positively charged current, which forms a current loop and gives the nucleus a magnetizable quantity. The magnetic moments are typically randomly oriented, however, when a subject (or rodent) enters the scanner and is exposed to an external magnetic field (B_0), the protons will align with (parallel) or against (antiparallel) to that external field. The parallel alignment requires the least amount of energy, and thus is the preferred state, thus most protons align with B_0 (longitudinal magnetization). The frequency of the procession of protons in the direction of the magnetic field is proportional to the strength of B_0 and can be calculated by the Larmor frequency equation: $f_0 = \gamma B_0$ where f_0 is the precession frequency, B_0 is the strength of the external magnetic field, and γ is the gyromagnetic ratio, a constant specific to each nucleus or particle. The precession frequency of protons is called the Larmor frequency (van Geuns et al. 1999).

In order to obtain MR signal, a RF pulse (typically referred to as B_1) is applied to the tissue by the RF coil in the scanner. This RF pulse transfers energy to the protons, causing them to fall out of alignment with B_0, and start processing in the transverse plane (xy) (Pykett 1984). This process can only occur when the RF pulse has the same processional frequency as the protons, thus it is set to the Larmor frequency. RF pulses are programmed at a specific flip angle, determined by the strength and duration of B_1, such that, for example, a 90° would tip the main magnetization vector perpendicularly to the static magnetic field. When the RF pulse is removed, the protons relax and return to their original state, processing, once again in line with B_0 (z-axis), and equilibrium is restored. As the protons relax, they emit energy, which causes variation in magnetic field; this generates measurable signal detectable in the RF receiver coil in the xy plane (Nishimura 1996) **(Figure 2.4)**.

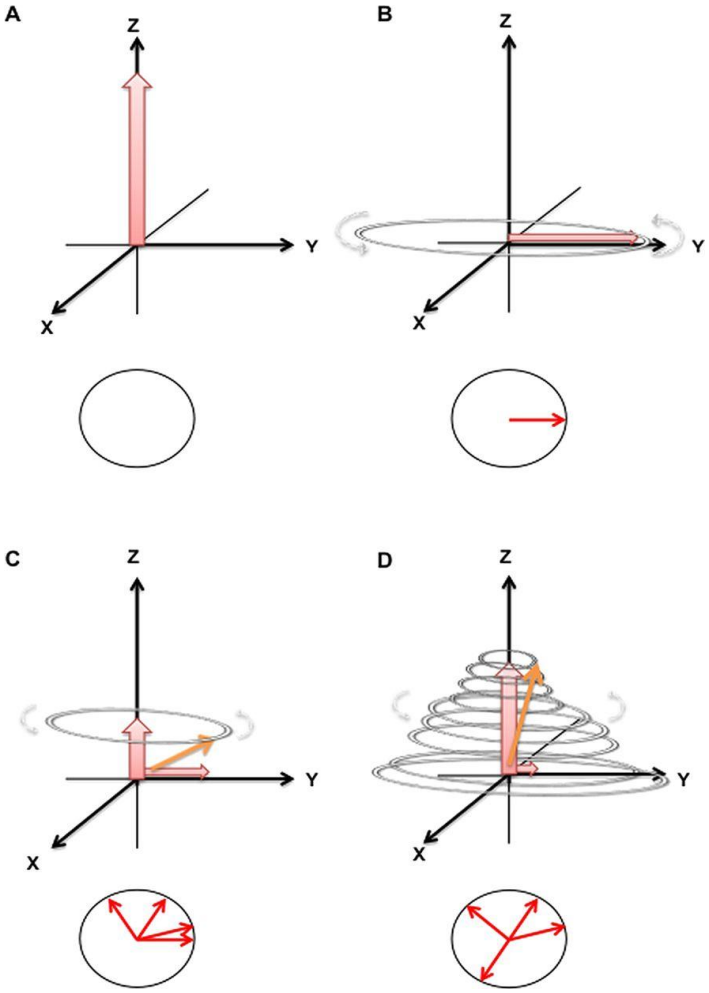


Figure 2.4. Diagram of longitudinal recovery following a 90-degree radiofrequency pulse. **A.** Protons are aligned with the main magnetic field B0. **B.** A 90 degrees RF pulse is applied causing proteins to align with the y direction. **C.** Following the pulse, protons fall out of phase, and process back towards the z direction in a spiraling motion, aligned with the main magnetic field B_0. **D.** The T1 relaxation (recovery of longitudinal magnetization) and T1 relaxation (loss of transverse magnetization). Figure from (Currie et al. 2013).

There are two types of relaxation. The first occurs in the longitudinal axis, called T1 relaxation, also known as longitudinal relaxation. This occurs when protons exchange energy with their environment, and slowly return to their original state of alignment with B_0, where longitudinal magnetization is restored (Currie et al. 2013; Nishimura 1996). T1 relaxation time refers to the time required for the magnetization to return to its original value in line with B_0. T1 relaxation efficiency varies for different molecules such that different tissue types have T1 relaxation time differences. These can be leveraged in order to visualize contrast between tissue types in MRI images. For example, protons in free water (unbound/unrestricted) relax too quickly to have an efficient T1 relaxation. Similarly, hydrogen protons bound to large macromolecules such as lipids relax very slowly and so also have low efficiency at T1 relaxation. In order to maximize the difference in signal obtained from different tissue types, T1-weighted images have short repetition

time (TR, time between two RF pulses) and echo time (TE, time between initial RF pulse and signal peak) (**Figure 2.5**) (Elmaoğlu and Çelik 2011). The second type of relaxation is T2 relaxation, or transverse relaxation. This occurs in the transverse plane; as magnetization decreases, protons lose their coherence and exchange energy between each other (Elmaoğlu and Çelik 2011). Typically, a refocusing pulse is applied to rephase the protons and counteract the effects of the external magnetic field inhomogeneity. Multiple 180° pulses are applied consecutively resulting in a chain of spin echoes, each one with a lower intensity than the previous one due to decaying signal. The curve connecting the spin echo intensities is the T2 curve, whereas the T2* curve is generated when 180° refocusing pulses are not used, resulting in a much faster decay in signal (**Figure 2.5**) (Currie et al. 2013). As with T1 relaxation, T2 relaxation time is also dependent on tissue type, leading to different contrasts per tissue. Protons that have slow T1 relaxation times will appear dark in T1-weighted images, but protons with a slow T2 relaxation appear bright in (most) T2-weighted images.

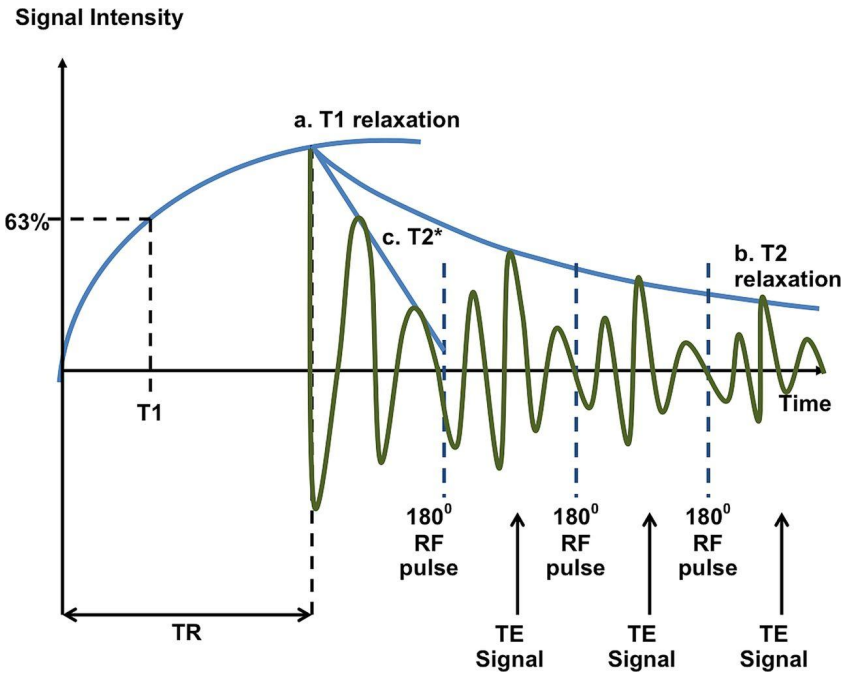


Figure 2.5. Diagram highlighting T1, T2, and T2* relaxation from (Currie et al. 2013). **A**. T1 relaxation curve (recovery of longitudinal magnetization) results from switching off of a radiofrequency (RF) pulse. curve. **B**. T2 relaxation curve: a 180° refocusing pulse is applied to rephrase the protons and counteract the effects of the external magnetic field inhomogeneity. The echo time (TE), also referred to as spin echo, a temporary gain in signal intensity is observed. Multiple 180° pulses are applied consecutively resulting in a chain of spin echoes, each one with a lower intensity than the previous one due to decaying signal. The curve connecting the spin echo intensities is the T2 curve. **C**. T2* curve is generated when 180° refocusing pulses are not used, resulting in a much faster decay in signal. TR, repetition time.

Spatially encoded gradient coils located inside the bore of the MRI scanner are used to obtain spatial location of the signal emitted by the relaxation of protons (after the secondary magnetic field is removed) (Currie et al. 2013). The field gradients, typically applied in the x and y directions, are used to alter the magnetization and the resulting field dependent Larmor frequency by a predictable quantity, spatial location, and phase of precession (Currie et al. 2013). This information is recorded in k-space, a raw matrix in which the data points represent both real and imaginary components (frequency and phase) of precession (Nishimura 1996). This allows for MR image reconstruction by applying the Fourier transformation to the data in K-space.

2.8.1 Small animal magnetic resonance imaging

Neuroimaging techniques provide an intriguing opportunity for translation between human and rodent research. This non-invasive imaging modality is useful for investigating longitudinal changes in brain development *in vivo*, allowing for examination of the neurodevelopmental trajectories. Further, it can also be applied *ex vivo* to investigate neuroanatomy at specific timepoints at very high resolution (further discussed in section **2.8.3**).

Compared to the standard human clinical strength MRI systems (1.5-3 T), small MRI systems typically operate using higher field strength in the range of 4.7-11.7 T, with most common use at 7 T. Certain considerations must be taken into account for small animal MRI. The size of the mouse, relative to humans, presents some difficulties for acquiring anatomical images of comparable quality. Voxel sizes must be as small as tens to hundreds of microns (compared to the millimeter scale used in humans). This is typically accomplished by the use of higher field strengths, as mentioned, as well as longer scan times, the use of contrast agents, and custom designed coils (Lerch et al. 2012). To achieve high quality images *in vivo,* animals must be kept immobilized. This challenge is overcome by anesthetizing the animals prior to placing them in the scanner and during the scan with an inhalant anesthesia such as isoflurane, or by administering another type of anesthetic prior to scanning such as dexmedetomidine, or with a combination of the two (Benveniste et al. 2017; Denic et al. 2011). The advantage of using inhaled anesthesia is that it can be calibrated to the specific requirements of the rodent. Animals should be monitored for core temperature, respiratory rate, electrocardiogram, oxygen saturation, and carbon dioxide levels to maintain consistent physiological conditions across experimental animals.

The use of paramagnetic contrast agents such as manganese chloride is widely employed in both structural and functional MRI of the nervous system (Vousden et al. 2018; Cloyd, Koren, and Abisambra 2018). It allows for greater contrast in the same or less imaging time, which is favourable both for decreasing time under anesthesia for animals, but also from a cost perspective. When manganese (Mn^{2+}) dissociates from chloride it becomes paramagnetic; it is taken up by the excitable cells in the brain via voltage-gated calcium channels, and so it can accumulate in active brain regions and is transported to axonal tracts (Lin and Koretsky 1997; Sloot and Gramsbergen 1994; Takeda et al. 1998). As this contrast metal is taken up, it shortens the longitudinal relaxation time (T1), and increases the relaxation rate (1/T1) of solvent water protons (Pan et al. 2011). The regions in which the Mn^{2+} accumulates become bright in a T1-weighted MRI (Pan et al. 2011). One of the main challenges of using this contrast agent is that it can be cytotoxic in high doses. However, at the doses typically used for *in vivo* studies, administration of manganese chloride has not been found to significantly interfere with behaviour, such as hippocampal dependent spatial learning, or neurodevelopment, but may lead to severe skin ulcerations over time (Vousden et al. 2018; Szulc et al. 2015; Rollins et al. 2019). Excessive doses, however, have been associated with organ toxicity in both humans and rodents (Cloyd, Koren, and Abisambra 2018). Another very commonly used paramagnetic contrast agent, in both clinical human and research applications, and rodent imaging, is gadolinium. This paramagnetic compound is more commonly used *ex vivo* in rodents, and works by reducing T1 relaxation times, also allowing for improved contrast and image resolution, and shorter acquisition times (Głodek, Adamiak, and Przeworski 2016; Cahill et al. 2012). Many of these considerations are made with adult or adolescent rodent imaging in mind. Special considerations should be taken for embryonic and neonatal mouse imaging, as discussed in the next section.

2.8.2 Embryonic and neonatal mouse neuroimaging

MRI is an inherently 3-dimensional imaging method, applicable for mouse phenotyping both *in vivo* and for *ex vivo* fixed samples. This modality has been well established for anatomical imaging of larger animals (and humans), however significant technical challenges are faced for applying these methods in embryonic and neonatal mice.

Ex vivo MRI, as well as optical projection tomography (OPT) and micro-computed tomography (micro-CT) have all been successfully used to produce highly detailed anatomical

images of mouse embryos (Wong et al. 2012; Wong, Spring, and Henkelman 2013; Wong et al. 2014). Work performed by Wong and colleagues using OPT yielded a high resolution 4D atlas staging mouse development from embryonic day (E) 11.5 to 14 (Wong et al. 2015). *Ex vivo* MRI and micro-CT have also been used to produce highly detailed 3D anatomical atlases of mouse embryos (Norris et al. 2015; Jacobs et al. 1999; Dhenain, Ruffins, and Jacobs 2001). Finally, diffusion tensor imaging (DTI) has also been used successfully to delineate the anatomy of the central nervous system (CNS) of fixed mouse embryos (Norris et al. 2015; Mori et al. 2001).

Imaging of neonatal mice has been successfully performed both *in vivo* and *ex vivo*. Pioneering work by Szulc and colleagues performed *in vivo* longitudinal T1-weighted MRI in neonatal mice from PND 1 to 11 (Szulc et al. 2015), while equally innovative work by Qiu and colleagues longitudinally imaged mice from PND 3 to adulthood (Qiu et al. 2018). *Ex vivo* neonatal imaging has been performed at high-resolution with T1 and T2 weighted MRI contrasts, as well as DTI, allowing for high resolution images of the developing brain (Wu et al. 2013). In this thesis, I leverage advances made in high-resolution imaging to examine both embryonic and neonatal brain development in response to MIA at different gestational times.

2.8.3 Considerations for *in vivo* vs *ex vivo* MRI

Neuroimaging of the rodent brain can be performed both *in vivo* and *ex vivo*, both techniques require special considerations, and have different drawbacks and advantages. *In vivo* MRI provides an opportunity to perform longitudinal evaluation of tissue changes, examine brain changes that index progressive brain development or aging, as well as responses to manipulations or treatments (Lerch et al. 2012). This is a great advantage as there is no need to sacrifice separate cohorts of mice to acquire pseudo-longitudinal data. Further, this type of work lends itself to more nuanced statistical analyses, such as linear mixed-effects modeling (as discussed in section **2.10.1**), which can increase statistical power (Mackenzie-Graham 2012). *Ex vivo* MRI of fixed samples permits a thorough examination with greater sensitivity and higher resolution typically, as there are no constraints on imaging time. The use of tighter fitting coils, higher concentration of contrast agents, and a lack of motion artifacts further improve image quality (Mackenzie-Graham 2012; Lerch et al. 2012). Furthermore, some have developed protocols in which multiple brains can be imaged simultaneously using standard MRI hardware, providing greater throughput on data

acquisition (Bock et al. 2005; Dazai et al. 2011). Unfortunately, *ex vivo* tissues are more susceptible to distortions and dehydration from fixation, which may disrupt their integrity; this is not the case for live tissue (Cahill et al. 2012).

Notably, there are situations in which one method favours another, as explored by Lerch and colleagues in their direct comparison of the two MRI contrasts (Lerch et al. 2012). *Ex vivo* imaging provided more precise images than *in vivo*, and thus may be favourable in scenarios in which time course data is not required. If change over time is of interest, then *in vivo* provides better information as one can acquire within subject data.

2.9 Image processing

Small animal MRI has been used to study normal development and aging, as well as many disease models. It offers many advantages as it is a non-invasive way to obtain a whole brain assay that can be repeated over time. Further, image analysis tools are comparable to those used in human MRI research (and often originate from techniques developed to study human MRI data), and can be performed in many different ways, as described below.

2.9.1 Deformation based morphometry

Deformation-based morphometry (DBM) allows for the characterization of gross and global structural differences in brain shape (Chung et al. 2003; Mietchen and Gaser 2009). DBM uses deformation, or vector, fields to compare the relative position of certain structures within a subject's brain. This requires the images of all subjects to be spatially normalized and registered in a group-wise fashion to create an average image or registered to a template image (Chung et al. 2001). The registration is typically performed in multiple steps. First, images undergo linear rigid alignment with 6 degrees of freedom. The work presented in this thesis uses a least squares fit (LSQ) function to perform the registration, and the linear rigid registration is often referred to as a LSQ6 fit which accounts for translations and rotations along x, y, and z dimensions (Ashburner and Friston 1998; Friedel et al. 2014). The next step would be an affine, or linear LSQ12 fit (i.e. 12 degrees of freedom that include those in LSQ6 as well as scales along the x, y, and z dimensions and shears over xy, xz, and yz planes), followed by nonlinear registration (Ashburner and Friston

1998; Friedel et al. 2014). This matching process ensures that all images conform to the same stereotaxic space, enabling the possibility of voxel-by-voxel comparisons (Chung et al. 2001; Lepore et al. 2006). Treating deformation fields as vector fields representing absolute displacement, or absolute Jacobian determinants, allows for investigation of all linear and nonlinear transformations required to register a subject to the study average or template. Relative Jacobian determinants explicitly model only the non-linear part of the deformations and remove residual global linear transformations (attributable to differences in total brain size) (Chung et al. 2001).

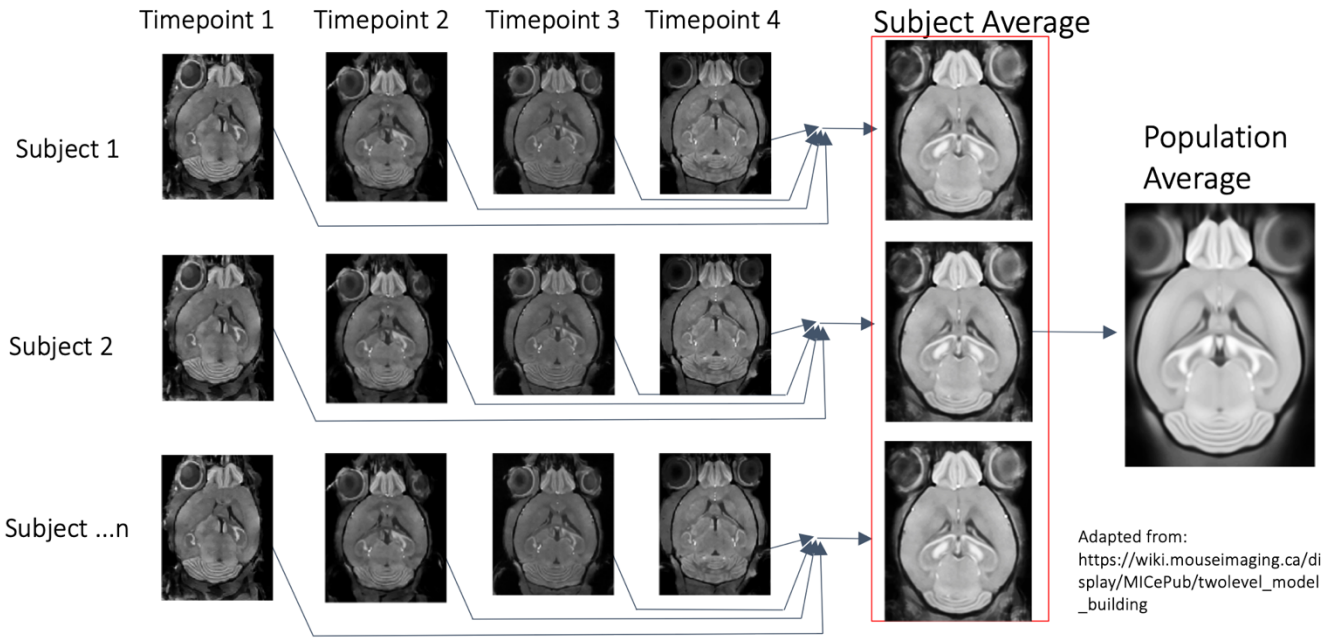


Figure 2.6. Diagram of the registration pipeline. All scans per subject (mouse) are registered to create a subject average. All subject averages were then registered to create s population average for the entire study.

2.9.2 Volumetric analysis

Volumetric analysis involves the estimation of volumes for brain structures of interest, often based on segmentation, amongst other methods. This can be done in a number of ways outlined in this section. Manual segmentation relies on trained anatomists manually segmenting regions of interest from the brain scans. This is the gold standard in segmentation, as the human rater performs a slice-by-slice identification of the given neuroanatomical structure of interest

based on a brain atlas or anatomical priors, following a specific protocol to ensure reliability and accuracy (Despotović, Goossens, and Philips 2015). Once the structure is segmented, the volume is estimated based on the number of voxels in the segmented region and their resolution. This process is difficult to scale to multiple scans and multiple brain regions as it is time consuming. Furthermore, there are limitations with regard to inter- and intra-rater reliability, as well as drift over time (Despotović, Goossens, and Philips 2015). Therefore, its primary use is in smaller studies (Sankar et al. 2015), or for the creation of atlases (Goerzen et al. 2020; Winterburn et al. 2013) that can then be used in automatic segmentation algorithms (Lerch et al. 2017).

Automatic segmentation algorithms provide a useful alternative to manual segmentation, and there are various semi- or fully automated algorithms used to achieve this. In some cases, the algorithms make use of a manually segmented atlas, or representative MRI with segmentations for the regions of interest. These are then used to parcellate the target dataset by using image registration (linear and nonlinear), or tissue classification based on image features. Once alignment is achieved, the segmentation can be transferred from to the target image via the inverse of the transformations required to achieve alignment (Lerch et al. 2017).

The accuracy of segmentation can be improved if there are multiple atlases or representative MRIs as anatomical variability in a given population may be better accounted for. This analysis technique is more robust to biases due to misregistration, and resampling error (Chakravarty et al. 2013). In this case, multiple atlases (with the same region of interest segmented) would be aligned to the target image, resulting in multiple possible segmentations for the target, allowing for biases to be distributed across the different segmentations, and have less of an impact on the final segmentation, which is created by a combination of the multiple atlas-to-target segmentations. There is a further improvement on the multi-atlas concept as there is an additional layer to the segmentation (Chakravarty et al. 2013; Pipitone et al. 2014). A subset of target images is selected to create a template library, onto which the atlas or atlases are propagated. This template library is then propagated onto all the target images, giving a much larger number of possible segmentations, and improving accuracy for the final segmentation.

2.10 Statistical analysis methods

2.10.1 Univariate analysis tools for longitudinal data

Neuroimaging provides a complex biological data set, requiring sophisticated statistical techniques to appropriately extract relevant signals. Over the past few decades, longitudinal neuroimaging studies have become increasingly widespread. These provide advantages such as increased statistical power by reducing the confounding effect of between-subject variability (Bernal-Rusiel et al. 2013). Outcome variables are measured repeatedly within the same subjects over multiple time-points, with the aim of characterizing changes in trajectories over time within-individual in association with experimental manipulation or clinical variables at play. In comparison, cross-sectional studies obtain measurements at a single occasion, and thus are subject to more between-subject variability (Bernal-Rusiel et al. 2013). Therefore, longitudinal studies provide unique insights into the temporal dynamics of underlying biology. Linear mixed-effects models extend traditional linear models to include a combination of fixed effects and random effects as predictor values and offer a powerful and versatile framework for analyzing real-life longitudinal neuroimaging data (Harrison et al., 2018). There are two alternatives to the use of linear mixed effects models: 1) repeated measures analysis of variance, and 2) cross-sectional general linear model-based analysis of summary measures (e.g., percent difference). However, these methods are sub-optimal for longitudinal data as they do not appropriately model the covariance structure of serial measurements, cannot handle missing data, and lose nuance of subject variability (Fitzmaurice, Laird, and Ware, 2011). Further, linear mixed effects models are particularly useful when there is non-independence in data due to either hierarchical structure or repeated measures.

Linear mixed effects models use linear regressions to describe temporal trajectory of various measurement's means. This trajectory is typically expressed as a linear combination of a set of independent variables (Bernal-Rusiel et al. 2013). Not all subjects are required to have a common set of measurements, which provides an advantage in that more data can be included, especially in cases where there is inconsistency in the number or frequency of samples for a given subject. These models are built with fixed effects, which can be thought of as grouping factors or variables of interest (e.g., treatment) that you expect to have an effect on the response variable, and random effects, which can be thought of as grouping factors for which we are trying to control

(e.g., litter, cohort, or subject). Choosing which predictors and interactions to include in models is an important question and should represent a specific *a priori* hypothesis (Harrison et al., 2018). Optimal model selection can also be performed by testing how accurately the model describes the data. Some tools with which one can do this include the Log Likelihood Ratio Test, which assess two models using the Chi square distribution (Lewis, Butler, and Gilbert 2011), or Akaike Information Criterion which provides a goodness-of-fit score, penalizing overfitting (Busemeyer and Diederich 2014). Thus, as was done in the statistical analyses performed in **Chapters 4** and **6**, if we model a treatment by time interaction as our fixed effects and subject is chosen as a random effect, the model will allow each subject to have its own intercept and examine how trajectories change over time with respect to treatment.

2.10.2 Multivariate analysis tools: behavioural partial least squares analysis

Neuroimaging data can give us complex signals from a large number of spatially distributed signals, in this case, voxels, often over multiple timepoints. While univariate analyses are critical for identifying signal changes at the level of individual elements, multivariate techniques may enable us to better capture neuroanatomical changes with a greater focus on spatiotemporal changes in the brain as a network and in relation to another modality, such as behaviour. The studies presented in this thesis recorded changes in neuroanatomy as measured by MRI not in isolation of, but in relation to a number of behavioural measures acquired in the same animals and adjacent to the scan time. It is highly probable that variation in a mouse's performance in one behaviour may be interrelated to their performance on another, and to their neuroanatomy. In order to optimally capture these distributed patterns of neuroanatomical and behavioural covariation across these different within-subject metrics, we can turn to multivariate analyses.

One such analysis is the partial least squares (PLS), which seeks to relate two sets of data, or variables, to each other. PLS seeks to find the optimal weighted linear combination of variables that maximally covary together (Zeighami et al. 2019; McIntosh and Mišić 2013; McIntosh and Lobaugh 2004). In a typical neuroimaging experiment, one would have a matrix of neuroimaging data (e.g., voxel-wise DBM values, or volumetric measures) in columns, and rows corresponding to participants, or subjects. The second matrix may represent a set of behaviour metrics and/or demographic measures organized in the same way, with participants or subjects as rows, and data

in columns (behaviour PLS). There are other types of PLS, such as seed PLS, which tries to relate the activity of a particular voxel to the rest of the voxels, or multi-block PLS, which is a combination of the behaviour and seed, amongst others. These are beyond the scope of this work (McIntosh and Mišić 2013).

The two input matrices are typically z-scored in order to standardize values, particularly if they come from different sources. A covariance matrix is then computed from these two matrices to represent all voxel deformation values and behavioural data per subject. Singular value decomposition (SVD) is then applied to the covariance matrix (Eckart and Young 1936) to yield a set of orthogonal latent variables (LVs), which are patterns that describe the brain-behaviour relationship, or covariation, in the data. Within the latent variables, there are three vectors. First is a singular vector which contains the saliences or weights that identify voxels that make the greatest contribution to the effects captured by the latent variable; this can be thought of as a brain loading or weight. Next, there is another singular vector which contains the behaviour or demographic saliences, yielding the contribution that each behaviour makes to the spatiotemporal pattern identified in the latent variable, which can be thought of as a behaviour loading or weight. The strength of the relationship extracted by each LV is reflected in the relative size of the singular value. The proportion of covariance accounted for by each latent variable is given by the ratio of the squared singular value to the sum of all other squared singular values. The expression of each latent variable can be calculated by taking the dot product of the singular vector and the original input data yielding both a brain and behaviour score. The number of LVs generated is dependent on the smallest dimension in the matrix (McIntosh and Mišić 2013; Zeighami et al. 2019; Patel et al. 2020).

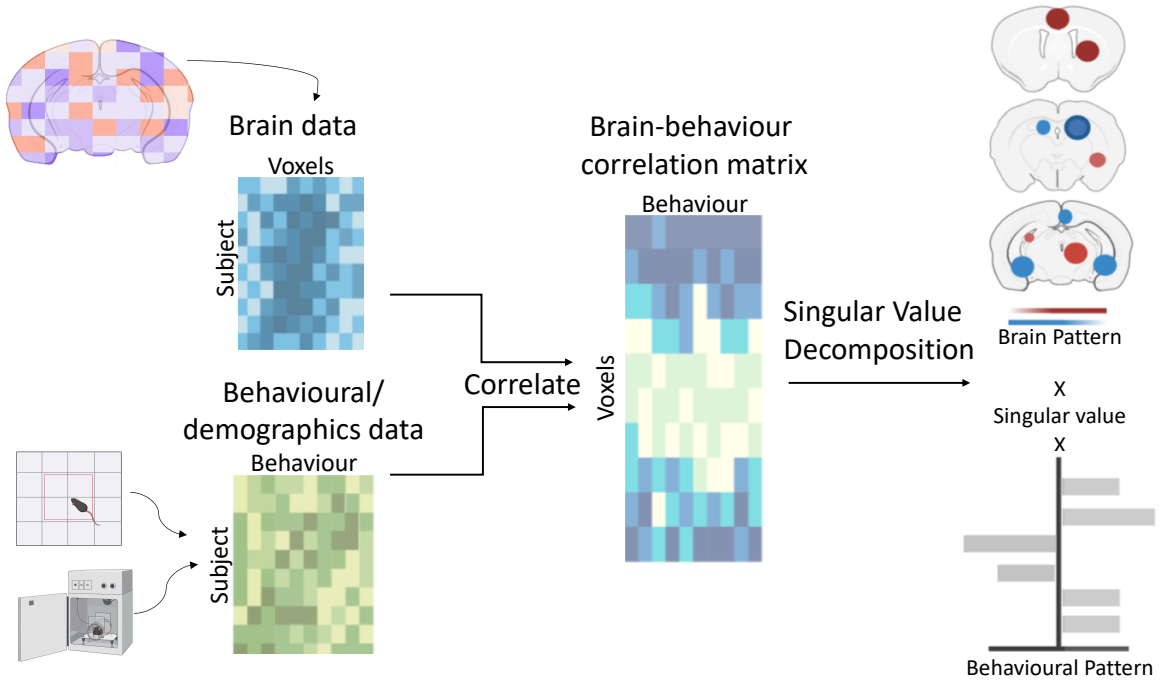


Figure 2.7. Workflow of the partial least-squares (PLS) analysis, which aims to relate two sets of variables to each other. The original variables (brain and behaviour z-scored matrices) are correlated across participants to create a brain-behaviour matrix and subjected to singular value decomposition. The decomposition yields multiple latent variables: linear combinations of the original variables, with the weights chosen to maximize the covariance between them. The contribution of individual variables to the latent variable is assessed by bootstrap resampling. The pairing of the deformation and clinical-cognitive pattern is assessed by permutation tests and cross-validation. Figure adapted from (Kirschner et al. 2020) created using BioRender.com.

In order to assess the statistical significance of each LV, permutations testing is typically used, wherein the rows (subjects) of the brain data matrix are randomly shuffled to nullify dependencies between brain and behaviour. The new data are then z-scored (or mean centered) and subjected to SVD as in the original analysis. This procedure is repeated multiple times (n=1000) to generate a null distribution of singular values under the null hypothesis. A p-value is then estimated as the probability that a singular value from the distribution of permuted samples exceeds the singular value from the original (non-permuted) data. (Zeighami et al. 2019; Patel et al. 2020). A threshold of $p<0.05$ is typically used (95% or greater chance that the singular value of the non-permuted data exceeds that of a permuted singular value).

Next, the reliability and stability of each latent variable must be assessed. This is typically done with bootstrap resampling. Subjects (rows for both input matrices) are randomly sampled and replaced (n=1000) to generate a set of resampled correlation matrices for which PLS is computed to generate a distribution of saliences. Again, SVD is applied to generate a sampling, or bootstrap, distribution for each weight of the singular vectors. The bootstrap distribution can be used to estimate the confidence intervals and standard errors for singular vector weights (McIntosh and Mišić 2013). The ratio of each singular vector weight and its bootstrap-estimated standard error were used to calculate a "bootstrap ratio" for each voxel. Voxels that make large contributions to certain patterns can therefore be identified by large bootstrap ratios, which have high stability. If a bootstrap distribution happens to be normally distributed, then the ratio can be thought of as a z-score. Bootstrap ratios are typically thresholded at values corresponding to 95% confidence interval (Zeighami et al. 2019; McIntosh and Mišić 2013).

2.11 Transcriptomics

The central dogma of molecular biology describes the two-step process by which information stored in genes as DNA is transcribed into RNA, and finally translated into proteins (Kukurba and Montgomery 2015). The transcription of certain genes into complementary RNA molecules specifies a cell's identity and regulates biological activities within the cell. Thus, RNA molecules present in a biological system can give us some indication of what cells are doing or capable of doing (Van den Berge et al. 2019). The term transcriptome has a variety of meanings but is typically used in reference to the quantity of messenger RNA (mRNA) content of the cell, however the transcriptome has a high degree of complexity and is made up of a variety of protein coding and noncoding RNA species (LaRossa 2013; Wang, Gerstein, and Snyder 2009).

Initial gene expression studies relied on low-throughput methods limited to measuring single transcripts such as quantitative polymerase chain reaction (qPCR) or northern blots (Kukurba and Montgomery 2015). Exciting technological advancements over the last few decades have allowed much higher-throughput transcriptomic investigations such as genome-wide quantification of gene expression (McGettigan 2013). RNA-sequencing provides a more precise measurement of transcript levels and their isoforms than other methods (Wang, Gerstein, and Snyder 2009)

The workflow for a typical RNA-sequencing experiment is as follows; the first step involves extraction and purification of RNA from a sample of interest, i.e., tissue from a brain region of interest, or organ, etc. To ensure a successful RNA sequencing experiment, there must be a sufficient quantity of RNA to produce a library for sequencing. Typically, one can obtain an RNA Integrity Number (RIN) between 1 and 10, with 10 being the highest quality with least degradation. Low-quality RNA (RIN<6) can substantially affect sequencing results potentially leading to erroneous conclusions. This is a challenge often faced when using human autopsy samples or paraffin embedded tissues as RNA may be degraded (Kukurba and Montgomery 2015).

Next, the total, or fractionated RNA is filtered and reverse transcribed to a library of cDNA fragments with adaptors attached to either one or both ends. The molecule may then be amplified, or not, and are then sequenced in a high-throughput manner to obtain short sequences from one end (single-end sequencing) or both ends (pair-sequencing). These reads are typically 30-400 base pairs (Z. Wang, Gerstein, and Snyder 2009). Library preparation may include choosing some types of RNA species to be removed. The ribosomal RNA pool accounts for 95% of the total cellular RNA, and so if they are not removed from library construction, they may consume the bulk of the sequencing reads, limiting detection of other less abundant RNAs that may be of greater interest to the experiment (Kukurba and Montgomery 2015). This is typically done by selecting for polyadenylated (poly-A) RNAs such that the 3' poly-A tail of mRNA molecules is targeted using poly-T oligos covalently attached to a substrate (e.g., magnetic beads). This approach is preferred if one is interested in coding RNA (LaRossa 2013). One way in which to make library construction more cost-effective is to assay multiple samples in one sequencing lane such that many reads are generated per sequencing run (e.g., Illumina HiSeq). Following sequencing, the reads are aligned to a reference genome (or reference transcripts) to create a genome-scale transcriptional map containing information about the expression of each gene. For the transcriptional analyses presented in this dissertation, we used NovaSeq S4 PE100. This means that the insert size of the RNA library preparations is 150bp, so the PE100 sequencing is able to generate a single consensus sequencing of the fragment with the forward and reverse reads of 100 nucleotides; further, there were 34M reads/sample.

CHAPTER 3: Characterizing the effects of maternal immune activation on neurodevelopmental trajectories: a cross-species systematic review of magnetic resonance imaging findings

3.1 Preface

This chapter includes a systematic review of the literature using magnetic resonance imaging (MRI) techniques to investigate the effects of exposure to maternal infection or immune activation (MIA) *in utero* on offspring brain development. This work sought to better understand the current state of the literature regarding how exposure to this risk factor affects brain morphology and neurodevelopmental trajectories in offspring, and to identify potential gaps in our knowledge in this field. By focusing on neuroimaging modalities, we can begin to interpret findings across multiple species in a unified framework; this is one of the few modalities that allows for homologous characterization of developmental trajectories across species.

As discussed in **Chapters 1 and 2**, and in this **Chapter**, significant efforts across human and animal research have deepened our understanding of the ways in which MIA-exposure *in utero* alters development, increasing risk for neurodevelopmental disorders. Despite many important advances, there are still significant gaps in our knowledge, as identified in this systematic review. The differential impact of gestational MIA-timing on neurodevelopmental trajectories, the effects of MIA-exposure in early life, its interaction with additional risk factors, and putative sex-differences remain poorly understood. The systematic review presented here aimed to discuss findings from human and animal studies that performed neuroimaging in offspring exposed to maternal infection, inflammation, or MIA, in the context of neurodevelopmental disorders.

CHAPTER 3

The role of maternal immune activation in altering the neurodevelopmental trajectories of offspring: a translational review of neuroimaging studies with implications for autism spectrum disorder and schizophrenia

Elisa Guma, MSc[1,2], Eric Plitman, PhD[2,3], M Mallar Chakravarty, PhD[1,2,3,4]

[1] Integrated Program in Neuroscience, McGill University, 845 Sherbrooke St W, Montreal, QC, H3A 0G4 Canada
[2] Computational Brain Imaging Lab, Cerebral Imaging Center, Douglas Mental Health University Institute, Verdun, Quebec, H4H 1R3, Canada
[3] Department of Psychiatry, McGill University, 845 Sherbrooke St W, Montreal, QC, H3A 0G4 Canada
[4] Department of Biological and Biomedical Engineering, McGill University, 845 Sherbrooke St W, Montreal, QC, H3A 0G4, Canada

Published: https://doi.org/10.1016/j.neubiorev.2019.06.020

3.2 Abstract

Exposure to maternal infection *in utero* increases the risk that offspring will develop neurodevelopmental disorders such as autism spectrum disorder (ASD) and schizophrenia. Research in animal models has confirmed this link and demonstrated that maternal immune activation (MIA) is sufficient to induce alterations in offspring neurodevelopment. Building homology between observations made in humans and animal models is a challenge; however, neuroimaging allows for homologous characterization of developmental trajectories across species. This systematic review aims to discuss findings from human and animal studies that performed neuroimaging in offspring exposed to maternal infection, inflammation, or MIA, in the context of neurodevelopmental disorders.

3.3 Introduction

Epidemiological evidence has established a relationship between *in utero* exposure to maternal infection and increased risk of developing neurodevelopmental disorders such as schizophrenia and autism spectrum disorder (ASD) later in life (Brown et al. 2004; Selten et al. 2010; Brown et al. 2001; Wright et al. 1995). Although primarily associated with ASD and schizophrenia, there have been less frequent associations with other neurodevelopmental disorders, such as attention deficit/hyperactivity disorder, cerebral palsy, and epilepsy (Knuesel et al. 2014). One of the first observations of this association dates back to the 1918 Spanish influenza pandemic, in which Karl A. Menninger documented an association between patients with psychotic disorders and exposure to maternal influenza (Yudofsky 2009). Further, after the 1964 rubella pandemic, the prevalence of schizophrenia and ASD rose from the expected ~1% to 20% and 13%, respectively, in affected areas (Estes and McAllister 2016a; Qi Li et al. 2009).

Interestingly, the link between prenatal exposure to infection and increased risk for neurodevelopmental disorders is not pathogen specific; there is evidence for exposure to *Toxoplasma Gondii* (Mortensen et al. 2007; Pedersen et al. 2011; Severance et al. 2016), reproductive, genital, and urinary tract infections (Clarke et al. 2009; Nielsen, Laursen, and Mortensen 2013), herpes simplex virus 2 (Buka et al. 2003; Buka et al. 2008; Buka et al. 2001; Mortensen et al. 2010), pneumonia, and others as potent risk factors. Common to this diverse group of pathogens is the activation of the maternal immune system and increased maternal serum

levels of pro-inflammatory cytokines such as IL-6, IL-1β, and tumor necrosis factor (TNF)-α (Miller et al. 2011; Potvin et al. 2008; Masi et al. 2015; Ricci et al. 2013; Molloy et al. 2006; Al-Asmari and Khan 2014).

Animal research is an essential tool for understanding neurodevelopment and developing new diagnostic tools and therapeutics. This is especially important given the challenges of studying maternal immune activation (MIA) in humans. Animal models have established causality by showing that MIA during pregnancy does disrupt early neurodevelopment of offspring, altering their developmental trajectories. Long-term behavioural, structural, and functional deficits relevant to schizophrenia and ASD are commonly observed in these offspring, including altered cognitive and social behaviour, impaired sensorimotor gating, and increased anxiety, as well as altered cell migration, microglial function, synaptic structure, and function (as reviewed in (Gumusoglu and Stevens 2019; Reisinger et al. 2015; Estes and McAllister 2016b; Knuesel et al. 2014; Boksa 2010)). These structural and functional alterations do not seem to depend on specific immune-activating agents; however, certain cytokines, such as IL-6 and IL-17a, have been identified as key players (Gumusoglu and Stevens 2019; Smolders et al. 2018; Choi et al. 2016; Wu et al. 2017; Bergdolt and Dunaevsky 2019). Based on the idea that increased maternal cytokine levels and not specific pathogens disrupt neurodevelopment of the offspring, the two most commonly used immune activators are lipopolysaccharide (LPS), a gram-negative bacterial cell wall component that mimics a bacterial infection by binding to toll like receptor (TLR)4, and polyinosinic:polycytidylic acid (poly I:C), a synthetic double stranded RNA analog that mimics a viral infection by binding to TLR3 (Dowling and Mansell 2016).

Despite many important efforts, there are still significant gaps in our knowledge regarding the precise mechanism by which prenatal MIA disrupts early brain development; further, the differential impact on neurodevelopmental trajectories of dose and timing of immunogens used to induce MIA remains elusive (Kentner et al. 2019; Estes and McAllister 2016a). The hemochorial placenta (occurring in mammals including humans and rodents) allows for direct contact between maternal and fetal compartments (Colucci et al. 2011). This suggests that maternal cytokines and chemokines may cross the placenta and enter the fetal compartment in the event of an immune challenge. The fetal immune system may not have the capacity to adequately respond to elevated levels of pro-inflammatory cytokines, which may disrupt the cytokine equilibrium and negatively impact fetal brain development (Reisinger et al. 2015). Microglia, the resident immune cells of the

central nervous system, are thought to be central in the MIA-induced neurodevelopmental disruptions given their regulatory role in pruning and maintenance of synapses, and evidence of their disruption in both schizophrenia and ASD (Smolders et al. 2018).

We are faced with a consistent problem across neuroscientific disciplines, specifically in the neurodevelopmental field, regarding how to build a homology between observations made in humans and animal models of neuropsychiatric disorders. Some of this difficulty stems from the limited assays that can be used to examine neurodevelopmental trajectories across species. Neuroimaging techniques (e.g., magnetic resonance imaging [MRI], positron emission tomography [PET]) are an intriguing exception in that they allow for neuroanatomical specificity and further lend themselves to longitudinal data acquisition and analyses that allow for examination of the nature and timing of the emergence of aberrant neurodevelopment. This type of work has been critical in furthering our understanding of normative brain development in both humans and animals (Hammelrath et al. 2016; Mengler et al. 2014; Raznahan et al. 2014; Giedd 2010; Reardon et al. 2018; Qiu et al. 2018), and of neurodevelopmental disorders in humans, and has led to the idea that these disorders are characterized by deviation from normative developmental trajectories (Shaw et al. 2008; Raznahan et al. 2014).

3.4 Methods

3.4.1 Literature search

Embase, Medline, and PsycINFO were used to search for published English-language human and animal studies using neuroimaging modalities to investigate the effects of prenatal MIA on offspring. The following search terms were used in Ovid: ("magnetic resonance imaging" or “MRI” or "functional magnetic resonance imaging" or "fMRI" or "positron emission tomography" or "PET" or "magnetic resonance spectroscopy" or "MRS" or "diffusion tensor imaging" or "DTI" or "Computed Tomography" or "CT") AND ("prenatal maternal immune activation" or "MIA" or "maternal infection" or "maternal inflammation" or "prenatal immune challenge"). Two authors (E.G. and E.P.) performed the search independently (last search: October 2018) and evaluated eligibility for inclusion based on titles and abstracts of all

publications. Authors also reviewed reference sections of major reviews (Reisinger et al. 2015; Meyer 2014).

3.4.2 Inclusion criteria

Full-length English language articles were included if: (1) the study investigated effects of exposure prenatal MIA on offspring development (animal or human) and (2) used an imaging modality to assay the brain.

3.4.3 Exclusion criteria

Case studies were excluded.

3.5 Results

The primary Ovid search resulted in 645 publications (871 prior to removing duplicates). Fifty-four articles were selected to undergo a full-text assessment for eligibility. Twenty-nine animal studies (Kannan et al. 2007; Saadani-Makki et al. 2009; Kannan, Saadani-Makki, Balakrishnan, Chakraborty, et al. 2011; Kannan, Saadani-Makki, Balakrishnan, Dai, et al. 2011; Zhang et al. 2018; Fatemi et al. 2008; Fatemi, Folsom, Reutiman, Abu-Odeh, et al. 2009; Fatemi, Folsom, Reutiman, Huang, et al. 2009; Qi Li et al. 2009, 2010; Q. Li et al. 2015; Piontkewitz, Assaf, and Weiner 2009; Piontkewitz, Arad, and Weiner 2011b, [a] 2011; Short et al. 2010; Willette et al. 2011; Girard et al. 2010; Beloosesky et al. 2013; Bergeron et al. 2013; Malkova et al. 2014; Arsenault et al. 2014; Vernon et al. 2015; Richetto et al. 2017; Crum et al. 2017; da Silveira et al. 2017; Ginsberg et al. 2017; Sharabi et al. 2018; Ooi et al. 2017; Bauman et al. 2013) and 10 human studies (Ellman et al. 2010; Graham et al. 2018; Rudolph et al. 2018; Rasmussen et al. 2018; Spann et al. 2018; Dhombres et al. 2017; Jenster et al. 2018; Birnbaum et al. 2017; Lipitz et al. 2010; Diebler, Dusser, and Dulac 1985) were deemed eligible for inclusion. The characteristics of the animal and human studies are reported in **Tables 3.1** and **3.2**, respectively.

Table 3.1. Summary of human studies that met inclusion criteria (n=10)

Study # *	Authors, journal, year	Offspring n, sex	Mean age (+/-SD)	Study Population	Design of brain imaging acquisition	Measure of maternal inflammation	Gestational timing	Neuro-imaging	Key MRI findings
1	Rudolph et al. (2018), *Nature Neuroscience*	84 (50% M)	3.97 weeks (+/- 1.84)	Healthy mothers' infants	CS	Maternal serum IL-6	All trimesters averaged	rs-fMRI & T1- and T2-weighted sMR (3T); resolution for rs-fMRI=NA, for T1=1x1x1mm^3, for T2=1x1x1mm^3	Maternal IL-6 concentration associated with: ● SUB, DAN, SAL, CER, VAN, VIS, cinguloopercular, and frontoparietal network connectivity ● Connectivity between SUB-CER, VIS-DAN, SAL-CON ● Meta-analysis defined WM fMRI mask ● Prediction of within-network SAL connectivity and between-network connectivity in DAN, VAN, SAL, SUB
2	Spann et al. (2018), *The Journal of Neuroscience*	72 (36 after QC; 66.7% M)	~42 weeks (+/- 1.9 weeks)	Nulliparous pregnant adolescent women's offspring (healthy)	CS	Maternal serum IL-6 and CRP	Third trimester (34-37 weeks)	rs-fMRI & T2-weighted sMR (3T); resolution for rs-fMRI=3.16x 3.16x5mm^3, for T2=1x1x1mm^3	● Higher maternal IL-6 concentration associated with stronger left insula mPFC and lateral occipital gyrus connectivity, weaker connectivity between dACC and dorsomedial PFC; ● Higher maternal CRP levels associated with greater connectivity between left insula and right temporoparietal junction, right insula and basal ganglia, dACC and cuneus, temperoparietal junction and extrastriate cortices, and weaker connectivity between dACC and dmPFC and right basal ganglia
3	Birnbaum R et al. (2017), *Prenatal Diagnosis*	81	32-33 weeks of gestation	Fetuses of women positive for CMV	CS	Maternal seroconversion for CMV	1st, 2nd, and 3rd trimesters	T2-weighted sMRI (1.5T); resolution=0 .625x1.46x3 -5mm^3	● Bilateral temporal cavitations ● Unilateral dilatation of right temporal horn ● Periventricular WM hyperintense signal (33 weeks) ● Subcortical hyperintense at 29 weeks improved by 33 weeks
4	Graham AM et al. (2017), *Biological Psychiatry*	86 sMRI, 70 fMRI (59.3% M)	3.97 (+/- 1.84) weeks	Healthy mothers' infants	CS	Maternal serum IL-6	All trimesters averaged	T1- and T2-weighted sMRI & fMRI (3T); resolution for T1=1x1x1mm^3, for T2=1x1x1mm^3, for fMRI=NA	Higher maternal IL-6 associated with: ● Larger right (not left) amygdala volume and amygdala connectivity ● Stronger connectivity between right amygdala and right anterior insula, fusiform gyrus/inferior temporal gyrus, caudate, and thalamus, left brainstem and weaker

									connectivity to left superior occipital gyrus; ● Stronger connectivity between left amygdala and right fusiform/ITG, parietal/somatosensory cortex, parahippocampal gyrus, and weaker connectivity to ITG
5	Rasmussen et al. (2017), *NeuroImage*	32 (55.8% M)	34.6-41.8 weeks (scan 1); 51.3-56.1 weeks (scan 2)	Healthy mothers' infants	LG	Maternal serum IL-6	All trimesters averaged	T1- and T2-weighted sMRI & DTI (3T); resolution for T1=1x1x1mm^3, for T2=1x1x1mm^3, for DTI=2x2x2mm^3, 42 encoding directions	● Higher maternal IL-6 concentration associated with lower UF FA at birth with no association at 12 months indicative of stronger increase in FA from 1-12 months of age
6	Dhombres F et al. (2015), *Fetal Diagnosis and Therapy*	10	23-34 weeks of gestation	Fetuses exposed to T. gondii	CS	Serum IgG and T. gondii in amniotic fluid	All trimesters averaged	T1- and T2-weighted sMRI (1.5T); resolution for T1=1.25x2.56x4mm^3, for T2=1.25x1.88x4mm^3	● Abnormal echogenicity and thickness of germinal matrix ● Fetal brain lesions in white matter of subcortical, periaqueductal and periventricular regions at 33 weeks ● Lesions of necrosis in periventricular WM surrounded by inflammatory lesions and calcifications
7	Jenster M et al. (2013), *International Pediatric Research Foundation*	42 (61% M) chorioamnionitis, 29 (48% M) neonatal sepsis, 193 (55% M) no infection	5 days of life	Offspring exposed to chorioamnionitis or neonatal sepsis	CS	Maternal or neonatal fever, uterine tenderness, maternal or fetal tachycardia, purulent amniotic fluid or vaginal discharge, maternal leukocytosis, histological chorioamnionitis, neonatal sepsis, positive blood culture for pathogenic species, low white blood cell count, low absolute neutrophil count	NA	T1- and T2-weighted sMRI & DWI (1.5T); resolution for T1=0.7x0.7x1mm^3, T2=0.92x0.7x4mm^3, DTI=1.4x1.4x3mm^3, 3, 6 or 30 encoding directions	● Watershed pattern of injury seen in 98 subjects ● 59 subjects with basal ganglia/thalamus patterns of damage ● Neonatal sepsis associated with more severe damage; maternal chorioamnionitis was associated with moderate-severe brain injury
8	Ellman LM et al. (2010),	SSD 17 (70% M),	SSD = 39.96	Schizophrenia spectrum	CS	Maternal serum IL-8	2nd, 3rd trimesters	T1-weighted sMRI	● Maternal IL-8 associated with increases in

	Schizophrenia Research	CTL 8 (75% M)	(1.78) Control = 41.17 (1.69)	disorder (from large birth cohort study)				(1.5T); resolution=1 x1x1.4mm^3	ventricular volume, decreased entorhinal cortex volumes and posterior cingulate in SSD individuals
9	Lipitz S et al (2010), *Ultrasound Obstet Gynecol*	10 1st semester, 19 2nd trimester, 9 3rd trimester	30 weeks' gestation (1st & 2nd trimester infections) , later for 3rd trimester infections	Fetuses of women positive for CMV	CS	Maternal serum IgG and IgM in the amniotic fluid	1st, 2nd, and 3rd trimesters	T1- and T2-weighted sMRI & DWI (1.5T); resolution for T1=0.75-0.93x1-1.3x3-4mm^3, for T2=1.7x1.25 x4mm^3, for DTI=1.7x1. 25x4mm^3, encoding direction NA	• CMV associated with frontal, temporal, and parietal lobe, right caudate nucleus hyperintensities;
10	Diebler at el. (1985), *Neuroradiology*	31	0-2 months (n=17), 2-12 months(n= 8); 1-2 years (n=6)	Infants and children exposed to T. gondii	CS	Maternal or infantile serology and or parasitological examination of placenta; significant elevation of antitoxoplasma titers	2nd, 3rd trimesters	CT scan, no resolution reported	• Toxoplasmosis associated with hydrocephalous, hemiplegia and diplegia, calcifications in the basal ganglia and periventricular WM, porencephalic cysts and multicystic encephalomalacia • Early infection (<20 weeks): ventricular dilatation, porencephalic cysts and extensive calcifications particularly in the basal ganglia. • Infection between 20 and 30 weeks resulted in extensive periventricular calcifications and ventricular dilatation. • Late infection (<30th week) associated with fewer periventricular and intracerebral

CER: cerebellar network, CS: cross sectional, CMV: cytomegalovirus, CT: computed tomography, dACC: dorsal anterior cingulate, DAN: dorsal attention network, dmPFC: dorsomedial prefrontal cortex, DTI: diffusion tensor imaging, DWI: diffusion weighted imaging, IL: interleukin, ITG: inferior temporal gyrus, LG: longitudinal, rs-fMRI: resting state functional magnetic resonance imaging, SAL: salience network, sMRI: structural magnetic resonance imaging, SSD: schizophrenia spectrum disorder, SUB: subcortical network, T: Tesla, VAN: ventral attention network, VIS: visual network, WM: white matter

Table 3.2. Summary of preclinical studies that met inclusion criteria (n=29)

Study # *	Author, year, journal	Species, Strain, Sex	n	Age	Design of brain imaging acquisition	Model	Timing (GD)	Neuroimaging type	Key MRI findings
11	Ooi Y, et al. (2018), *Magnetic Resonance in Medical Science*	Rats, Wistar, M	14 (PND 35) & 10 (PND 70)	PND 35 and 70	CS	IP LPS; 100 μg/kg	16	T2-weighted sMRI (*in vivo*) (11.7T); resolution=78x 78x250μm^3	• Number of dilated VRSs significantly increased in LPS-offspring at PND35 but not PND70
12	Sharabi H, et al.(2018), *Neuroscien ce Research Article (IBRO)*	Rats, Sprague-Dawley, M	6/conditio n (LPS-NAC; LPS-SAL; SAL-SAL; 18 litters)	PND 25	CS	IP LPS; 500 μg/kg	18	DTI (*in vivo*) (9.4T); resolution=156 x156x800μm^3	• MD: LPS-SAL > SAL: pontine-tract/spinal-tract, medial lemniscus/external capsule, entorhinal ctx, corpus callosum/external capsule, geniculate body, auditory ctx, mammillary body, posterior thalamic nucleus, sub thalamic nucleus, sensory ctx, thalamus ventroposterior-medial, amygdala, hypothalamus, thalamus, fimbria, CA1 and CA3 • MD: LPS-NAC = SAL: callossum/external capsule, auditory ctx, mammillary body • RD: LPS-NAC < LPS-SAL = CTL: medial lemniscus, entorhinal ctx, inferior superior colliculi, corpus callosum/external capsule, deep mesencephalic nucleus, auditory ctx, mammillary body, posterior thalamic nucleus, hypothalamus, thalamus, fimbria, CA1 and CA3
13	Zhang Z et al. (2018), *Neurobiolo gy of Disease*	Rabbits, New Zealand White, NS	2-3/group (LPS & SAL) (5 litters);	PND 1, 5, 7-9	LG	IU LPS; 20 μg/kg	28	PET: [11C]-(R)-PK1195 tracer (TSPO) & T2-weighted sMRI (*in vivo*) (4.7T and microPET R4 tomograph); resolution=NA	• Increased TSPO binding in LPS exposed kits vs. SAL kits at all ages.
14	Crum WR et al. (2017), *Brain, Behaviour, and Immunity*	Rats, Sprague-Dawley, M	10 POL (8 dams) & 10 SAL (3 litters)	PND 50, 100, 180	LG	IV POL; 4 mg/kg	15	T2-weighted sMRI *(in vivo*) (7T); resolution=234 x234x600μm^3	• POL had smaller ACC and HP volume. • TBM: PND50-100: POL < SAL in prefrontal, motor, somatosensory, auditory and visual ctx, dorsal thalamic nuclei, ventral midbrain and brainstem; POL>SAL in ventricular, striatal, HP, ventral thalamic, and WM volumes.

									• No differences TBV or STR.
15	da Silveira VT, et al. (2017), *International Journal of Developmental Neuroscience*	Mice, C57BL/6, M	12 POL (6 GD9 and 6 GD17; 8 litters); 12 SAL (6 GD9 and 6 GD17; 6 litters)	1 year of age	CS	IV POL; 5 mg/kg	9 or 17	T2-weighted sMRI (*in vivo*) (4.7T); resolution= NA (1mm thick slices)	• TBV reduced in both GD9 and GD17 POL groups • No differences on normalized LV volume (to TBV)
16	Ginsberg Y et al. (2017), *Neuroscience*	Rats, Sprague-Dawley, F	6/ condition (LPS-MG, LPS-SAL, SAL-MG, SAL-SAL; 18 litters)	PND 25	CS	IP LPS; 500 μg/kg	18	DTI & T2-mapping (*in vivo*) (7T); resolution=150 x200x1000μm^3; 15 encoding directions	• ADC: LPS>SAL: entorhinal ctx, superior colliculus, cingulate ctx, corpus callosum, external capsule, auditory ctx, hypothalamus, thalamus, CA1 of HP • T2 levels: LPS>SAL: periventricular fiber system (i.e. corpus callosum, sub thalamic radiation, external capsule, forceps major), ctx, thalamus • MG pre-treatment resulted in T2 and ADC levels similar to CTLs
17	Richetto et al. (2016), *Cerebral Cortex*	Mice, C57BL6/ N, M	8 POL(8 litters); 6 SAL (6 litters)	PND 84	CS	IV POL; 5 mg/kg	17	T2-weighted sMRI, mcDESPOT (includes T1 and T2 with B1 correction and MWF (*ex vivo*; 7T); resolution for T2-weighted=112.5μm^3, mcDESPOT=150μm^3	• POL > SAL: R primary motor ctx, somatosensory ctx, and visual ctx, crus1 ansiform lobule, simple lobule, and inferior cerebellar peduncles • POL < SAL: bilaterally in piriform ctx, anterior commissure, interfascicular nucleus, third ventricle, L periaqueductal gray nucleus, L external capsule, L fimbria, R amygdala, R ventral mesencephalon, cerebellar lobule, paraflocculus and paramedian lobule of cerebellum • T1-mapping: POL>SAL: nucleus accumbens, inferior cerebellar peduncles • T2(spin-spin) relaxation: POL<SAL: piriform, PFC, ACC, insular, retrospelinal granular, motor, somatosensory, visual, and auditory cortices, hypothalamus, ventral thalamus, HP, ventral mesencephalon, cerebellum • MWF increased significantly in POL in ctx, HP, cerebellar gray and white matter

18	Li Q et al. (2015), *Translational Psychiatry*	Mice, C57BL6/N, M	21 POL (8 for n-3 and 7 for n-6; 3 litters), 17 SAL (6 n-3 and 11 for n-6; 3 litters)	PND84-89	CS	IV POL; 5 mg/kg	9	1H-MRS and T2-weighted sMRI (*in vivo*) (7T); resolution=109 x109x480μm^3; 1H-MRS voxel size=1.2x2.6x2.5mm^3	• Increase in NAA/Cr and decrease in mIns/Cr in n6-POL group (vs. n6-SAL) • Both NAA/Cr and mIns/Cr values normalized in n3-POL groups (no difference with SAL)
19	Vernon AC, et al.(2015), *European Neuropsychopharmacology*	Rats, Sprague-Dawley, M	10 poly I:C (8 litters); 10 saline (3 litters)	PND50, 100, 180	LG	IV POL; 4 mg/kg	15	1H-MRS in PFC T2-weighted sMRI *(in vivo*; 7T); resolution=234 x234x600um^3, 1H-MRS voxel size=3.8x2.2x2.0mm^3	• PND 50-100 POL>SAL: NAA+NAAG, Glu:tCR, GLX:tCr (not statistically significant after post-hoc correction), and decreased levels of Tau:tCr
20	Arsenault D et al. (2014), *Open Journal of Medical Psychology*	Mice, C57BL/6, both	39-40 LPS; 47-52 SAL (30 litters)	PND37-39 [18F]FPEB; PND42-44 [11C]PBR28	CS	IP LPS; 120 μg/kg (3 days)	15, 16, 17	PET: [18F]FPEB tracer (mGluR5) and [11C]PBR28 tracer (inflammation) (*in vivo*; NA); resolution=170 x170x170μm^3	• LPS did not change [11C]PBR85 binding in any ROIs (inflammation) • [18F]FPEB binding (mGluR5) reduced in LPS offspring HP
21	Malkova NV, et al. (2014), *PNAS*	Mice, C57BL/6J, M	6 poly I:C (3 litters); 6 saline (3 litters)	PND 70-84	CS	IP POL; 5 mg/kg	10, 12, 14	"functional" MnCl2 enhanced RARE (*in vivo*; 11.7T); resolution=100 μm^3	• POL: greater manganese (Mn2+) accumulation due to DOI in STR, somatosensory ctx, primary and secondary motor ctx, somatosensory (upper and lower limb), orbital ctx, infralimbic ctx, dorsal ACC, dorsal tenia tecta, medial dorsal thalamus • Parafascicular thalamic nucleus only activated by DOI in POL offspring
22	Bauman MD et al. (2013), *Translational Psychiatry*	Rhesus macaque, Both	4 IgG-ASD, 2 IgG-CON, 5 untreated	1, 3 and 6 months, 1 and 2 years	L	IV IgG antibody (15-20mg)[1]	30, 44, 58, 72, 86, 100	T1- weighted sMRI (*in vivo*; 1.5T); resolution=625 x625x700μm^3	• IgG-ASD offspring had faster TBV growth than IgG-Controls for TBV (3 to 6 months) • Male IgG-ASD offspring had larger frontal, occipital, but not parietal or temporal lobes at 2 years • WM volume increase in frontal, occipital, and parietal lobes
23	Beloosesky R et al. (2013), *American Journal of Obstetrics and Gynecology*	Rats, Sprague-Dawley, F	5 LPS-NAC, 5 NAC-LPS-NAC, 8 SAL-LPS-SAL, 6 SAL-SAL-SAL;	PND 25	CS	IP LPS; 500 μg/kg	18	DTI & T2-weighted sMRI (*in vivo*; 7T); resolution for DTI=150x200 x1000μm^3, 15 encoding	• T2 levels: LPS > controls: visual ctx, cingulate ctx, periaqueductal gray, dorsal hippocampal commissure, corpus callosum, external capsule, dentate gyrus, substantia nigra, geniculate body, HP, auditory ctx,

			(1 females/litter/group)					directions, T2=75x150x1000μm^3	piriform ctx, cingulum, thalamus, reticular thalamic nucleus, STR, insular ctx, and CA3 ● T2 levels: LPS-NAC > LPS in visual ctx, cingulate ctx, periaqueductal gray, dorsal HP, corpus callosum, entorhinal ctx, dentate gyrus, substantia nigra, HP, auditory and piriform ctx, cingulum, thalamus, STR, insular ctx, CA3 ● ADC: LPS >CTL: posterior thalamic nucleus, ventroposterior-medial nucleus of thalamus, hypothalamus, motor ctx (M1/M2) ● ADC: LPS-NAC > LPS post. thalamic nucleus, hypothalamus, motor ctx; CTL similar to LPS-NAC
24	Bergeron et al. (2013), *Developmental Neuroscience*	Rats, strain unclear, both	3 GBS; 13 controls (26 litters)	PND150	CS	IP GBS serotype Ia; 1 × 109 CFU/100 µl	19, 20, 21, 22	T2-weighted sMRI (*in vivo*; 7T); resolution=125x125x1000μm^3	● GBS-exposed males (not females) had enlarged lateral ventricles ● Both sexes had reduced thickness of periventricular WM
25	Kannan S et al. (2011), *Developmental Neuroscience*	Rabbits, New Zealand White, NS	8 LPS (6 litters); 6 (5 litters) SAL; 4 (3 litters) no-surgery CTL,	PND 1	CS	IU LPS; 20 μg/kg	28	PET: [11C]-(R)-PK1195 tracer (TSPO) & T2-weighted sMRI (*in vivo*; 4.7T and microPET R4 tomograph); resolution=NA	● Increased TSPO binding in the LPS exposed kits compared to control kits
26	Kannan et al. (2011), *Journal of Cerebral Blood Flow and Metabolism*	Rabbits, New Zealand White, NS	5 (5 litters) LPS, 5 (4 litters) SAL, 4 kits (3 litters) non-surgical CTL	PND 1	CS	IU LPS; 20 μg/kg	28	PET:[11C]-a[11C]methyl-L-tryptophan (AMT; tryptophan metabolism) tracer & T2-weighted sMRI (*in vivo*; 4.7T and microPET R4 tomograph); resolution=156x156x800μm^3	● Decreased binding in LPS group compared to both control groups ● SAL group was lower than no intervention controls
27	Piontkewitz Y et al. (2011), *Biological Psychiatry*	Rats, Wistar, both	158 POL (32 litters);164 SAL (28 litters)	PND 35, 46, 56, 70, 90	LG + CS	IV POL; 5 mg/kg	15	T2-weighted sMRI (*in vivo*; 7T); resolution=117x117x1000μm^3	● Smaller HP volume in POL over time (except at PND 35) ● LV volume significantly larger in male POL group starting at PND 56 ● PFC volume decline began on PND 56 in POL males, PND70 in SAL males and

									POL females, and was absent in SAL females ● STR volume smaller in POL offspring ● No TBV differences
28	Willette AA et al. (2011), *Behavioural Brain Research*	Rhesus macaque, both	9 (1 at 2ng/kg, 8 at 4ng/kg) LPS; 9 CTL (2 IV SAL injection and 7 not handled)	1 year	CS	IV LPS; 2 or 4 ng/kg	125 and 126	T1- and T2-weighted sMRI (*in vivo*; 3T); resolution=234 x234x498μm^3	● TBV of LPS monkeys was slightly larger (5.9%) ● Mean global WM significantly increased (8.8%) ● Marginally thicker GM in R parietal and frontal lobes ● Thinner GM in medial temporal lobe
29	Girard S et al. (2010), *The Journal of Immunology*	Rats, Lewis, NS	6 LPS; 6 SAL (imaged dams)	GD17 (pre-LPS) and GD20 (post-LPS) for placenta	LG	IP LPS; 200 μg/kg	18, 19, 20 every 12 hours; 6 total)	T1- and T2-weighted sMRI (*in vivo*; 7T); resolution=234 x234x1500μm	● Decreased T2-weighted signal intensity and clearance rate (10%) in placentas (GD20) LPS-exposed dams
30	Li Q et al. (2010), *Neuroimage*	Mice, C57BL6/N, M	14 POL (GD9=8, GD17=6); 8 (GD9=3, GD17=5) SAL	PND 84	CS	IV POL; 5 mg/kg	9 or 17	DTI (*ex vivo*; 7T); resolution=125 x125x350μm^3, 30 encoding directions	● GD9 and GD17 POL: lower FA in the L amygdala and cerebral peduncles and R fimbria, and higher FA in L stria medullaris ● GD9 POL: lower FA in ACC, ventral STR, external capsule, and higher FA in L fimbria, lateral septal area, and PFC ● GD17 POL: lower FA in the R ventral subicular regions and increased FA in R stria medullaris and amygdala/piriform ctx
31	Short JS, et al. (2010), *Biological Psychiatry*	Rhesus macaque, both	12 H3N2; 7 CTL	1 year	CS	IN H3N2[3]	119	T1- and T2-weighted sMRI (*in vivo*; 3T); resolution=273 x273x500μm^3	H3N2 exposure: ● Decreased TBV and cortical GM, cerebellar WM, trend decrease in TBV and WM increases in LV ● Decrease in cingulate and parietal ctx GM ● Decreased WM volume in L parietal region ● Greater WM volume in cingulate ctx ● Decreased bilateral amygdala volume (uncorrected) ● No differences in HP or STR
32	Fatemi SH, et al. (2009), *Schizophrenia Research*	Mice, C57BL/6, M	4 H1N1 (4 litters); 3 CTL (3 litters) (per age)	PND 0, 14, 35, 56	CS	IN H1N1[2]	16	DTI & T2-weighted sMRI (*ex vivo*; 11.8T); resolution for PND0=70x70x74μm^3, PND14-56=117x117x78μm^3; 6	● Decreased cerebellar volume (PND 14) and ventricular volume (PND 0) ● Decreased FA in R internal capsule (PND 0) ● Increased FA in corpus callosum (PND 14) and R middle cerebellar peduncle (PND 56)

								encoding directions	
33	Fatemi SH et al. (2009), *European Neuropsychopharmacology*	Mice, C57BL/6, M	4 H1N1 (4 litters), 3 CTL (3 litters) (per age)	PND 0, 14, 35, 56	CS	IN H1N1[2]	16	DTI & T2-weighted sMRI (*ex vivo*; 11.8T); resolution for PND0=70x70x74μm^3, PND14-56=117x117x78μm^3; 6 encoding directions	• Decreased TBV (7%; PND 14) and HP volume (6%; PND 35). • No differences in FA in HP white matter (trending increase at PND 14)
34	Li Q et al. (2009), *PLoS One*	Mice, C57BL6/N, M	14 POL (GD9=8, GD17=6), n=8 SAL (3GD9, 5 GD17)	PND 84	CS	IV POL; 5 mg/kg	9 or 17	T2-weighted sMRI (*in vivo*; 7T); resolution=97x97x250μm^3	• No differences in TBV and WMV for GD9 or GD17 POL • GD9 POL: larger LV volume • GD17 POL: larger 4th ventricle
35	Piontkewitz Y et al. (2009), *Schizophrenia Bulletin*	Rats, Wistar, M	16 POL (8-16 litters), 12 SAL (6-12) (1-2 rats per litter)	PND35 and 120	LG	IV POL; 5 mg/kg	15	T2-weighted sMRI (*in vivo*; 7T); resolution=117x234x1500μm^3	• No HP or LV volume differences at PND 35 • Decreased HP and increased LV at PND 120 • RIS treatment (high and low) prevented volume alterations in POL groups • High risperidone had decreased TBV compared to CTL
36	Piontkewitz Y et al. (2009), *Biological Psychiatry*	Rats, Wistar, M	81 POL (28 litters), 72 SAL (26 litters)	PND35 and 120	LG	IV POL; 5 mg/kg	15	T2-weighted sMRI (*in vivo*; 7T); resolution=117x234x1500μm^3	• No volume differences at PND 35 • Decreased HP and increased LV volume in at PND 120 • No differences at PND 120 following adolescent CLZ pre-treatment
37	Saadani-Makki F, et al. (2009), *Journal of Child Neurology*	Rabbits, New Zealand White, NS	5 LPS (3 litters), 5 SAL (4 litters)	PND 1	CS	IU LPS; 20 μg/kg	28	DTI & T2-weighted sMRI (*ex vivo*; 4.7T); resolution=250x250x700μm^3, 6 encoding directions	• Decrease in e1 &in FA in LPS group in periventricular WM
38	Fatemi SH et al. (2008), *Schizophrenia Research*	Mice, C57BL/6, M	4 H1N1 (4 litters), 3 CTL (3 litters) (per age)	PND 0, 14, 35, 56	CS	IN H1N1[2],	19	DTI & T2-weighted sMRI (*ex vivo*; 11.8T); resolution for PND0=70x70x74μm^3, PND14-56=117x117x78μm^3; 6 encoding directions	• Decreased TBV (4%) at PND 35 • Decreased FA in the corpus callosum at PND 35
39	Kannan S et al. (2007),	Rabbits, New Zealand	4 (3 litters) 30ug/kg, 4 (4 litters)	PND 1	CS	IU LPS; 20, 30, 0r 40 μg/kg	28	PET: [11C]-(R)-PK1195 tracer (TSPO)	• Increase TSPO binding in the LPS exposed (20 and 30ug/kg) kits (greater

	Journal of Nuclear Medicine	White, NS	20 ug/kg LPS; 4 (3 litters) SAL					& T2-weighted sMRI (*in vivo*; 4.7T and microPET R4 tomograph); resolution=156 x156x500μm^3	increase for 30ug/kg) relative to controls kits

ACC: anterior cingulate cortex, ADC: apparent diffusion coefficient, AMT: α[11C]methyl--tryptophan, CA: cornu Ammonis, CS: cross sectional, CTL: control, ctx: cortex, DOI: 2,5-Dimethoxy-4-iodoamphetamine, DTI: diffusion tensor imaging, DWI: diffusion weighted imaging, F: female, GBS: group B streptococcus, GD: gestational day, GM: gray matter, ^{1}H-MRS: proton magnetic resonance spectroscopy, HP: hippocampus, IP: intraperitoneal, IU: intrauterine, IV: intravenous, L: left, LG: longitudinal, LPS: lipopolysaccharide, LV: lateral ventricle, M: male, mcDESPOT: multicomponent-driven equilibrium single pulse observation of $T_1$and T_2, MD: medial diffusivity, MG: magnesium sulfate, NAC: N-acetyl aspartate, NS: not specified, PET: positron emission tomography, PFC: prefrontal cortex, POL: polyinosinic:polycytidylic acid, R: right, RD: radial diffusivity, rs-fMRI - resting state functional magnetic resonance imaging, SAL: saline, sMRI- structural magnetic resonance imaging, STR: striatum, T - Tesla, TBV: total brain volume, TSPO: Translocator protein, VRS: Vichrow-Robin spaces, WM: white matter

[1] IgG antibody isolated from women whose children had ASD Women also tested positive for maternal autoantibody reactivity to fetal brain proteins at 73 kiloDalton

[2] Dilution of $10^{-4.5}$ of 6.5 log 10 (CCID50) per 0.1ml human influenza virus in 90ul of minimum essential medium

[3] 10^7 EID50 of virus via 1-mL infusion

* Study number aligns with **Figure 3.1**

3.5.1 Human studies

As summarized in **Figure 3.1**, 3 studies performed neuroimaging (structural MRI [sMRI] and diffusion weighted imaging [DWI]) on human fetuses in the 1st, 2nd, and 3rd trimesters of mothers who had cytomegalovirus (Birnbaum et al. 2017) (dark green) and in the 3rd trimester of mothers who had *Toxoplasma gondii* infection (dark blue) (Diebler, Dusser, and Dulac 1985; Dhombres et al. 2017). Five studies performed neuroimaging within the first year of life, one study imaged (sMRI & DWI) neonates at 5 days old (sMRI & DWI) exposed to chorioamnionitis (Jenster et al. 2018) (light orange). Three studies imaged offspring within the first 2 months of life. In 2 of these, neuroimaging (sMRI or resting state functional MRI [rs-fMRI]) was performed in 4-week-old neonates for whom maternal IL-6 measures were taken (Graham et al. 2018; Rudolph et

al. 2018) (pink). One study imaged (computed tomography [CT]) offspring exposed to *toxoplasma gondii* between 0-2 months of life (Diebler, Dusser, and Dulac 1985). These offspring were imaged again at 34-42 weeks, and 1-2 years of age. Two other studies also investigated offspring within the first year of life (34-42 weeks), 2 of which had measures of maternal IL-6 and CRP (rs-fMRI or sMRI or diffusion tensor imaging [DTI]) (Spann et al. 2018; Rasmussen et al. 2018). Finally, one study (sMRI) was performed in adults with schizophrenia (mean age of 40) for whom maternal IL-8 measures were recorded (Ellman et al. 2010).

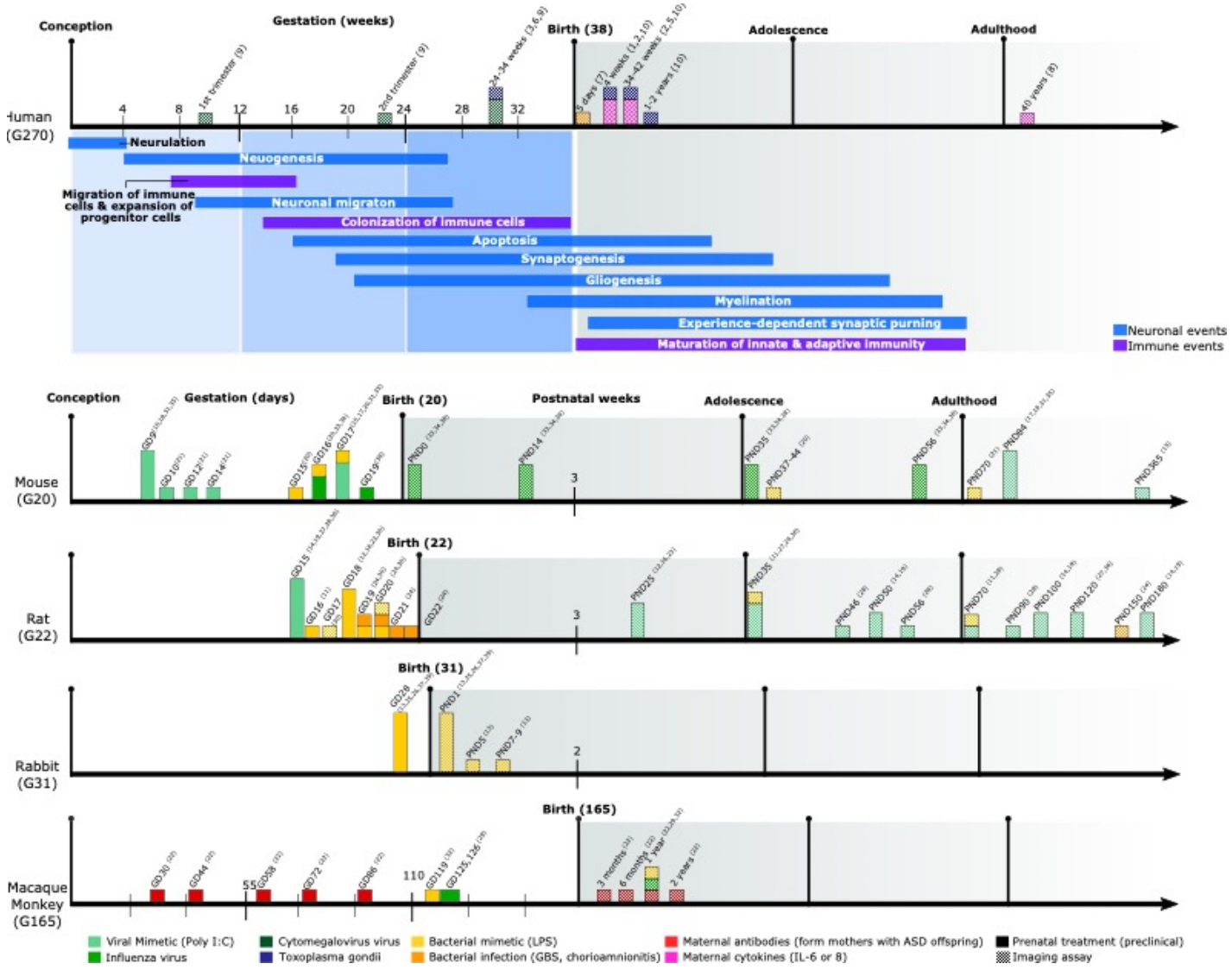


Figure 3.1 (caption on next page).

Figure 3.1 (continued). Summary of MRI-based MIA-exposure findings across 5 species. The steps of brain (blue bars) and immune system (purple bars) development are highly organized from gestation to early postnatal life; however, the timing of these neurodevelopmental processes differs between species. The short- and long-term effects of maternal immune activation depend on many factors, including the time window of development in which they are experienced, thus understanding homologies and differences between human and animal model development is critical. This figure summarizes the 39 reviewed studies in terms of the gestational timing of MIA and the timing at which neuroimaging was performed in the offspring. Studies are numbered 1-39 (see tables 3.1 and 3.2); this number is used as reference in the figure. Different pathogens or immune activators are color coded as follows: viral infections are green, with light green for viral mimetics (poly I:C), green for influenza virus, dark green for cytomegalovirus. Bacterial infections are in yellow for the mimetic LPS, and orange for group B streptococcus (GBS) and chorioamnionitis. Maternal antibodies are in red and maternal cytokines are in pink. Solid bars represent prenatal treatment and are relevant for the preclinical studies, whereas the hatched bars refer to timing of neuroimaging of the offspring (pre- or postnatally).

3.5.2 Animal studies

As outlined in **Figure 3.1** and **Table 3.2**, 10 studies modelled MIA in mice, 4 of which induced MIA at gestational day (GD) 9 using poly I:C (green) and imaged offspring (sMRI, DTI, or proton magnetic resonance spectroscopy [^{1}H-MRS]) in adulthood (3 studies on postnatal day (PND) 84 and 1 at PND 365) (Qi Li et al. 2009, 2010; Q. Li et al. 2015; da Silveira et al. 2017). One study induced MIA with poly I:C at GD10, 12, and 14, and also imaged offspring in adulthood (PND 70-84) using manganese chloride enhanced MRI (MEMRI) (Malkova et al. 2014). One study induced MIA using LPS (yellow) at GD 15, 16, and 17, and assessed neuroinflammation (PND 37-39) and glutamate receptor function (PND 42-44) in adolescence using PET imaging (Arsenault et al. 2014). MIA was induced at GD 16 in 2 studies, and GD 19 in 1 study using H1N1 influenza (light green), and offspring were imaged using DTI or sMRI *ex vivo* at PNDs 0, 14, 35, 56 (Fatemi et al. 2008; Fatemi, Folsom, Reutiman, Abu-Odeh, et al. 2009; Fatemi, Folsom, Reutiman, Huang, et al. 2009). Finally, 4 studies investigated the effects of poly I:C injection at GD 17 on adult offspring (PND 84 using sMRI, DTI, or ^{1}H-MRS) (Qi Li et al. 2009, 2010; Richetto et al. 2017; da Silveira et al. 2017).

Ten rat studies are reviewed, as shown in **Figure 3.1**. Five studies investigated the effects of MIA at GD 15 using poly I:C in adolescent and adult offspring; one study performed sMRI at PNDs 35, 46, 56, 70, 90 (Piontkewitz, Arad, and Weiner 2011b), 2 did sMRI at PND 35, 120

(Piontkewitz, Arad, and Weiner 2011a; Piontkewitz, Assaf, and Weiner 2009), and 2 did sMRI and ^{1}H-MRS at PNDs 50, 100, 180 (Vernon et al. 2015; Crum et al. 2017). Many studies used LPS to induce MIA: 1 at GD16 with sMRI in offspring at PND 35 and 70 (Ooi et al. 2017), 3 at GD18 with offspring imaged (sMRI & DTI) at PND25 (Beloosesky et al. 2013; Ginsberg et al. 2017; Sharabi et al. 2018), and 1 investigated effects repeated LPS administration (GD 18, 19, 20) on placentas *in utero* (GD 17 and 20) (Girard et al. 2010). Finally, one study investigated MIA (induced by Group B streptococcus [GBS] serotype) late in gestation (GD 19, 20, 21, and 22), and imaged (sMRI) offspring in adulthood (PND 150) (Bergeron et al. 2013).

In New Zealand White Rabbits, 5 studies investigated the effects of MIA late in gestation (GD28 of 31) on offspring at PND 1, 5, and 7-9; 4 studies used PET imaging (3 measured inflammation with a radioligand for Translocator protein (TSPO) (Kannan et al. 2007; Zhang et al. 2018; Kannan, Saadani-Makki, Balakrishnan, Chakraborty, et al. 2011), 1 measured tryptophan metabolism with α[11C]methyl--tryptophan (AMT) tracer) (Kannan, Saadani-Makki, Balakrishnan, Dai, et al. 2011), and 1 used DTI (Saadani-Makki et al. 2009). Finally, in the rhesus monkey, 1 study investigated the effects of MIA exposure using human IgG antibodies isolated from mothers with ASD offspring (red) in the first 2 trimesters (GD 30, 44, 58, 72, 86, 100), and imaged (sMRI) offspring at 1, 3, and 6 months, and 1 and 2 years of age (Bauman et al. 2013). Two other studies investigated MIA at week 17 (GD 119 with H3N2 and GD 126 & 126 with LPS) and imaged offspring at 1 year of age (Short et al. 2010; Willette et al. 2011).

3.6 Discussion

3.6.1 Summary of key findings in humans

Studying MIA in humans is challenging; epidemiologic studies have limitations in defining individual exposures, whereas birth cohort studies may lack serial serologic measurements to verify recent infections, and thus few studies exist (Minakova and Warner 2018; Brown and Meyer 2018). Recent advances in neuroimaging allow for detailed investigation of structure and function in the developing brain. Using these methods, recent studies have demonstrated that exposure to *Toxoplasma gondii* in the first 2 trimesters leads to abnormal thickness of the germinal matrix (a highly vascularized region where neuronal and glial cells migrate from during 8-28 weeks'

gestation (Gleason and Back 2005)); it is also associated with severe neurological signs, including microcephaly, hydrocephalus, mental retardation, and blindness, and can result in a termination of pregnancy (McAuley 2014). Late infection (>30 weeks) is associated with less severe outcomes, but periventricular and intracerebral calcifications are still observed (Dhombres et al. 2017; Diebler, Dusser, and Dulac 1985). Infections with cytomegalovirus demonstrate some homologies as they also lead to hyperintensities in the periventricular WM, temporal, frontal, and parietal lobes, and caudate nucleus (Birnbaum et al. 2017; Lipitz et al. 2010). However, the association between gestational timing of cytomegalovirus infection and fetal outcomes is less clear. Further work is required to better understand if the gestational timing of infection and severity of neuroanatomical alterations is similar to toxoplasmosis. Similar to the other infections, chorioamnionitis was also found to damage periventricular WM and alter basal ganglia and thalamus development (Birnbaum et al. 2017).

Elegant work from the groups of Buss, Fair, and Spann has shown that maternal inflammation (serum IL-6 levels in all trimesters) is associated with subtle alterations in offspring. Fronto-limbic circuitry of the neonate (4 week old) brain was found to be altered, with observations of larger right amygdala volume, and greater bilateral amygdala connectivity to regions involved in sensory processing and integration (fusiform, somatosensory cortex, thalamus), learning and memory (caudate and parahippocampal gyrus), and salience detection (insula) (Graham et al. 2018); decreased fractional anisotropy (FA) of the uncinate fasciculus, a key fronto-limbic WM bundle, was also observed (Rudolph et al. 2018). Additionally, maternal IL-6 concentration was predictive of neonatal functional brain connectivity in networks important for social, emotional, and cognitive development, and known to be impaired in neuropsychiatric disorders, such as the dorsal attention, salience, and subcortical networks (Woodward and Cascio 2015; Rudolph et al. 2018). Third trimester MIA (IL-6 and CRP) was also associated with strength of salience network connectivity in the mPFC, temporoparietal junction, and basal ganglia (Spann et al. 2018).

Only one retrospective study investigated the relationship between maternal pro-inflammatory cytokine (IL-8) levels during pregnancy and brain morphology of adults with schizophrenia spectrum disorders. They report higher IL-8 levels *in utero* to be associated with increased ventricular volume and decreased entorhinal and posterior cingulate volume (Ellman et al. 2010). Thus, MIA-exposure plays a key role in either inducing or exacerbating morphological alterations.

3.6.2 Summary of structural changes following prenatal MIA in preclinical studies

Preclinical studies investigate the effects of prenatal MIA at different gestational windows on offspring development from birth to adulthood. This provides us with a greater understanding of the long-term changes due to MIA. The majority of preclinical studies in the literature, and those included in this review, model MIA in mice and rats (69%). These adequately capture many features of embryogenesis and fetal brain development, such as neurulation, neurogenesis, neuronal differentiation and migration, and migration and colonization of immune cells (**Figure 3.1**). However, synaptogenesis, gliogenesis, and myelination begin postnatally in rodents, but prenatally (third trimester) in humans (Shoykhet and Clark 2011; Lebel and Deoni 2018), thus investigating the effects of MIA on these processes may be better modeled in other species such as nonhuman primates, whose neurodevelopment is similar to the human (Gumusoglu and Stevens 2019). The discussion of preclinical findings has been divided below by gestational timing of MIA.

3.6.2.1 Early gestation (rodents <GD10, rhesus monkey < ~GD82)

The time window we refer to as early gestation in rodents corresponds to the end of the first trimester in primate gestation. During this time, the developing brain is undergoing critical neurodevelopmental processes such as the initiation of neuronal and immune cell migration, neurogenesis, cortical plate formation, and microglial colonization (Selemon and Zecevic 2015; Semple et al. 2013; Clancy, Darlington, and Finlay 2001). Five studies investigated the effects of MIA in early gestation on offspring neonatal (1 rhesus monkey study) and adult (4 mouse studies) development. Rhesus monkeys exposed to maternal immunoglobulin G isolated from mothers whose offspring had ASD in the first 2 trimesters were found to have accelerated growth in total brain volume (TBV) between 3 and 6 months of life, and increased frontal and occipital lobe volume, driven by WM volume expansion at 1 and 2 years of life (Bauman et al. 2013).

Adult mouse offspring prenatally exposed to poly I:C early in gestation (GD 9) were found to have larger lateral ventricle volume (PND 84) (Qi Li et al. 2009), lower fractional anisotropy (FA) in the anterior cingulate, ventral striatum, external capsule, and amygdala (amongst other regions), and higher FA in the PFC, stria medullaris, fimbria, and lateral septum (Qi Li et al. 2010); increased N-acetylaspartate (NAA) and decreased myo-inositol (mIns) were observed in the PFC, indicative of neuronal and astrocytic dysfunction, respectively (Q. Li et al. 2015). Finally, only 1

study reported TBV reductions in very old mice (PND 365) following GD 9 MIA (poly I:C) (da Silveira et al. 2017). Effects on the adolescent brain remain to be elucidated.

3.6.2.2 Mid gestation in Rodents (GD10-14)

Rodent mid-gestation corresponds to the early-middle second trimester in primate gestation; during this time, the blood-brain barrier is forming, immune cell and neuronal migration is ongoing as is neurogenesis in many midbrain and subcortical regions, and sex determination occurs (Matcovitch-Natan et al. 2016; Eggers and Sinclair 2012; Clancy, Darlington, and Finlay 2001; Semple et al. 2013; Selemon and Zecevic 2015). Several studies have investigated the effects of prenatal poly I:C challenge during mid-gestation (GD 15) in rats. Adolescent male rats were found to have no volume alterations in regions of interest such as the hippocampus, lateral ventricles, and TBV; however, smaller striatal volume was observed in both male and female rats (Piontkewitz, Assaf, and Weiner 2009; Piontkewitz, Arad, and Weiner 2011a, [b] 2011).

Many alterations become apparent in both early and late adulthood, including decreased anterior cingulate (ACC) cortex, dorsal thalamic nuclei (Crum et al. 2017), hippocampal, striatal, and PFC volume (with 1 report of earlier decline in males) (Piontkewitz, Assaf, and Weiner 2009; Piontkewitz, Arad, and Weiner 2011a, [b] 2011). Similar regions (ACC, infralimbic area, caudate, dorsomedial thalamus) were also found to be more activated (using MEMRI) in adult poly I:C exposed offspring in response the hallucinogen 2,5-Dimethoxy-4-iodoamphetamine (Malkova et al. 2014). Interestingly, the parafascicular thalamic nuclei, which play a role in the pathogenesis of hallucinations, were only activated in MIA offspring (Malkova et al. 2014). Finally, aberrant neuronal function and glutamate signaling were also observed in the PFC (elevations in total NAA, glutamate, and glutamate+glutamine, and reductions in Tau (Vernon et al. 2015)). In summary, infection in mid-gestation leads to structural, functional, and neurochemical alterations to the PFC, amongst other key regions, which appear in adulthood, and may present earlier in males, in line with schizophrenia symptomatology (Meyer, Feldon, and Dammann 2011; Lieburg et al. 2016). However, the neonatal and pre-adolescent period requires further investigation.

3.6.2.3 Late gestation (GD15-21 mouse/rat; GD 28 rabbit)

Late gestation in rodents corresponds roughly to the end of the second trimester in primate development, where corticogenesis and cortical layer organization, synapto- and gliogenesis, and

apoptosis are beginning; furthermore, neurogenesis of the hippocampus and cortical layers is peaking (Estes and McAllister 2016a; Knuesel et al. 2014; Clancy, Darlington, and Finlay 2001). The effects of MIA in late gestation have been investigated at the level of the placenta, which plays an important role in modulating the effects of inflammation on the fetus (Hsiao and Patterson 2012; Goeden et al. 2016). One study found that LPS exposure (GD 18-20) decreased T2-signal intensity and clearance rate (~10%) in the placentas (GD 20), indicative of placental damage and inflammation similar to chorioamnionitis (Girard et al. 2010).

A large number of studies investigated the effects of intrauterine LPS administration in late gestation on the neonatal New Zealand white rabbit brain using PET and DTI. Interestingly, increased neuroinflammation, assessed using the TSPO tracer, is observed in a dose-dependent manner as early as PND 1 and can persist up to PND 17 (Zhang et al. 2018; Kannan et al. 2007; Kannan, Saadani-Makki, Balakrishnan, Chakraborty, et al. 2011). Conversely, decreased cortical (i.e. frontal and parietal) serotonin was also observed at PND 1, as assessed by the AMT tracer (Kannan, Saadani-Makki, Balakrishnan, Dai, et al. 2011), as well as decreased FA in the periventricular WM (i.e. corpus callosum, anterior commissure, internal capsule, and corona radiata) (Saadani-Makki et al. 2009). Similarly, mouse offspring prenatally exposed to the human influenza strain H1N1 at GD 16 also had decreased FA in the right internal capsule at P 0, coupled with decreased ventricular volume.

Three studies investigated rats in peri-adolescence (PND 25) prenatally exposed to LPS at GD 18, and found widespread changes using both DWI and quantitative imaging. LPS exposure was again found to negatively impact periventricular WM, as well as the entorhinal, auditory, sensory cortices, hippocampus, caudate-putamen, hypothalamus, and thalamus (increased medial diffusivity (MD), apparent diffusion coefficient, and T2-signal intensity) (Sharabi et al. 2018; Ginsberg et al. 2017; Beloosesky et al. 2013). Similarly, mice who were prenatally exposed to the H1N1 influenza virus at GD 16 or GD 19 had decreased TBV and cerebellar volume, and increased corpus callosum volume, at PND 14 (Fatemi et al. 2008; Fatemi, Folsom, Reutiman, Abu-Odeh, et al. 2009; Fatemi, Folsom, Reutiman, Huang, et al. 2009).

Adolescent offspring prenatally exposed to LPS (GD 15, 16, 17) had reduced binding potential of the PET tracer [18F]FPEB, a radioligand for metabotropic glutamate receptor 5, in the hippocampus. Interestingly, no signs of neuroinflammation were found using [11C]PBR85, a radioligand for peripheral benzonitrile receptor 28 (Arsenault et al. 2014); neuroinflammation

observed in the early postnatal period (Kannan et al. 2007; Z. Zhang et al. 2018) may normalize by adolescence, although different tracers were used. Adolescent rats were also found to have decreased hippocampal volume and a higher number of dilated Vichrow-Robin spaces, often associated with neurodevelopmental and neurodegenerative diseases, following MIA at GD 16 (H1N1, LPS respectively) (Fatemi, Folsom, Reutiman, Huang, et al. 2009; Ooi et al. 2017).

Three studies investigated the effects of prenatal poly I:C exposure at GD 17 in adulthood and found widespread alterations using sMRI and DTI. At PND 84, poly I:C exposed offspring had smaller anterior commissure, external capsule, and piriform cortex volumes (Richetto et al. 2017). Volume increases were observed largely in the cerebellum, a region sensitive to neurodevelopmental insult (Wang, Kloth, and Badura 2014), and the 4th ventricle (Richetto et al. 2017; Qi Li et al. 2009). Decreased T2-relaxation time was observed in similar regions such as the cerebellum, hippocampus, and piriform cortex, as well as many other cortical regions (PFC, ACC, insular, motor, somatosensory, visual, auditory cortices). WM tract integrity of the cerebral peduncle, fimbria, and subiculum was comprised following poly I:C exposure at GD 17 (decreased FA); some gray matter (GM) regions were also found to have decreased FA such as the amygdala and piriform area (Richetto et al. 2017; Qi Li et al. 2010). Finally, MIA induced by GBS exposure (GD 19, 20, 21, 22) decreased forebrain volume and increased lateral ventricle volume mainly in male offspring, with reduced periventricular external capsule thickness in older adult (PND150) rat offspring of both sexes (Bergeron et al. 2013). Thus, evidence suggests that MIA in late gestation may compromise placental function and lead to neuroanatomical and neurochemical alterations throughout the offspring lifespan.

3.6.2.4 Late gestation exposure in primates (>GD110)

Synaptogenesis and myelination are actively occurring in the human third trimester, which corresponds to postnatal rodent development (Gumusoglu and Stevens 2019). Thus, the effects of MIA on these processes may be better studied in non-human primates given their similarly protracted intrauterine periods. Interestingly, 2 studies have investigated the effects of the bacterial mimetic LPS, and the influenza virus H2N3 on neonatal offspring development in the rhesus monkey. They found that offspring exposed to LPS at 17 weeks' gestation had increased global WM volume, and thicker GM in the right parietal and frontal lobes, with thinner GM volume in the medial temporal lobe (Willette et al. 2011). Conversely, H2N3 exposure at 17 weeks of

gestation resulted in increased LV volume, and a decrease in total and cortical GM volume and cerebellar WM. Decreased GM volume was observed in the cingulate and parietal cortex, whereas WM volume was also increased. Finally, decreased bilateral amygdala volume was also observed (Short et al. 2010).

3.6.3 Can structural brain abnormalities be prevented?

The current body of literature on prenatal MIA exposure provides compelling evidence for progressive neuroanatomical and behavioural alterations appearing throughout the lifespan, mimicking many neuropsychiatric disorders. A handful of preclinical studies included in this review have investigated various therapeutic manipulations administered either to the mother, or to the offspring, and found them to rescue some MIA-induced brain phenotypes (Sharabi et al. 2018; Piontkewitz, Assaf, and Weiner 2009; Piontkewitz, Arad, and Weiner 2011a; Ginsberg et al. 2017; Q. Li et al. 2015). Treatment with either antioxidants (e.g. N-acetyl cysteine) or anti-inflammatory compounds (e.g. IL-1Ra, magnesium sulfate) around the time of the maternal immune challenge were found to normalize offspring deficits, including alterations to WM/tissue integrity (MD or radial diffusivity [RD], or T2-signal) in young (PND 25) rats (Sharabi et al. 2018; Ginsberg et al. 2017). It is possible that these rescuing effects are a result of normalizing placental clearance ability and tissue integrity (T2 signal), as shown by Girard and colleagues (Girard et al. 2010).

Investigation of therapeutic interventions in the offspring has also been a point of interest, as they may be used during prodromal or high-risk phases to prevent the emergence of neuropsychiatric disorders. For example, treatment with atypical antipsychotic drugs, which have been shown to increase anti- and decrease pro-inflammatory cytokine production, in adolescent humans and in cell culture (Al-Amin, Nasir Uddin, and Mahmud Reza 2013; Kato et al. 2011) during an asymptomatic phase, successfully prevented lateral ventricle and hippocampal volume alterations in adult mice following prenatal poly I:C challenge (Piontkewitz, Assaf, and Weiner 2009; Piontkewitz, Arad, and Weiner 2011a). Similarly, administration of a diet rich in omega-3 polyunsaturated-fatty acids was shown to normalize MIA-induced NAA and mIns alterations (Q. Li et al. 2015). Several other therapies, such as probiotics (Hsiao et al. 2013), environmental

enrichment (Connors et al. 2014), or maternal zinc (Chua et al. 2012; Coyle et al. 2009) have also shown promise in reducing schizophrenia or ASD behavioural or neurochemical aberrations.

3.6.4 Parallels between clinical and preclinical findings

Both clinical and preclinical studies included in this review suggest that prenatal exposure to maternal inflammation leads to widespread neuroanatomical alterations detectable throughout the lifespan (fetus to adult). Periventricular WM is sensitive to damage following infection late in gestation in rabbit, rat, and mouse studies, and in human fetuses exposed to toxoplasmosis *in utero*. This gestational timing coincides with the beginning of WM development. Damage to this region may be a result of pro-inflammatory cytokines and diffuse activation of microglia within immature WM, leading to death or injury or pre-myelinating oligodendrocytes (Khwaja and Volpe 2008). Further, development of periventricular vasculature may also be impaired, resulting in further damage to this region (Dammann and O'Shea 2008). Periventricular WM is also in close proximity of ventricles and striatum where alterations are consistently observed in many clinical and preclinical studies.

Some human studies suggest that maternal infection early in gestation leads to more severe deficits in the offspring frontal, temporal, and parietal cortices and periventricular WM than exposure in late gestation. The preclinical findings do suggest that the timing of MIA may influence the severity or evolution of neuroanatomical changes; however, very few studies actually employed longitudinal designs to be able to adequately investigate this question. Even so, it appears that MIA in early gestation leads to accelerated brain growth early in life, and neurochemical alterations in the PFC (impaired neuronal and astrocytic function), as well as diffuse WM alterations in adulthood. Conversely, MIA in mid-gestation leads to changes that only appear in the adult brain, such as increased lateral ventricle, decreased hippocampus, and PFC volume, with more pronounced effects in males. Finally, MIA in late gestation seems to induce neuroinflammation and decreased cortical serotonin early in the lifespan, decrease glutamate receptor function in adolescence, followed by diffuse structural changes in adolescence and adulthood in the cortex and cerebellum.

There is also the possibility for a dose-dependent effect. In human studies in which mothers were infected with viral or bacterial pathogens, fetal and neonatal brain structure alterations were

more severe than in studies investigating effects of IL-6 or CRP in healthy mothers on offspring. In the latter group, higher pro-inflammatory cytokine levels were associated with broader alterations to fronto-limbic and salience network connectivity. However, there is no long-term follow-up in these studies, making it difficult to determine how MIA affects developmental trajectories. Only 1 study investigated the relationship between maternal cytokine levels and brain anatomy in individuals with schizophrenia spectrum disorders and did indeed find associations between IL-8 and brain structure suggesting that *in utero* cytokine exposure does cause enduring neuroanatomical changes. The clinical and preclinical studies included in this review provide strong evidence for the idea that exposure to maternal infection or inflammation *in utero* leads to neurodevelopmental changes. There is a wide body of literature confirming that behavioural, neurochemical, and cellular changes are also detectable, but this work is outside of the scope of this review (Gumusoglu and Stevens 2019; Meyer 2014; Knuesel et al. 2014; Estes and McAllister 2016a; Solek et al. 2018).

3.6.5 Parallels between MIA-induced brain alterations and those observed in patients with ASD and schizophrenia

MIA-exposure is associated with deficits in neuroanatomy and behaviour relevant to both schizophrenia and ASD pathophysiology. Notably, these disorders are both of prenatal origin and overlap to some extent in terms of symptomatology (e.g. difficulties with social interaction, emotion, verbal and nonverbal communication, and odd or inflexible behaviour (Stone and Iguchi 2011; Hommer and Swedo 2015; Gumusoglu and Stevens 2019; Park et al. 2018)). Further, there are similarities with respect to the brain regions affected in each illness.

Neuroanatomical alterations commonly observed in patients with schizophrenia include: widespread cortical thinning, most pronounced in the frontal and temporal lobes, decreased hippocampal, thalamic, amygdala, nucleus accumbens and total brain volume, increased pallidum and ventricular volume, and WM abnormalities in the corpus callosum and corona radiata (Kelly et al. 2018; van Erp et al. 2016; van Erp et al. 2018). Furthermore, aberrant glutamatergic signaling has often been observed, most commonly in the unmedicated state, in brain regions such as the ACC/medial PFC, hippocampus, and basal ganglia (Hu et al. 2015; Plitman et al. 2014; Iwata et al. 2018). Interestingly, comparable deficits are observed in MIA offspring, including lateral

ventricle enlargement, volume decreases and glutamate dysregulation in the PFC and hippocampus in adulthood, and alterations to the periventricular WM in many of the rodent studies reviewed here (Piontkewitz, Assaf, and Weiner 2009; Piontkewitz, Arad, and Weiner 2011a; Arsenault et al. 2014; Piontkewitz, Arad, and Weiner 2011b; Crum et al. 2017; Vernon et al. 2015; Bergeron et al. 2013; Malkova et al. 2014).

Individuals with ASD have abnormalities in similar regions but with different directionality; cortical overgrowth in frontal, temporal, cingulate, and parietal lobes is commonly observed in the first 2 years of life, as well as enlarged amygdala and cerebellum volumes (Schumann et al. 2010; Amaral, Schumann, and Nordahl 2008). Cortical overgrowth in the first 2 years of the rhesus monkey lifespan was also observed following MIA (Bauman et al. 2013) and cerebellar abnormalities were observed in mouse models (Fatemi et al. 2008). Finally, enlarged amygdala volume, as well aberrant fronto-limbic pathways, were associated with increased pro-inflammatory cytokine exposure in human neonates (Graham et al. 2018; Rasmussen et al. 2018).

There are many additional parallels between schizophrenia, ASD, and MIA models at the cellular level, including deficits in Purkinje cells, impaired expression of parvalbumin and reelin, excessive microglial activation, and altered dendritic morphology and synaptic pruning mechanisms (Careaga, Murai, and Bauman 2017; Keshavan et al. 2014; Canetta et al. 2016; Gao and Penzes 2015). It is noteworthy that this section of the review focuses specifically on similarities between MIA-induced brain alterations, as per the results of the included studies, and well-accepted findings from ASD and schizophrenia literature. Notwithstanding, there are several dissimilarities between the neuroanatomical alterations rendered by MIA and the literature surrounding ASD and schizophrenia. These discrepancies could in part be accounted for by an accumulation of risk factors, of which MIA is only one; however, a detailed discussion of these dissimilarities is beyond the scope of the review.

3.6.6. Male bias in preclinical studies

The human studies reviewed here were fairly balanced in their inclusion of males and female offspring; however, none of the studies investigated sex as a variable of interest to determine whether exposure to maternal inflammation affected male and female offspring differently. Moreover, of the preclinical studies reviewed, 6 did not specify offspring sex, 16 only

studied males, 2 only studied females, and only 4 studies included both sexes in equal numbers. Unfortunately, this is representative of the general lack of balanced groups in preclinical research. Of the 4 studies that did include males and females, interesting sex differences emerged longitudinally. Male MIA-exposed offspring developed neuroanatomical deficits either earlier than females, such as decreased PFC volume, or had deficits that females did not, such as lateral ventricle enlargement (Piontkewitz, Arad, and Weiner 2011b). The consideration of sex as a variable would seem self-evident given the strong sex bias in prevalence, symptom presentation, and treatment response in many neurodevelopmental disorders, yet very few preclinical studies include both males and females, with even fewer explicitly investigating sex differences (Coiro and Pollak 2019; Prata et al. 2017). A recent policy by the National Institute of Health aims to address this concern by mandating the consideration of using female cells and animals in preclinical research; hopefully this will balance the sex bias present in the literature.

3.6.7 Limitations

With respect to the included studies, there are a few noteworthy limitations. First, MIA models have a wide range of protocols that vary based on gestational timing, mode of delivery, dose, and immunogen used. Further, some less obvious, yet important, sources of variability include animal strain and genetic background, animal vendor, breeding, housing, amongst others reviewed by Weber-Stabdlbauer and Meyer, and by Kentner and colleagues (Kentner et al. 2019; Weber-Stadlbauer and Meyer 2019). All of these factors may lead to different downstream effects; the immunogen used determines the nature of the immune response, whereas the timing of exposure may interfere with different neurodevelopmental processes, altering the nature and severity of outcomes (Estes and McAllister 2016a; Knuesel et al. 2014). Further, only a few studies confirmed MIA or sickness behaviours in mothers (da Silveira et al. 2017; Crum et al. 2017; Vernon et al. 2015; Bergeron et al. 2013; Girard et al. 2010; Willette et al. 2011; Short et al. 2010), which is challenging to do in smaller species (i.e. taking a blood sample or increased handling could affect pregnancies (Kentner et al. 2019). Some ways to use variability as an opportunity for research, as suggested by Weber-Stadlbauer and Meyer, include investigating different MIA immunogens in different species, investigating susceptible and resilient mothers or placental placement to try to understand within- and between-litter phenotypic variation, and understanding

the influence of microbiota on outcomes of MIA models (Kentner et al. 2019). Notably, it is critical for researchers to report methodological details of their chosen model to enhance transparency and comparability of these models across laboratories and species (Weber-Stadlbauer and Meyer 2019; Kentner et al. 2019).

Although animal models can never recapitulate a full spectrum of human behaviour and acknowledging the fact that there are pronounced differences between rodent and primate brain development, there are also considerable cross-species alignment in terms of key developmental milestones, well captured by a translational modality such as whole-brain imaging. The advantages to using preclinical MIA models to study the effects of maternal cytokines on brain development are that the immune response induced by viral and bacterial mimetics allow for precise timing of immunogenic impact, providing a better understanding of the links between immune activation and embryonic brain development. Further, preclinical brain imaging lends itself well to dense sampling, often a challenge in humans, and allows for post-hoc behavioural and post-mortem evaluation of these findings. However, it is noteworthy that much of the human work on elevated cytokine (IL-6) concentrations during pregnancy by the groups of Buss, Fair, and Peterson reflect chronic systemic low-grade inflammation within a normal range, potentially due to poor nutrition, obesity, chronic stress amongst other factors, rather than an acute increase in inflammation due to infection or trauma. This work suggests that even modest variations in cytokines affect neonatal brain function; that being said, it does not lend itself well to study sensitive time windows of exposure as is done in the preclinical literature. There are a considerable number of human studies investigating maternal infection; however, these do not correlate offspring outcomes with specific cytokine levels.

There is also great variation in the MR image quality and image analysis methods, particularly within the preclinical studies. Many of the studies use low resolution images, with particularly thick slices (as thick as 1500 μm^3 in some rodent studies (Piontkewitz, Arad, and Weiner 2011a; Piontkewitz, Assaf, and Weiner 2009; da Silveira et al. 2017)), which prevents careful delineation of many structures within the rodent brain (see **Table 3.2**). Additionally, many rely on coarse structural volume estimations, relying on manual segmentation of these low-resolution images. Many of these studies may thus have insufficient power to detect subtle volume changes, as outlined by the power analysis by Lerch and colleagues, either based on low resolution or images, or low samples size, or both; in order to detect a within-subject 3% volume change in

the hippocampus, it is recommended to have 10 mice per group at 4 timepoints with a resolution of 125um (Lerch et al. 2012). Further, many DWI studies included in this review use low resolution images with very few encoding directions and small sample sizes; however, the majority of these studies are over a decade old and were performed when DWI was emerging as a technique (Fatemi, Folsom, Reutiman, Huang, et al. 2009; Fatemi, Folsom, Reutiman, Abu-Odeh, et al. 2009; Fatemi et al. 2008; Saadani-Makki et al. 2009). More recent work has employed 3D whole-brain voxel-wise approaches to investigate higher resolution images. Finally, considerations regarding sample preparation should be noted, as *ex vivo* MRI is sensitive to perfusion artifacts (Cahill et al. 2012). Even so, some of the findings discussed in this review require replication with higher resolution data from different modalities including structural and functional MRI, as well as DWI, and potentially more quantitative MRI sensitive to myelin such as magnetization transfer ratio imaging, in addition to larger samples.

Offspring deficits are often measured cross-sectionally at different timepoints, using different modalities, making it challenging to determine the neurodevelopmental origins of specific deficits related to MIA-exposure. Our synthesis of these 39 studies suggests that more work is needed to understand the long-term consequences of MIA-exposure on offspring brain development. More specifically, there are very few studies that take advantage of the non-invasive nature of brain imaging to examine offspring longitudinally. This is critical as the consensus within the human literature is that to fully understand neurodevelopmental abnormalities, understanding how risk factors may impact developmental trajectories is critical. Without these homologous trajectories, it will be increasingly difficult to build translational "bridges" between observations from preclinical and clinical studies. Furthermore, performing *in vivo* MRI *in utero* or *ex vivo* MRI of embryos in models of maternal inflammation would provide us with crucial information regarding the evolution of brain structural changes following insult (Pedroni et al. 2014; Turnbull and Mori 2007; Zhang, Wu, and Turnbull 2018; Wu and Zhang 2016).

This review should also be considered in light of its own limitations. It is possible that relevant articles may have been omitted from the search due to the selection of search terms. Furthermore, this review focused on studies performing neuroimaging; however, in many cases, this was not their only assay. Discussing neuroanatomical changes in light of behavioural and cellular alterations is critical to furthering our understanding of how MIA primes the brain to be more sensitive to neurodevelopmental insult. A further limitation is lack of examination of a

common pathway (or pathways) that may emerge due to MIA-exposure. In addition, it would be extremely interesting to extend this work by conducting a meta-analysis on reported volumes of certain structures, such as the hippocampus or lateral ventricles. However, we would certainly need some element of neuroanatomical standardization across species and gestational periods, as well as standardization of inflammatory agent, dose, and gestational timing used, which currently does not exist. We feel that this review draws attention to these nuances in the literature, and in future work, a meta-analysis could add significant value to this literature.

3.6.8 Conclusions and future directions

Converging evidence from clinical and preclinical studies suggests that MIA is a disease primer for various neurodevelopmental disorders. Better characterizations of age-, region-, and gender-specific effects on neuroanatomical development are critical to furthering our understanding of this risk factor. Careful characterization of MIA using high-resolution, multi-model longitudinal neuroimaging studies may help to elucidate many of these questions and identify regions of heightened vulnerability in which therapeutic interventions may be targeted. It may be interesting to combine MIA models with other relevant risk factors, such as schizophrenia- or ASD-relevant genetic mutations, or other environmental risk factors such as adolescent stress or drug use. Further, better integration of behavioural findings with brain structural changes (macro- and microscopic) may further our understanding of brain-behaviour relationships, and how they might be disrupted by MIA. Finally, improving the reproducibility of the model, with precise reporting and transparency, is of the utmost importance for moving the field forward.

3.7 Acknowledgements

The authors would like to thank Dr. Gabriel A Devenyi for his guidance in the creation of **Figure 3.1**. EG and MMC receive financial support from Fonds de la recherche en santé du Québec and EP from Healthy Brains for Healthy Lives Fellowship from McGill University.

3.8 Disclosures

Authors have no disclosures to report.

References

Al-Amin, Md Mamun, Mir Muhammad Nasir Uddin, and Hasan Mahmud Reza. 2013. "Effects of Antipsychotics on the Inflammatory Response System of Patients with Schizophrenia in Peripheral Blood Mononuclear Cell Cultures." *Clinical Psychopharmacology and Neuroscience: The Official Scientific Journal of the Korean College of Neuropsychopharmacology* 11 (3): 144–51.

Al-Asmari, A. K., and Md W. Khan. 2014. "Inflammation and Schizophrenia: Alterations in Cytokine Levels and Perturbation in Antioxidative Defense Systems." *Human & Experimental Toxicology* 33 (2): 115–22.

Amaral, David G., Cynthia Mills Schumann, and Christine Wu Nordahl. 2008. "Neuroanatomy of Autism." *Trends in Neurosciences* 31 (3): 137–45.

Arsenault, Dany, Aijun Zhu, Chunyu Gong, Kun-Eek Kil, Sreekanth Kura, Ji-Kyung Choi, and Anna-Liisa Brownell. 2014. "Hypo-Anxious Phenotype of Adolescent Offspring Prenatally Exposed to LPS Is Associated with Reduced mGluR5 Expression in Hippocampus." *Open Journal of Medical Psychology* 3 (3): 202–11.

Bauman, M. D., A-M Iosif, P. Ashwood, D. Braunschweig, A. Lee, C. M. Schumann, J. Van de Water, and D. G. Amaral. 2013. "Maternal Antibodies from Mothers of Children with Autism Alter Brain Growth and Social Behavior Development in the Rhesus Monkey." *Translational Psychiatry* 3 (July): e278.

Beloosesky, Ron, Yuval Ginsberg, Nizar Khatib, Nir Maravi, Michael G. Ross, Joseph Itskovitz-Eldor, and Zeev Weiner. 2013. "Prophylactic Maternal N-Acetylcysteine in Rats Prevents Maternal Inflammation-Induced Offspring Cerebral Injury Shown on Magnetic Resonance Imaging." *American Journal of Obstetrics and Gynecology* 208 (3): 213.e1–6.

Bergdolt, Lara, and Anna Dunaevsky. 2019. "Brain Changes in a Maternal Immune Activation Model of Neurodevelopmental Brain Disorders." *Progress in Neurobiology* 175 (April): 1–19.

Bergeron, J. D. L., J. Deslauriers, S. Grignon, L. C. Fortier, M. Lepage, T. Stroh, C. Poyart, and G. Sébire. 2013. "White Matter Injury and Autistic-like Behavior Predominantly Affecting Male Rat Offspring Exposed to Group B Streptococcal Maternal Inflammation." *Developmental Neuroscience* 35 (6): 504–15.

Birnbaum, Roee, Liat Ben-Sira, Tally Lerman-Sagie, and Gustavo Malinger. 2017. "The Use of Fetal Neurosonography and Brain MRI in Cases of Cytomegalovirus Infection during Pregnancy: A Retrospective Analysis with Outcome Correlation." *Prenatal Diagnosis* 37 (13): 1335–42.

Boksa, Patricia. 2010. "Effects of Prenatal Infection on Brain Development and Behavior: A Review of Findings from Animal Models." *Brain, Behavior, and Immunity* 24 (6): 881–97.

Brown, Alan S., Melissa D. Begg, Stefan Gravenstein, Catherine A. Schaefer, Richard J. Wyatt, Michaeline Bresnahan, Vicki P. Babulas, and Ezra S. Susser. 2004. "Serologic Evidence of Prenatal Influenza in the Etiology of Schizophrenia." *Archives of General Psychiatry* 61 (8): 774–80.

Brown, Alan S., Patricia Cohen, Jill Harkavy-Friedman, Vicki Babulas, Dolores Malaspina, Jack M. Gorman, and Ezra S. Susser. 2001. "Prenatal Rubella, Premorbid Abnormalities, and Adult Schizophrenia." *Biological Psychiatry* 49 (6): 473–86.

Brown, Alan S., and Urs Meyer. 2018. "Maternal Immune Activation and Neuropsychiatric Illness: A Translational Research Perspective." *The American Journal of Psychiatry*,

September, appiajp201817121311.
Buka, S. L., M. T. Tsuang, J. M. Goldstein, L. J. Seidman, E. F. Torrey, M. A. Klebanoff, and R. H. Yolken. 2003. "Maternal Exposure to Herpes Simplex Virus Type 2 and Psychosis among Adult Offspring: Replication and Specificity." *Schizophrenia Research* 60 (1): 35.
Buka, S. L., M. T. Tsuang, E. F. Torrey, M. A. Klebanoff, D. Bernstein, and R. H. Yolken. 2001. "Maternal Infections and Subsequent Psychosis among Offspring." *Archives of General Psychiatry* 58 (11): 1032–37.
Buka, Stephen L., Tyrone D. Cannon, E. Fuller Torrey, Robert H. Yolken, and Collaborative Study Group on the Perinatal Origins of Severe Psychiatric Disorders. 2008. "Maternal Exposure to Herpes Simplex Virus and Risk of Psychosis among Adult Offspring." *Biological Psychiatry* 63 (8): 809–15.
Cahill, L. S., C. L. Laliberté, J. Ellegood, and S. Spring. 2012. "Preparation of Fixed Mouse Brains for MRI." *NeuroImage*. https://www.sciencedirect.com/science/article/pii/S1053811912001188.
Canetta, S., S. Bolkan, N. Padilla-Coreano, L. J. Song, R. Sahn, N. L. Harrison, J. A. Gordon, A. Brown, and C. Kellendonk. 2016. "Maternal Immune Activation Leads to Selective Functional Deficits in Offspring Parvalbumin Interneurons." *Molecular Psychiatry* 21 (7): 956–68.
Careaga, Milo, Takeshi Murai, and Melissa D. Bauman. 2017. "Maternal Immune Activation and Autism Spectrum Disorder: From Rodents to Nonhuman and Human Primates." *Biological Psychiatry* 81 (5): 391–401.
Choi, G. B., Y. S. Yim, H. Wong, S. Kim, H. Kim, S. V. Kim, C. A. Hoeffer, D. R. Littman, and J. R. Huh. 2016. "The Maternal Interleukin-17a Pathway in Mice Promotes Autism-like Phenotypes in Offspring." *Science*. https://doi.org/10.1126/science.aad0314.
Chua, Joanne S. C., Carina J. Cowley, Jim Manavis, Allan M. Rofe, and Peter Coyle. 2012. "Prenatal Exposure to Lipopolysaccharide Results in Neurodevelopmental Damage That Is Ameliorated by Zinc in Mice." *Brain, Behavior, and Immunity* 26 (2): 326–36.
Clancy, B., R. B. Darlington, and B. L. Finlay. 2001. "Translating Developmental Time across Mammalian Species." *Neuroscience* 105 (1): 7–17.
Clarke, Mary C., Antti Tanskanen, Matti Huttunen, John C. Whittaker, and Mary Cannon. 2009. "Evidence for an Interaction between Familial Liability and Prenatal Exposure to Infection in the Causation of Schizophrenia." *The American Journal of Psychiatry* 166 (9): 1025–30.
Coiro, Pierluca, and Daniela D. Pollak. 2019. "Sex and Gender Bias in the Experimental Neurosciences: The Case of the Maternal Immune Activation Model." *Translational Psychiatry*. https://doi.org/10.1038/s41398-019-0423-8.
Colucci, F., S. Boulenouar, J. Kieckbusch, and A. Moffett. 2011. "How Does Variability of Immune System Genes Affect Placentation?" *Placenta* 32 (8): 539–45.
Connors, E. J., A. N. Shaik, M. M. Migliore, and A. C. Kentner. 2014. "Environmental Enrichment Mitigates the Sex-Specific Effects of Gestational Inflammation on Social Engagement and the Hypothalamic Pituitary Adrenal Axis-Feedback System." *Brain, Behavior, and Immunity* 42 (November): 178–90.
Coyle, Peter, Nancy Tran, Jenny N. T. Fung, Brooke L. Summers, and Allan M. Rofe. 2009. "Maternal Dietary Zinc Supplementation Prevents Aberrant Behaviour in an Object Recognition Task in Mice Offspring Exposed to LPS in Early Pregnancy." *Behavioural Brain Research* 197 (1): 210–18.
Crum, William R., Stephen J. Sawiak, Winfred Chege, Jonathan D. Cooper, Steven C. R.

Williams, and Anthony C. Vernon. 2017. “Evolution of Structural Abnormalities in the Rat Brain Following in Utero Exposure to Maternal Immune Activation: A Longitudinal in Vivo MRI Study.” *Brain, Behavior, and Immunity* 63 (July): 50–59.

Dammann, Olaf, and T. Michael O’Shea. 2008. “Cytokines and Perinatal Brain Damage.” *Clinics in Perinatology* 35 (4): 643–63, v.

Dhombres, Ferdinand, Stéphanie Friszer, Paul Maurice, Marie Gonzales, François Kieffer, Catherine Garel, and Jean-Marie Jouannic. 2017. “Prognosis of Fetal Parenchymal Cerebral Lesions without Ventriculomegaly in Congenital Toxoplasmosis Infection.” *Fetal Diagnosis and Therapy* 41 (1): 8–14.

Diebler, C., A. Dusser, and O. Dulac. 1985. “Congenital Toxoplasmosis.” *Neuroradiology* 27 (2): 125–30.

Dowling, Jennifer K., and Ashley Mansell. 2016. “Toll-like Receptors: The Swiss Army Knife of Immunity and Vaccine Development.” *Clinical & Translational Immunology* 5 (5): e85.

Eggers, Stefanie, and Andrew Sinclair. 2012. “Mammalian Sex Determination—insights from Humans and Mice.” *Chromosome Research: An International Journal on the Molecular, Supramolecular and Evolutionary Aspects of Chromosome Biology* 20 (1): 215–38.

Ellman, Lauren M., Raymond F. Deicken, Sophia Vinogradov, William S. Kremen, John H. Poole, David M. Kern, Wei Yann Tsai, Catherine A. Schaefer, and Alan S. Brown. 2010. “Structural Brain Alterations in Schizophrenia Following Fetal Exposure to the Inflammatory Cytokine Interleukin-8.” *Schizophrenia Research* 121 (1-3): 46–54.

Erp, T. G. M. van, D. P. Hibar, J. M. Rasmussen, D. C. Glahn, G. D. Pearlson, O. A. Andreassen, I. Agartz, et al. 2016. “Subcortical Brain Volume Abnormalities in 2028 Individuals with Schizophrenia and 2540 Healthy Controls via the ENIGMA Consortium.” *Molecular Psychiatry* 21 (4): 585.

Erp, Theo G. M. van, Esther Walton, Derrek P. Hibar, Lianne Schmaal, Wenhao Jiang, David C. Glahn, Godfrey D. Pearlson, et al. 2018. “Cortical Brain Abnormalities in 4474 Individuals With Schizophrenia and 5098 Control Subjects via the Enhancing Neuro Imaging Genetics Through Meta Analysis (ENIGMA) Consortium.” *Biological Psychiatry* 84 (9): 644–54.

Estes, Myka L., and A. Kimberley McAllister. 2016a. “Maternal Immune Activation: Implications for Neuropsychiatric Disorders.” *Science* 353 (6301): 772–77.

Fatemi, S. Hossein, Timothy D. Folsom, Teri J. Reutiman, Desiree Abu-Odeh, Susumu Mori, Hao Huang, and Kenichi Oishi. 2009. “Abnormal Expression of Myelination Genes and Alterations in White Matter Fractional Anisotropy Following Prenatal Viral Influenza Infection at E16 in Mice.” *Schizophrenia Research* 112 (1-3): 46–53.

Fatemi, S. Hossein, Timothy D. Folsom, Teri J. Reutiman, Hao Huang, Kenichi Oishi, and Susumu Mori. 2009. “Prenatal Viral Infection of Mice at E16 Causes Changes in Gene Expression in Hippocampi of the Offspring.” *European Neuropsychopharmacology: The Journal of the European College of Neuropsychopharmacology* 19 (9): 648–53.

Fatemi, S. Hossein, Teri J. Reutiman, Timothy D. Folsom, Hao Huang, Kenichi Oishi, Susumu Mori, Donald F. Smee, et al. 2008. “Maternal Infection Leads to Abnormal Gene Regulation and Brain Atrophy in Mouse Offspring: Implications for Genesis of Neurodevelopmental Disorders.” *Schizophrenia Research* 99 (1): 56–70.

Gao, R., and P. Penzes. 2015. “Common Mechanisms of Excitatory and Inhibitory Imbalance in Schizophrenia and Autism Spectrum Disorders.” *Current Molecular Medicine* 15 (2): 146–67.

Giedd, J. 2010. “Neuroimaging of Human Development and Neurodevelopmental Disorders.”

International Journal of Developmental Neuroscience. https://doi.org/10.1016/j.ijdevneu.2010.07.008.

Ginsberg, Yuval, Nizar Khatib, Boaz Weiss, Shay Arison, Michael G. Ross, Zeev Weiner, and Ron Beloosesky. 2017. "Magnesium Sulfate (MG) Prevents Maternal Inflammation Induced Offspring Cerebral Injury Evident on MRI but Not via IL-1β." *Neuroscience* 353 (June): 98–105.

Girard, Sylvie, Luc Tremblay, Martin Lepage, and Guillaume Sébire. 2010. "IL-1 Receptor Antagonist Protects against Placental and Neurodevelopmental Defects Induced by Maternal Inflammation." *Journal of Immunology* 184 (7): 3997–4005.

Gleason, Christine A., and Stephen A. Back. 2005. "Chapter 61 - Developmental Physiology of the Central Nervous System." In *Avery's Diseases of the Newborn (Eighth Edition)*, edited by H. William Taeusch, Roberta A. Ballard, and Christine A. Gleason, 903–7. Philadelphia: W.B. Saunders.

Goeden, Nick, Juan Velasquez, Kathryn A. Arnold, Yen Chan, Brett T. Lund, George M. Anderson, and Alexandre Bonnin. 2016. "Maternal Inflammation Disrupts Fetal Neurodevelopment via Increased Placental Output of Serotonin to the Fetal Brain." *The Journal of Neuroscience: The Official Journal of the Society for Neuroscience* 36 (22): 6041–49.

Graham, Alice M., Jerod M. Rasmussen, Marc D. Rudolph, Christine M. Heim, John H. Gilmore, Martin Styner, Steven G. Potkin, et al. 2018. "Maternal Systemic Interleukin-6 During Pregnancy Is Associated with Newborn Amygdala Phenotypes and Subsequent Behavior at 2 Years of Age." *Biological Psychiatry* 83 (2): 109–19.

Gumusoglu, Serena B., and Hanna E. Stevens. 2019. "Maternal Inflammation and Neurodevelopmental Programming: A Review of Preclinical Outcomes and Implications for Translational Psychiatry." *Biological Psychiatry* 85 (2): 107–21.

Hammelrath, Luam, Siniša Škokić, Artem Khmelinskii, Andreas Hess, Noortje van der Knaap, Marius Staring, Boudewijn P. F. Lelieveldt, Dirk Wiedermann, and Mathias Hoehn. 2016. "Morphological Maturation of the Mouse Brain: An in Vivo MRI and Histology Investigation." *NeuroImage* 125 (January): 144–52.

Hommer, Rebecca E., and Susan E. Swedo. 2015. "Schizophrenia and Autism-Related Disorders." *Schizophrenia Bulletin* 41 (2): 313–14.

Hsiao, Elaine Y., Sara W. McBride, Sophia Hsien, Gil Sharon, Embriette R. Hyde, Tyler McCue, Julian A. Codelli, et al. 2013. "Microbiota Modulate Behavioral and Physiological Abnormalities Associated with Neurodevelopmental Disorders." *Cell* 155 (7): 1451–63.

Hsiao, Elaine Y., and Paul H. Patterson. 2012. "Placental Regulation of Maternal-Fetal Interactions and Brain Development." *Developmental Neurobiology* 72 (10): 1317–26.

Hu, Wei, Matthew L. MacDonald, Daniel E. Elswick, and Robert A. Sweet. 2015. "The Glutamate Hypothesis of Schizophrenia: Evidence from Human Brain Tissue Studies." *Annals of the New York Academy of Sciences* 1338 (March): 38–57.

Iwata, Yusuke, Shinichiro Nakajima, Eric Plitman, Yukiko Mihashi, Fernando Caravaggio, Jun Ku Chung, Julia Kim, et al. 2018. "Neurometabolite Levels in Antipsychotic-Naïve/free Patients with Schizophrenia: A Systematic Review and Meta-Analysis of 1H-MRS Studies." *Progress in Neuro-Psychopharmacology & Biological Psychiatry* 86 (August): 340–52.

Jenster, Meike, Sonia L. Bonifacio, Theodore Ruel, Elizabeth E. Rogers, Emily W. Tam, John Colin Partridge, Anthony James Barkovich, Donna M. Ferriero, and Hannah C. Glass. 2018. "Maternal or Neonatal Infection: Association with Neonatal Encephalopathy Outcomes."

Pediatric Research 83 (3): 747.
Kannan, Sujatha, Fadoua Saadani-Makki, Bindu Balakrishnan, Pulak Chakraborty, James Janisse, Xin Lu, Otto Muzik, Roberto Romero, and Diane C. Chugani. 2011. "Magnitude of [(11)C]PK11195 Binding Is Related to Severity of Motor Deficits in a Rabbit Model of Cerebral Palsy Induced by Intrauterine Endotoxin Exposure." *Developmental Neuroscience* 33 (3-4): 231–40.
Kannan, Sujatha, Fadoua Saadani-Makki, Bindu Balakrishnan, Hui Dai, Pulak K. Chakraborty, James Janisse, Otto Muzik, Roberto Romero, and Diane C. Chugani. 2011. "Decreased Cortical Serotonin in Neonatal Rabbits Exposed to Endotoxin in Utero." *Journal of Cerebral Blood Flow and Metabolism: Official Journal of the International Society of Cerebral Blood Flow and Metabolism* 31 (2): 738–49.
Kannan, Sujatha, Fadoua Saadani-Makki, Otto Muzik, Pulak Chakraborty, Thomas J. Mangner, James Janisse, Roberto Romero, and Diane C. Chugani. 2007. "Microglial Activation in Perinatal Rabbit Brain Induced by Intrauterine Inflammation: Detection with 11C-(R)-PK11195 and Small-Animal PET." *Journal of Nuclear Medicine: Official Publication, Society of Nuclear Medicine* 48 (6): 946–54.
Kato, T. A., A. Monji, Y. Mizoguchi, S. Hashioka, H. Horikawa, Y. Seki, M. Kasai, H. Utsumi, and S. Kanba. 2011. "Anti-Inflammatory Properties of Antipsychotics Via Microglia Modulations: Are Antipsychotics a 'Fire Extinguisher' in the Brain of Schizophrenia?" *Mini-Reviews in Medicinal Chemistry*. https://doi.org/10.2174/138955711795906941.
Kelly, S., N. Jahanshad, A. Zalesky, P. Kochunov, I. Agartz, C. Alloza, O. A. Andreassen, et al. 2018. "Widespread White Matter Microstructural Differences in Schizophrenia across 4322 Individuals: Results from the ENIGMA Schizophrenia DTI Working Group." *Molecular Psychiatry* 23 (5): 1261–69.
Kentner, Amanda C., Staci D. Bilbo, Alan S. Brown, Elaine Y. Hsiao, A. Kimberley McAllister, Urs Meyer, Brad D. Pearce, Mikhail V. Pletnikov, Robert H. Yolken, and Melissa D. Bauman. 2019. "Maternal Immune Activation: Reporting Guidelines to Improve the Rigor, Reproducibility, and Transparency of the Model." *Neuropsychopharmacology: Official Publication of the American College of Neuropsychopharmacology* 44 (2): 245–58.
Keshavan, Matcheri S., Jay Giedd, Jennifer Y. F. Lau, David A. Lewis, and Tomáš Paus. 2014. "Changes in the Adolescent Brain and the Pathophysiology of Psychotic Disorders." *The Lancet. Psychiatry* 1 (7): 549–58.
Khwaja, O., and J. J. Volpe. 2008. "Pathogenesis of Cerebral White Matter Injury of Prematurity." *Archives of Disease in Childhood. Fetal and Neonatal Edition* 93 (2): F153–61.
Knuesel, Irene, Laurie Chicha, Markus Britschgi, Scott A. Schobel, Michael Bodmer, Jessica A. Hellings, Stephen Toovey, and Eric P. Prinssen. 2014. "Maternal Immune Activation and Abnormal Brain Development across CNS Disorders." *Nature Reviews. Neurology* 10 (11): 643–60.
Lebel, Catherine, and Sean Deoni. 2018. "The Development of Brain White Matter Microstructure." *NeuroImage* 182 (November): 207–18.
Lerch, Jason P., Lisa Gazdzinski, Jürgen Germann, John G. Sled, R. Mark Henkelman, and Brian J. Nieman. 2012. "Wanted Dead or Alive? The Tradeoff between in-Vivo versus Ex-Vivo MR Brain Imaging in the Mouse." *Frontiers in Neuroinformatics* 6 (March): 6.
Liemburg, Edith, Anita Sibeijn-Kuiper, Leonie Bais, Gerdina Pijnenborg, Henderikus Knegtering, Jorien van der Velde, Esther Opmeer, et al. 2016. "Prefrontal NAA and Glx

Levels in Different Stages of Psychotic Disorders: A 3T 1H-MRS Study." *Scientific Reports* 6 (February): 21873.

Lipitz, S., C. Hoffmann, B. Feldman, M. Tepperberg-Dikawa, E. Schiff, and B. Weisz. 2010. "Value of Prenatal Ultrasound and Magnetic Resonance Imaging in Assessment of Congenital Primary Cytomegalovirus Infection." *Ultrasound in Obstetrics & Gynecology: The Official Journal of the International Society of Ultrasound in Obstetrics and Gynecology* 36 (6): 709–17.

Li, Qi, Charlton Cheung, Ran Wei, Vinci Cheung, Edward S. Hui, Yuqi You, Priscilla Wong, Siew E. Chua, Grainne M. McAlonan, and Ed X. Wu. 2010. "Voxel-Based Analysis of Postnatal White Matter Microstructure in Mice Exposed to Immune Challenge in Early or Late Pregnancy." *NeuroImage* 52 (1): 1–8.

Li, Qi, Charlton Cheung, Ran Wei, Edward S. Hui, Joram Feldon, Urs Meyer, Sookja Chung, et al. 2009. "Prenatal Immune Challenge Is an Environmental Risk Factor for Brain and Behavior Change Relevant to Schizophrenia: Evidence from MRI in a Mouse Model." *PloS One* 4 (7): e6354.

Li, Q., Y. O. Leung, I. Zhou, L. C. Ho, W. Kong, P. Basil, R. Wei, et al. 2015. "Dietary Supplementation with N-3 Fatty Acids from Weaning Limits Brain Biochemistry and Behavioural Changes Elicited by Prenatal Exposure to Maternal Inflammation in the Mouse Model." *Translational Psychiatry* 5 (September): e641.

Malkova, Natalia V., Joseph J. Gallagher, Collin Z. Yu, Russell E. Jacobs, and Paul H. Patterson. 2014. "Manganese-Enhanced Magnetic Resonance Imaging Reveals Increased DOI-Induced Brain Activity in a Mouse Model of Schizophrenia." *Proceedings of the National Academy of Sciences of the United States of America* 111 (24): E2492–2500.

Masi, A., D. S. Quintana, N. Glozier, A. R. Lloyd, I. B. Hickie, and A. J. Guastella. 2015. "Cytokine Aberrations in Autism Spectrum Disorder: A Systematic Review and Meta-Analysis." *Molecular Psychiatry* 20 (4): 440–46.

Matcovitch-Natan, O., D. R. Winter, A. Giladi, S. Vargas Aguilar, A. Spinrad, S. Sarrazin, H. Ben-Yehuda, et al. 2016. "Microglia Development Follows a Stepwise Program to Regulate Brain Homeostasis." *Science*. https://doi.org/10.1126/science.aad8670.

McAuley, James B. 2014. "Congenital Toxoplasmosis." *Journal of the Pediatric Infectious Diseases Society* 3 Suppl 1 (September): S30–35.

Mengler, Luam, Artem Khmelinskii, Michael Diedenhofen, Chrystelle Po, Marius Staring, Boudewijn P. F. Lelieveldt, and Mathias Hoehn. 2014. "Brain Maturation of the Adolescent Rat Cortex and Striatum: Changes in Volume and Myelination." *NeuroImage* 84 (January): 35–44.

Meyer, Urs. 2014. "Prenatal poly(i:C) Exposure and Other Developmental Immune Activation Models in Rodent Systems." *Biological Psychiatry* 75 (4): 307–15.

Meyer, Urs, Joram Feldon, and Olaf Dammann. 2011. "Schizophrenia and Autism: Both Shared and Disorder-Specific Pathogenesis via Perinatal Inflammation?" *Pediatric Research* 69 (5 Pt 2): 26R – 33R.

Miller, Brian J., Peter Buckley, Wesley Seabolt, Andrew Mellor, and Brian Kirkpatrick. 2011. "Meta-Analysis of Cytokine Alterations in Schizophrenia: Clinical Status and Antipsychotic Effects." *Biological Psychiatry* 70 (7): 663–71.

Minakova, Elena, and Barbara B. Warner. 2018. "Maternal Immune Activation, Central Nervous System Development and Behavioral Phenotypes." *Birth Defects Research* 110 (20): 1539–50.

Molloy, Cynthia A., Ardythe L. Morrow, Jareen Meinzen-Derr, Kathleen Schleifer, Krista Dienger, Patricia Manning-Courtney, Mekibib Altaye, and Marsha Wills-Karp. 2006. "Elevated Cytokine Levels in Children with Autism Spectrum Disorder." *Journal of Neuroimmunology* 172 (1-2): 198–205.

Mortensen, Preben Bo, Bent Nørgaard-Pedersen, Berit Lindum Waltoft, Tina L. Sørensen, David Hougaard, E. Fuller Torrey, and Robert H. Yolken. 2007. "Toxoplasma Gondii as a Risk Factor for Early-Onset Schizophrenia: Analysis of Filter Paper Blood Samples Obtained at Birth." *Biological Psychiatry* 61 (5): 688–93.

Mortensen, Preben Bo, Bent Nørgaard-Pedersen, Berit L. Waltoft, Tina L. Sørensen, David Hougaard, and Robert H. Yolken. 2007. "Early Infections of Toxoplasma Gondii and the Later Development of Schizophrenia." *Schizophrenia Bulletin* 33 (3): 741–44.

Mortensen, Preben B., Carsten B. Pedersen, David M. Hougaard, Bent Nørgaard-Petersen, Ole Mors, Anders D. Børglum, and Robert H. Yolken. 2010. "A Danish National Birth Cohort Study of Maternal HSV-2 Antibodies as a Risk Factor for Schizophrenia in Their Offspring." *Schizophrenia Research* 122 (1-3): 257–63.

Nielsen, Philip R., Thomas M. Laursen, and Preben B. Mortensen. 2013. "Association between Parental Hospital-Treated Infection and the Risk of Schizophrenia in Adolescence and Early Adulthood." *Schizophrenia Bulletin* 39 (1): 230–37.

Ooi, Yasuhiro, Chizuko Inui-Yamamoto, Yoshichika Yoshioka, Akitoshi Seiyama, and Junji Seki. 2017. "11.7 T MR Imaging Revealed Dilatation of Virchow-Robin Spaces within Hippocampus in Maternally Lipopolysaccharide-Exposed Rats." *Magnetic Resonance in Medical Sciences: MRMS: An Official Journal of Japan Society of Magnetic Resonance in Medicine* 16 (1): 54–60.

Park, Min Tae M., Armin Raznahan, Philip Shaw, Nitin Gogtay, Jason P. Lerch, and M. Mallar Chakravarty. 2018. "Neuroanatomical Phenotypes in Mental Illness: Identifying Convergent and Divergent Cortical Phenotypes across Autism, ADHD and Schizophrenia." *Journal of Psychiatry & Neuroscience: JPN* 43 (3): 201–12.

Pedersen, Marianne Giørtz, Hanne Stevens, Carsten Bøcker Pedersen, Bent Nørgaard-Pedersen, and Preben Bo Mortensen. 2011. "Toxoplasma Infection and Later Development of Schizophrenia in Mothers." *The American Journal of Psychiatry* 168 (8): 814–21.

Pedroni, Silvia M. A., Juan M. Gonzalez, Jean Wade, Maurits A. Jansen, Andrea Serio, Ian Marshall, Ross J. Lennen, and Guillermina Girardi. 2014. "Complement Inhibition and Statins Prevent Fetal Brain Cortical Abnormalities in a Mouse Model of Preterm Birth." *Biochimica et Biophysica Acta* 1842 (1): 107–15.

Piontkewitz, Yael, Michal Arad, and Ina Weiner. 2011a. "Risperidone Administered during Asymptomatic Period of Adolescence Prevents the Emergence of Brain Structural Pathology and Behavioral Abnormalities in an Animal Model of Schizophrenia." *Schizophrenia Bulletin* 37 (6): 1257–69.

Piontkewitz, Yael, Michal Arad, and Ina Weiner. 2011b. "Abnormal Trajectories of Neurodevelopment and Behavior Following in Utero Insult in the Rat." *Biological Psychiatry* 70 (9): 842–51.

Piontkewitz, Yael, Yaniv Assaf, and Ina Weiner. 2009. "Clozapine Administration in Adolescence Prevents Postpubertal Emergence of Brain Structural Pathology in an Animal Model of Schizophrenia." *Biological Psychiatry* 66 (11): 1038–46.

Plitman, Eric, Shinichiro Nakajima, Camilo de la Fuente-Sandoval, Philip Gerretsen, M. Mallar Chakravarty, Jane Kobylianskii, Jun Ku Chung, et al. 2014. "Glutamate-Mediated

Excitotoxicity in Schizophrenia: A Review." *European Neuropsychopharmacology: The Journal of the European College of Neuropsychopharmacology* 24 (10): 1591–1605.
Potvin, Stéphane, Emmanuel Stip, Amir A. Sepehry, Alain Gendron, Ramatoulaye Bah, and Edouard Kouassi. 2008. "Inflammatory Cytokine Alterations in Schizophrenia: A Systematic Quantitative Review." *Biological Psychiatry* 63 (8): 801–8.
Prata, Joana, Susana G. Santos, Maria Inês Almeida, Rui Coelho, and Mário A. Barbosa. 2017. "Bridging Autism Spectrum Disorders and Schizophrenia through Inflammation and Biomarkers - Pre-Clinical and Clinical Investigations." *Journal of Neuroinflammation* 14 (1): 179.
Qiu, Lily R., Darren J. Fernandes, Kamila U. Szulc-Lerch, Jun Dazai, Brian J. Nieman, Daniel H. Turnbull, Jane A. Foster, Mark R. Palmert, and Jason P. Lerch. 2018. "Mouse MRI Shows Brain Areas Relatively Larger in Males Emerge before Those Larger in Females." *Nature Communications* 9 (1): 2615.
Rasmussen, Jerod M., Alice M. Graham, Sonja Entringer, John H. Gilmore, Martin Styner, Damien A. Fair, Pathik D. Wadhwa, and Claudia Buss. 2018. "Maternal Interleukin-6 Concentration during Pregnancy Is Associated with Variation in Frontolimbic White Matter and Cognitive Development in Early Life." *NeuroImage*, April. https://doi.org/10.1016/j.neuroimage.2018.04.020.
Raznahan, Armin, Phillip W. Shaw, Jason P. Lerch, Liv S. Clasen, Deanna Greenstein, Rebecca Berman, Jon Pipitone, Mallar M. Chakravarty, and Jay N. Giedd. 2014. "Longitudinal Four-Dimensional Mapping of Subcortical Anatomy in Human Development." *Proceedings of the National Academy of Sciences of the United States of America* 111 (4): 1592–97.
Reardon, P. K., Jakob Seidlitz, Simon Vandekar, Siyuan Liu, Raihaan Patel, Min Tae M. Park, Aaron Alexander-Bloch, et al. 2018. "Normative Brain Size Variation and Brain Shape Diversity in Humans." *Science* 360 (6394): 1222–27.
Reisinger, Sonali, Deeba Khan, Eryan Kong, Angelika Berger, Arnold Pollak, and Daniela D. Pollak. 2015. "The Poly(I:C)-Induced Maternal Immune Activation Model in Preclinical Neuropsychiatric Drug Discovery." *Pharmacology & Therapeutics* 149 (May): 213–26.
Ricci, S., R. Businaro, F. Ippoliti, V. R. Lo Vasco, F. Massoni, E. Onofri, G. M. Troili, et al. 2013. "Altered Cytokine and BDNF Levels in Autism Spectrum Disorder." *Neurotoxicity Research* 24 (4): 491–501.
Richetto, Juliet, Robert Chesters, Annamaria Cattaneo, Marie A. Labouesse, Ana Maria Carrillo Gutierrez, Tobias C. Wood, Alessia Luoni, Urs Meyer, Anthony Vernon, and Marco A. Riva. 2017. "Genome-Wide Transcriptional Profiling and Structural Magnetic Resonance Imaging in the Maternal Immune Activation Model of Neurodevelopmental Disorders." *Cerebral Cortex* 27 (6): 3397–3413.
Rudolph, Marc D., Alice M. Graham, Eric Feczko, Oscar Miranda-Dominguez, Jerod M. Rasmussen, Rahel Nardos, Sonja Entringer, Pathik D. Wadhwa, Claudia Buss, and Damien A. Fair. 2018. "Maternal IL-6 during Pregnancy Can Be Estimated from Newborn Brain Connectivity and Predicts Future Working Memory in Offspring." *Nature Neuroscience* 21 (5): 765–72.
Saadani-Makki, Fadoua, Sujatha Kannan, Malek Makki, Otto Muzik, James Janisse, Roberto Romero, and Diane Chugani. 2009. "Intrauterine Endotoxin Administration Leads to White Matter Diffusivity Changes in Newborn Rabbits." *Journal of Child Neurology* 24 (9): 1179–89.
Schumann, Cynthia M., Cinnamon S. Bloss, Cynthia Carter Barnes, Graham M. Wideman, Ruth

A. Carper, Natacha Akshoomoff, Karen Pierce, et al. 2010. “Longitudinal Magnetic Resonance Imaging Study of Cortical Development through Early Childhood in Autism.” *The Journal of Neuroscience: The Official Journal of the Society for Neuroscience* 30 (12): 4419–27.

Selemon, L. D., and N. Zecevic. 2015. “Schizophrenia: A Tale of Two Critical Periods for Prefrontal Cortical Development.” *Translational Psychiatry* 5 (August): e623.

Selten, Jean-Paul, Aleida Frissen, Gerty Lensvelt-Mulders, and Vera A. Morgan. 2010. “Schizophrenia and 1957 Pandemic of Influenza: Meta-Analysis.” *Schizophrenia Bulletin* 36 (2): 219–28.

Semple, Bridgette D., Klas Blomgren, Kayleen Gimlin, Donna M. Ferriero, and Linda J. Noble-Haeusslein. 2013. “Brain Development in Rodents and Humans: Identifying Benchmarks of Maturation and Vulnerability to Injury across Species.” *Progress in Neurobiology* 106-107 (July): 1–16.

Severance, E. G., J. Xiao, L. Jones-Brando, S. Sabunciyan, Y. Li, M. Pletnikov, E. Prandovszky, and R. Yolken. 2016. “Toxoplasma Gondii-A Gastrointestinal Pathogen Associated with Human Brain Diseases.” *International Review of Neurobiology* 131 (October): 143–63.

Sharabi, Hila, Nizar Khatib, Yuval Ginsberg, Zeev Weiner, Michael G. Ross, Blumenfeld-Katzir Tamar, Sasson Efrat, Hallak Mordechai, and Ron Beloosesky. 2018. “Therapeutic N-Acetyl-Cysteine (Nac) Following Initiation of Maternal Inflammation Attenuates Long-Term Offspring Cerebral Injury, as Evident in Magnetic Resonance Imaging (MRI).” *Neuroscience*, February. https://doi.org/10.1016/j.neuroscience.2018.01.013.

Shaw, Philip, Pietro De Rossi, Bethany Watson, Amy Wharton, Deanna Greenstein, Armin Raznahan, Wendy Sharp, Jason P. Lerch, and M. Mallar Chakravarty. 2014. “Mapping the Development of the Basal Ganglia in Children with Attention-Deficit/hyperactivity Disorder.” *Journal of the American Academy of Child and Adolescent Psychiatry* 53 (7): 780–89.e11.

Shaw, Philip, Noor J. Kabani, Jason P. Lerch, Kristen Eckstrand, Rhoshel Lenroot, Nitin Gogtay, Deanna Greenstein, et al. 2008. “Neurodevelopmental Trajectories of the Human Cerebral Cortex.” *The Journal of Neuroscience: The Official Journal of the Society for Neuroscience* 28 (14): 3586–94.

Shaw, Philip, Meaghan Malek, Bethany Watson, Deanna Greenstein, Pietro de Rossi, and Wendy Sharp. 2013. “Trajectories of Cerebral Cortical Development in Childhood and Adolescence and Adult Attention-Deficit/hyperactivity Disorder.” *Biological Psychiatry* 74 (8): 599–606.

Short, Sarah J., Gabriele R. Lubach, Alexander I. Karasin, Christopher W. Olsen, Martin Styner, Rebecca C. Knickmeyer, John H. Gilmore, and Christopher L. Coe. 2010. “Maternal Influenza Infection during Pregnancy Impacts Postnatal Brain Development in the Rhesus Monkey.” *Biological Psychiatry* 67 (10): 965–73.

Shoykhet, Mish, and Robert S. B. Clark. 2011. “Structure, Function, and Development of the Nervous System.” *Pediatric Critical Care*. https://doi.org/10.1016/b978-0-323-07307-3.10057-6.

Silveira, Vivian T. da, Daniel de Castro Medeiros, Jivago Ropke, Patricia A. Guidine, Gustavo H. Rezende, Marcio Flavio D. Moraes, Eduardo Mazoni A. M. Mendes, Danielle Macedo, Fabricio A. Moreira, and Antonio Carlos P. de Oliveira. 2017. “Effects of Early or Late Prenatal Immune Activation in Mice on Behavioral and Neuroanatomical Abnormalities Relevant to Schizophrenia in the Adulthood.” *International Journal of Developmental*

Neuroscience: The Official Journal of the International Society for Developmental Neuroscience 58 (May): 1–8.
Smolders, Silke, Tina Notter, Sophie M. T. Smolders, Jean-Michel Rigo, and Bert Brône. 2018. "Controversies and Prospects about Microglia in Maternal Immune Activation Models for Neurodevelopmental Disorders." *Brain, Behavior, and Immunity* 73 (October): 51–65.
Solek, Cynthia M., Nasr Farooqi, Myriam Verly, Tony K. Lim, and Edward S. Ruthazer. 2018. "Maternal Immune Activation in Neurodevelopmental Disorders." *Developmental Dynamics*. https://doi.org/10.1002/dvdy.24612.
Spann, Marisa N., Catherine Monk, Dustin Scheinost, and Bradley S. Peterson. 2018. "Maternal Immune Activation During the Third Trimester Is Associated with Neonatal Functional Connectivity of the Salience Network and Fetal to Toddler Behavior." *The Journal of Neuroscience: The Official Journal of the Society for Neuroscience* 38 (11): 2877–86.
Stone, William S., and Lisa Iguchi. 2011. "Do Apparent Overlaps between Schizophrenia and Autistic Spectrum Disorders Reflect Superficial Similarities or Etiological Commonalities?" *North American Journal of Medicine & Science* 4 (3): 124–33.
Turnbull, Daniel H., and Susumu Mori. 2007. "MRI in Mouse Developmental Biology." *NMR in Biomedicine* 20 (3): 265–74.
Vernon, Anthony C., Po-Wah So, David J. Lythgoe, Winfred Chege, Jonathan D. Cooper, Steven C. R. Williams, and Shitij Kapur. 2015. "Longitudinal in Vivo Maturational Changes of Metabolites in the Prefrontal Cortex of Rats Exposed to Polyinosinic-Polycytidylic Acid in Utero." *European Neuropsychopharmacology: The Journal of the European College of Neuropsychopharmacology* 25 (12): 2210–20.
Wang, Samuel S-H, Alexander D. Kloth, and Aleksandra Badura. 2014. "The Cerebellum, Sensitive Periods, and Autism." *Neuron* 83 (3): 518–32.
Weber-Stadlbauer, Ulrike, and Urs Meyer. 2019. "Challenges and Opportunities of a-Priori and a-Posteriori Variability in Maternal Immune Activation Models." *Current Opinion in Behavioral Sciences* 28 (August): 119–28.
Willette, Auriel A., Gabriele R. Lubach, Rebecca C. Knickmeyer, Sarah J. Short, Martin Styner, John H. Gilmore, and Christopher L. Coe. 2011. "Brain Enlargement and Increased Behavioral and Cytokine Reactivity in Infant Monkeys Following Acute Prenatal Endotoxemia." *Behavioural Brain Research* 219 (1): 108–15.
Woodward, Neil D., and Carissa J. Cascio. 2015. "Resting-State Functional Connectivity in Psychiatric Disorders." *JAMA Psychiatry* 72 (8): 743–44.
Wright, P., N. Takei, L. Rifkin, and R. M. Murray. 1995. "Maternal Influenza, Obstetric Complications, and Schizophrenia." *The American Journal of Psychiatry* 152 (12): 1714–20.
Wu, Dan, and Jiangyang Zhang. 2016. "Recent Progress in Magnetic Resonance Imaging of the Embryonic and Neonatal Mouse Brain." *Frontiers in Neuroanatomy* 10 (March): 18.
Wu, Wei-Li, Elaine Y. Hsiao, Zihao Yan, Sarkis K. Mazmanian, and Paul H. Patterson. 2017. "The Placental Interleukin-6 Signaling Controls Fetal Brain Development and Behavior." *Brain, Behavior, and Immunity* 62 (May): 11–23.
Yudofsky, Stuart C. 2009. "Contracting Schizophrenia." *JAMA: The Journal of the American Medical Association* 301 (3): 324–26.
Zhang, Jiangyang, Dan Wu, and Daniel H. Turnbull. 2018. "In Utero MRI of Mouse Embryos." *Methods in Molecular Biology* 1718: 285–96.
Zhang, Zhi, Amar Jyoti, Bindu Balakrishnan, Monica Williams, Sarabdeep Singh, Diane C.

Chugani, and Sujatha Kannan. 2018. “Trajectory of Inflammatory and Microglial Activation Markers in the Postnatal Rabbit Brain Following Intrauterine Endotoxin Exposure.” *Neurobiology of Disease* 111 (March): 153–62.

CHAPTER 4: Early or late gestational exposure to maternal immune activation alters neurodevelopmental trajectories in mice: an integrated neuroimaging, behavioural, and transcriptional study

4.1 Preface

The work presented in **Chapter 4** represents the first longitudinal characterization of mice prenatally exposed to MIA in two distinct gestational windows. The work was motivated by the systematic review presented in **Chapter 3**, wherein a dearth of longitudinal studies was identified (Guma et al. 2019). Longitudinal brain imaging has been a critical method for assessing normative trajectories of human brain development (Shaw et al. 2010; Raznahan et al. 2014). Additionally, it has allowed for the characterization of the timing and nature of deviations in developmental trajectories of individuals experiencing neurodevelopmental or neuropsychiatric disorders relative to those of healthy individuals (Shaw et al. 2010). In order to better understand how MIA-exposure *in utero* affects offspring development we undertook a longitudinal *in vivo* whole-brain imaging and behavioural study. In addition, a data-driven approach was used to identify regions of interest for post-mortem transcriptional profiling, based on shared covariation between brain anatomy and behaviour. This framework, seldomly implemented in the preclinical literature, stands to advance our understanding of when and how exposure to risk factors may induce deviations in development across multiple modalities, and should be applied to the study of other risk factors.

Additionally, the work presented here characterized the effects of MIA-exposure at two different gestational timepoints. Although debated, evidence from human epidemiological studies highlights exposure in the first two trimesters as conferring greater risk for offspring outcomes (Brown et al. 2004). Additionally, only a handful of animal studies have investigated the differential impact of MIA-exposure at different gestational timings. Those that have, do report interesting differences in behavioural outcomes, albeit cross-sectionally, which indicates that this may be an important modulatory factor. Here, early exposure to MIA was found to induce accelerated brain volume increases and the emergence of anxiety-like, stereotypic, and

sensorimotor gating deficits in adolescence/early-adulthood that normalized in later adulthood. In contrast, late exposure only resulted in subtle alterations to neuroanatomical trajectories. Leveraging the integrative methodology described above, key regions were identified in which transcriptional changes associated with fibroblast growth factor regulation, autistic behaviours, inflammatory pathways, and microRNA regulation were identified. Finally, although significant sex differences were not detected, this study included both male and female offspring, and statistically examined the potential for sex-dependent differences, which is an important consideration, often overlooked in preclinical studies (Coiro and Pollak 2019).

CHAPTER 4

Early or late gestational exposure to maternal immune activation alters neurodevelopmental trajectories in mice: an integrated neuroimaging, behavioural, and transcriptional study

Elisa Guma, MSc [1,2], Pedro do Couto Bordignon, MSc [3,5], Gabriel A. Devenyi PhD [2,4], Daniel Gallino, MSc [2], Chloe Anastassiadis, MSc [2,6], Vedrana Cvetkovska, PhD[5], Amadou Barry, PhD [3,11], Emily Snook, BSc [2,7], Jurgen Germann, PhD [2,8], Celia M.T. Greenwood, PhD [3,9,10,11], Bratislav Misic, PhD[12], Rosemary C. Bagot, PhD [3,5], M. Mallar Chakravarty, PhD [1,2,4,13]

[1] Integrated Program in Neuroscience, McGill University, Montreal, QC, Canada
[2] Computational Brain Imaging Lab, Cerebral Imaging Center, Douglas Mental Health University Institute, Montreal, QC, Canada
[3] Ludmer Center for Neuroinformatics and Mental Health, Montréal, QC, Canada
[4] Department of Psychiatry, McGill University, Montreal, QC, Canada
[5] Department of Psychology, McGill University, Montreal, QC, Canada
[6] Institute of Medical Science & Collaborative Program in Neuroscience, University of Toronto
[7] Faculty of Medicine, University of Toronto, Toronto, ON, Canada
[8] University Health Network, Toronto, Canada
[9] Lady Davis Institute for Medical Research, Jewish General Hospital, McGill University, Montreal, QC, Canada
[10] Department of Oncology, McGill University, Montreal, QC, Canada
[11] Departments of Human Genetics and Epidemiology, Biostatistics and Occupational Health, McGill University, Montreal, QC, Canada
[12] Montreal Neurological Institute, McGill University, Montreal, QC, Canada
[13] Department of Biological and Biomedical Engineering, McGill University, Montreal, QC, Canada

Published: https://doi.org/10.1016/j.biopsych.2021.03.017

4.2 Abstract

Background: Exposure to maternal immune activation (MIA) *in utero* is a risk factor for neurodevelopmental disorders later in life. The impact of gestational timing of MIA-exposure on downstream development remains unclear.

Methods: We characterized neurodevelopmental trajectories of mice exposed to viral mimetic, polyinosinic:polycytidylic acid (poly I:C), either on gestational day 9 (early) or 17 (late) using longitudinal structural magnetic resonance imaging from weaning to adulthood. Using multivariate methods, we related imaging and behavioural variables for the time of greatest alteration (adolescence/early adulthood) and identified regions for further investigation using RNA sequencing.

Results: Early MIA-exposure was associated with accelerated brain volume increases in adolescence/early-adulthood that normalized in later adulthood, in the striatum, hippocampus, and cingulate cortex. Similarly, alterations in anxiety-like, stereotypic, and sensorimotor gating behaviours observed in adolescence normalized in adulthood. MIA-exposure in late gestation had less impact on anatomical and behavioural profiles. Multivariate maps associated anxiety-like, social, and sensorimotor gating deficits with volume of the dorsal and ventral hippocampus, and anterior cingulate cortex, amongst others. The most transcriptional changes were observed in the dorsal hippocampus, with genes enriched for fibroblast growth factor regulation, autistic behaviours, inflammatory pathways, and microRNA regulation.

Conclusions: Leveraging an integrated hypothesis- and data-driven approach linking brain-behaviour alterations to the transcriptome, we find that MIA-timing differentially impacts offspring development. Exposure in late gestation leads to subthreshold deficits, whereas exposure in early gestation perturbs brain development mechanisms implicated in neurodevelopmental disorders.

4.3 Introduction

Prenatal brain development is a complex process orchestrated by interacting genetic, environmental, and immune factors. During this period offspring are highly vulnerable to a variety of risk factors for neurodevelopmental disorders that may only emerge later in childhood or adolescence (Knuesel et al. 2014; Thomason 2020; Selemon and Zecevic 2015). Epidemiological and preclinical evidence supports maternal infection as a risk factor for neurodevelopmental disorders such as autism spectrum disorder (ASD) and schizophrenia (Wormser and Tolan 2006; Knuesel et al. 2014; Estes and McAllister 2016; Brown and Meyer 2018).

The effects of maternal infection are most often attributed to the maternal immune activation (MIA) rather than the specific pathogen. Increases in maternal pro-inflammatory cytokines disrupt the delicate immune balance between maternal and fetal environments, altering developmental processes (Meyer, Feldon, and Yee 2009; Smith et al. 2007; Meyer et al. 2008). Studying MIA-exposure in animal models has been critical to establishing causality between MIA-exposure and downstream neurodevelopmental disruptions (Brown and Meyer 2018; Reisinger et al. 2015; Gumusoglu and Stevens 2019), demonstrating enduring behavioural, neuroanatomical, and transcriptional alterations relevant to ASD and schizophrenia (Boksa 2010; Guma, Plitman, and Chakravarty 2019; Gumusoglu and Stevens 2019; Haddad, Patel, and Schmid 2020; Kreitz et al. 2020; Piontkewitz, Arad, and Weiner 2011b).

Neurodevelopmental processes and maternal cytokine responsiveness vary across gestation. Thus, the gestational timing of MIA-exposure may influence the nature and severity of disruptions in offspring (Meyer et al. 2006; Dekel et al. 2010; Mor and Cardenas 2010; Guma, Plitman, and Chakravarty 2019). Although epidemiological studies suggest early gestation exposure confers greater risk for offspring (Brown et al. 2004; Atladóttir et al. 2010; Ellenbroek and Youn 2016; Cope, Powell, and Young 2016), there are variable reports regarding MIA-timing effects in both human and animal studies (Meyer et al. 2006; Meyer, Nyffeler, Yee, et al. 2008; Qi Li et al. 2009; Q. Li et al. 2015).

Here, we examine the impact of early (E; gestational day [GD] 9) or late (L; GD 17) prenatal MIA-exposure using the viral mimetic, polyinosinic:polycytidylic acid (poly I:C), on developmental trajectories in mice using longitudinal whole-brain magnetic resonance imaging (MRI) (Shaw, Gogtay, and Rapoport 2010; Qiu et al. 2018; Rapoport et al. 2005; Piontkewitz, Arad, and Weiner 2011b; Cannon et al. 2003; Pantelis et al. 2009) and multi-behavioural

characterization. We identify a deviation from normative trajectories in early-exposed offspring in the adolescent/early-adult period. We further examined brain-behaviour covariation, wherein greater anxiety-like, stereotypy, and sensorimotor gating impairments were associated with volumetric decreases in the hippocampus, cingulate cortex, striatum, thalamus, and cerebellum. Transcriptional profiling of this brain-behaviour pattern identified dysregulation of genes regulating fibroblast growth factor signaling, immune signaling and autistic behaviours in the dorsal hippocampus. Using a multi-modal approach, we construct a comprehensive understanding of how MIA-exposure, particularly in early gestation, shapes offspring neurodevelopment, and provide key insights into how MIA-exposure increases susceptibility for neurodevelopmental disorders.

4.4 Materials and methods

4.4.1. Animals

Timed-mating procedures were used to generate pregnant dams, who were injected intraperitoneally with either poly I:C (POL; P1530-25MG sodium salt TLR ligand tested; Sigma Aldrich) (5mg/kg) or vehicle (SAL; 0.9% sterile NaCl solution) at GD 9 (~the end of the human first trimester) or 17 (~the end of the human second trimester (37)) (4 groups: POL E, SAL E, POL L, SAL L; 5, 4, 6, 5 dams, respectively; see **supplement 4.8.1**). See **Figure 4.1** for experimental design. Immunostimulatory potential of poly I:C was confirmed in separate dams (see **supplement 4.8.6, 4.9.1 & supplementary table 4.S2**).

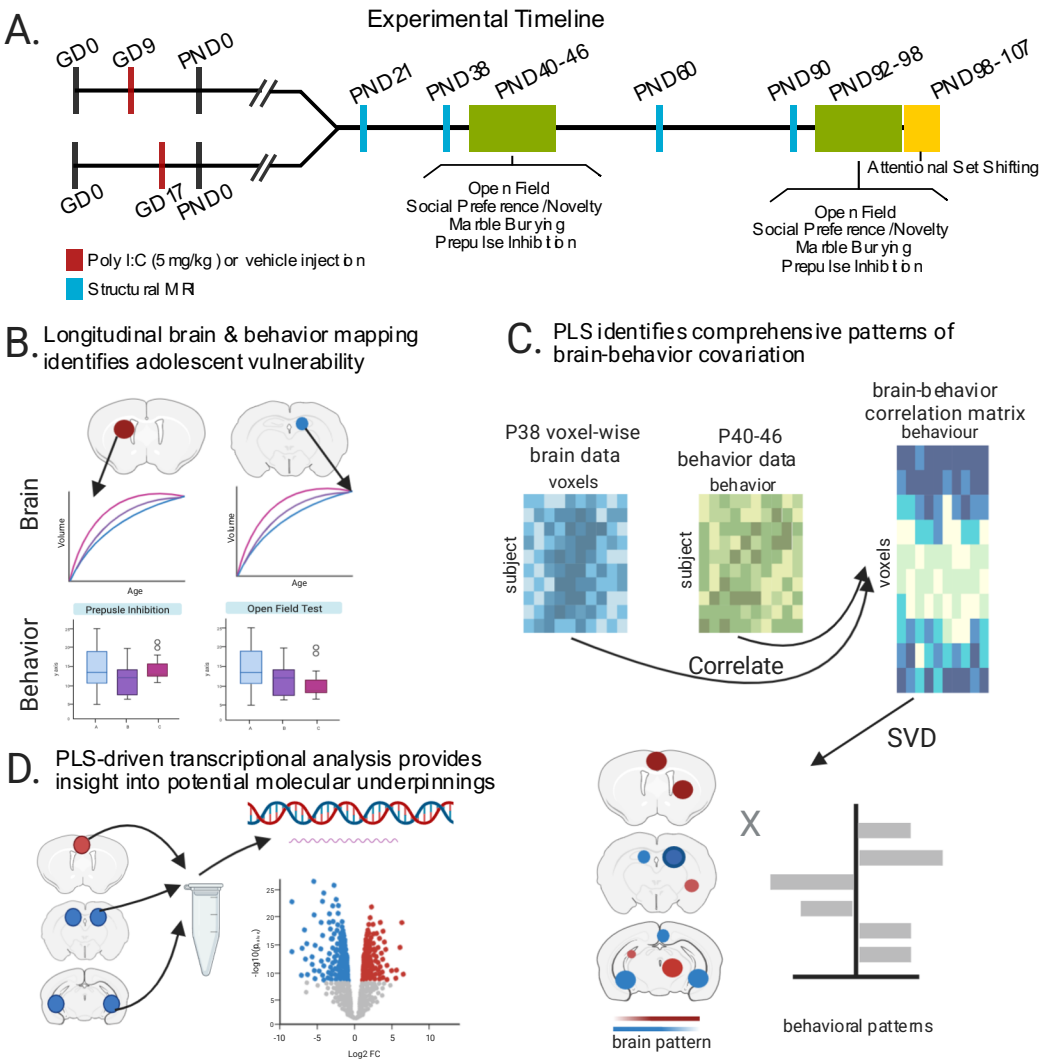


Figure 4.1. Experimental timeline. **A.** Pregnant dams were either injected (i.p.) with poly I:C (5m/kg) or vehicle (0.9% sterile NaCl solution) at gestational day (GD) 9 or 17 (red bars). Offspring were weaned and sexed on postnatal day (PND) 21. Longitudinal structural magnetic resonance imaging (MRI) was performed at PND 21, 38, 60, 90 (light blue bars). Two days following the PND38 (adolescence) and PND 90 (adulthood) scans, mice were assessed in open field test, social preference/novelty test (3 chambered social approach), marble burying task, and prepulse inhibition (green bars). The attentional set shifting task was also performed following the last adult behaviour (yellow bar). **B**. Univariate analyses were performed to assess group differences in neuroanatomy over time and behaviour for offspring exposed to MIA at GD 9, the early poly I:C (POL E) group, and offspring exposed at GD17, the late poly I:C group (POL L) group, relative to our combined vehicle exposed group, SAL (GD9 + 17). **C.** Partial least squares (PLS) was used to identify patterns of brain-behaviour covariation at the adolescent timepoint (PND38) where we observed our greatest group differences. This was used to identify regions of interest for RNA sequencing (**D**) used to probe potential molecular underpinnings of the observed changes. Figure made with BioRender https://biorender.com/.

4.4.2. Magnetic resonance imaging

Longitudinal T1-weighted (100 μm^3) manganese-enhanced (50 mg/kg (Lerch et al. 2012; Vousden et al. 2018)) structural MRIs were acquired *in vivo* at postnatal day (PND) 21 (~childhood), 38 (~adolescence), 60 (~young adulthood), and 90 (~adulthood) (Semple et al. 2013; Barbara Clancy et al. 2007) in offspring (7 Tesla Bruker Biospec 70/30; matrix size of 180 x 160 x 90; 14.5 minutes, 2 averages; 5% isoflurane for induction, 1.5% for maintenance) (Kong et al. 2018; Gallino et al. 2019; Rollins et al. 2019; Guma et al. 2018)**; see supplement 4.8.3**.

Images (n=376) were exported as DICOM, converted to MINC format, preprocessed, and visually inspected for quality control (QC; n=27 scans excluded). Deformation based morphometry (DBM) analysis was performed using the two-level Pydpiper toolkit (Friedel et al. 2014; van Eede et al. 2013) (**supplementary figure 4.S1**) to create subject-specific averages (first-level) that were registered to create a study average (Friedel et al. 2014) (second-level; sample size in **table 4.1**). Jacobian determinants (Chung et al. 2001) of the first-level deformation fields were resampled into final average space and blurred (0.2 mm Gaussian kernel) prior to statistical analyses (Friedel et al. 2014; van Eede et al. 2013); see **supplement 4.8.3.**

A voxel-wise linear mixed-effects model (R-3.5.1, RMINC-1.5.2.2, lme4 1.1-21) was used to examine a group-by-age (age as a third order natural spline) interaction covarying for sex (subject and litter as random intercepts; details in **supplement 4.8.7.1, 4.8.7.2, 4.9.2**, & **supplementary figure 4.S3** (Szulc et al. 2015; Qiu et al. 2018)) and corrected for False Discovery Rate (FDR) (Benjamini and Hochberg 1995). SAL E and L offspring were merged (SAL) as they were not significantly different (**supplement 4.8.7.2**). Sex-by-group-by-age interaction was investigated post-hoc (**supplement 4.9.3**).

Table 4.1. Sample per timepoint following quality control. Postnatal day (PND); poly I:C (POL); saline (SAL); late (L; gestational day 17 exposure); early (E; gestational day 9 exposure); male (M); female (F).

	PND 21 scan	**PND 38 scan**	**PND 60 scan**	**PND 90 scan**
SAL E + L (9 litters)	37 (19M/18F)	38 (20M/18F)	41 (21M/20F)	40 (20M/20F)
POL E (5 litters)	21 (8M/13F)	21 (9M/12F)	20 (8M/12F)	20 (8M/12F)
POL L (6 litters)	27 (12M/15F)	29 (13M/16F)	27 (14M/13F)	28 (14M/14F)

4.4.3. Behavioural testing

Following adolescent (PND 38) and adult (PND 90) scans anxiety-like and exploratory behaviour (**open field test**), social behaviour (**three chambered social approach**), stereotypic/repetitive behaviour (**marble burying task** (Deacon 2006)), and sensorimotor gating (**prepulse inhibition**) were assessed with 2 days between tests. Videos were analyzed using Ethovision XT12 (Noldus, Leesburg, VA, USA). A cognitive flexibility and reversal learning measure (**attentional set shifting task [ASST]** (Colacicco et al. 2002)) was performed following behaviours to evaluate enduring effects of MIA-exposure (Meyer et al. 2006). Detailed description for each test available in **supplement 4.8.4**.

Group differences (covarying for sex; litter as a random effect) were evaluated on adolescent and adult data using linear mixed-effects models. A Bonferroni correction was applied (5 adolescent, 6 adulthood tests: $\alpha = 0.05/11 = 0.0045$, uncorrected p-values, and corrected q-values reported). Sex differences were investigated post-hoc (**supplement 4.9.7.2)**.

4.4.4 Partial Least Squares Analysis

Partial Least Squares (PLS) is a multivariate analysis which allows us to find the optimal weighted linear combination of two variables (whole-brain voxel-wise DBM and 21 z-scored behavioural metrics from PND38) that maximally covary together (Zeighami et al. 2019; McIntosh

and Mišić 2013; McIntosh and Lobaugh 2004). This yields a set of orthogonal latent variables (LV) comprising 'brain weights' and 'behaviour weights' describing how each voxel or behavioural variable weighs onto a given LV, and a singular value, describing the proportion of covariance explained by the LV (Eckart and Young 1936). Permutation testing (n=1000) and bootstrap resampling (n=1000) were used to assess statistical significance, and contribution of original variables to each LV (details in **supplementary materials 4.8.7.3**).

4.4.5 Transcriptional analysis

Transcriptomic profiles were assessed in a separate cohort of mice (PND 38; POL E n=6M/6F, 5 litters, SAL E n=6M/6F, 6 litters) in the ACC (bregma +0.14mm), dHIP (bregma -2.80mm), and vHIP (bregma -3.08mm) using next-generation RNA sequencing. **Differential expression** (DE) between POL E and SAL E offspring at each ROI (calculated with Bioconductor's limma package (Version 3.40.6 (Ritchie et al. 2015)) was assessed using linear mixed-effects models (covarying for sex; FDR corrected (Benjamini and Hochberg 1995)). **Pathway enrichment analysis** was performed using g:Profiler (Reimand et al. 2019, 2007) to identify significantly enriched pathways or overrepresented genes from our gene lists ranked by the -log10P value relative to a background gene list for each ROI (and for pooled ROIs in **supplement 2.9.1**). The **rank rank hypergeometric overlap** test (RRHO) was used to measure concordance of genome wide DE patterns in pairs of ROIs between POL E and SAL E (Plaisier et al. 2010); a pathway enrichment analysis was performed on overlap lists. Finally, a **gene overlap analysis** was performed to compare our dHIP and vHIP DEGs to those identified by human post-mortem studies in the HIP of individuals with schizophrenia (Lanz et al. 2019). Similarly, DEGs across ROIs were compared to schizophrenia and ASD pancortical DEGs (Gandal et al. 2018). Further details in **supplement 4.8.8. & supplementary figure 4.S2.** Sex differences reported in **supplement 4.8.8.2 & 4.9.9**.

4.5 Results

4.5.1 Early and late gestational MIA-exposure differentially alters neurodevelopmental trajectory

We observed the most significant deviations in developmental trajectories in POL E offspring relative to SAL (second order natural spline of age term, t=3.835, <1% FDR); they had smaller brain volumes at PND 21, which overshot between PND 38 and PND 60, and normalized at PND 90. Many of these regions are implicated in neuro-psychiatric and -developmental disorders such as the hippocampus, cingulate cortex, striatum, as well as the subiculum, nucleus accumbens, septal nucleus, periaqueductal gray, and cerebellar vermis/crus I (van Erp et al. 2016; Lieberman et al. 2018; Nicolson et al. 2006; Bedford et al. 2019; Brisch et al. 2011) **(Figure 4.2; supplementary figure 4.S4 & supplementary table 4.S3)**.

POL L had a flatter developmental trajectory relative to SAL (third order natural spline of age term, t=5.286, <1%FDR), in the nucleus accumbens, auditory cortex, reticular nucleus, subiculum and hypothalamus, whereas amygdala volume decreased in later adulthood (**Figure 4.2 & supplementary figure 4.S4**). POL E trajectories were significantly different from POL L, confirming that early MIA-exposure had the largest effect on neuroanatomy (**supplement 4.9.5, supplementary figures 4.S9 & 4.S10).** We also observed a significant increase in volume of cortical regions in POL E offspring particularly in the early adult period (first order natural spline of age fit); significant effects for all age fits described in **supplement 4.9.3, 4.9.4, & supplementary figures 4.S5-4.S8**. We explored sex differences as a post-hoc analysis; POL E (vs POL L) males were more affected than females in a number of regions, but none were observed relative to SAL (**supplement 4.9.6, & supplementary figure 4.S11**).

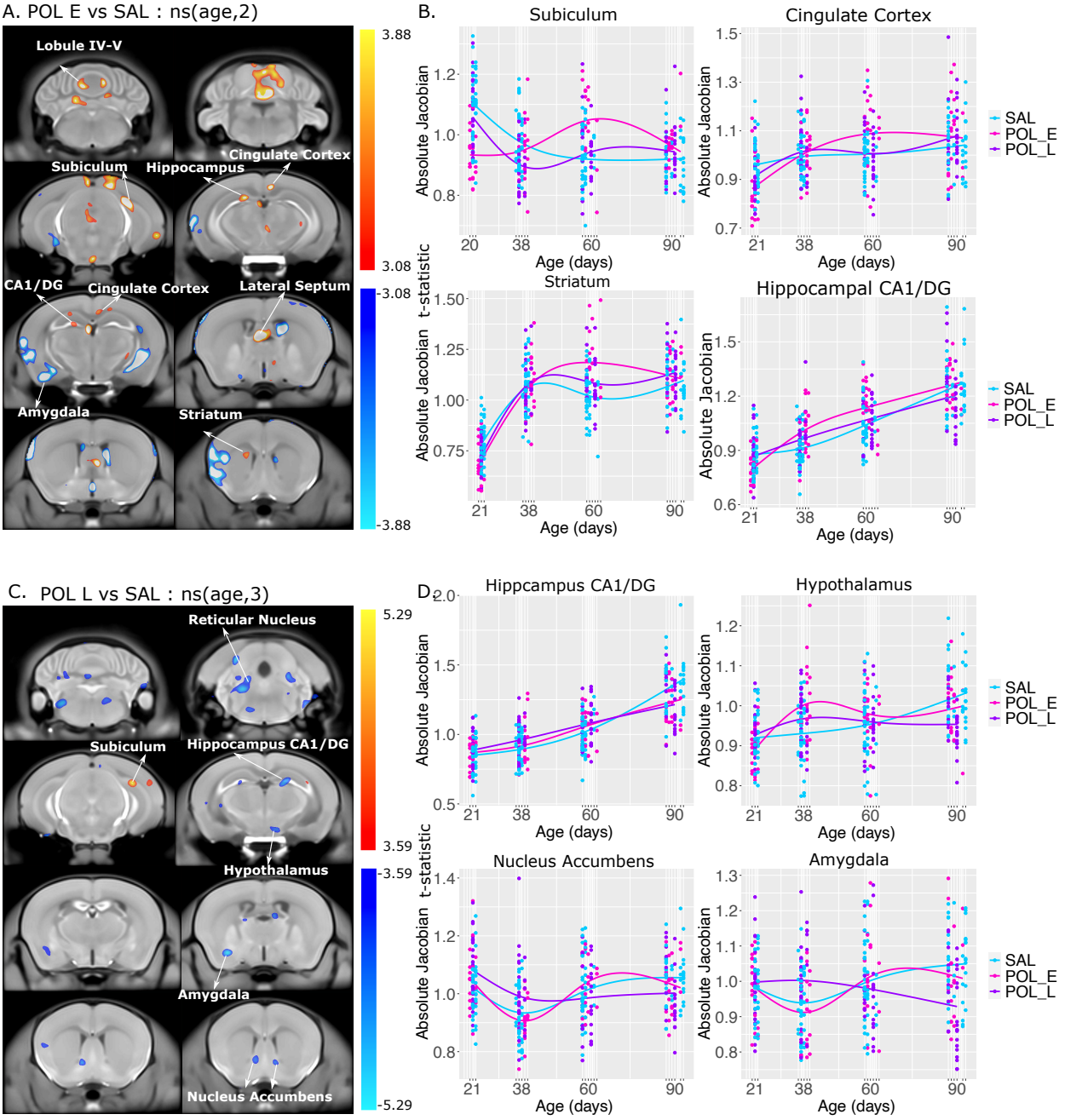


Figure 4.2 (caption continued on next page). Developmental trajectories differ between early poly I:C group (POL E) vs saline controls (SAL) & the late poly I:C group (POL L) vs SAL (thresholded at 5% False discovery rate (FDR)). **A**. t-statistic map of group (POL E vs SAL) by age (second order natural spline) thresholded between 5% FDR (bottom, t=3.08) and 1% FDR (top, t=3.83) overlaid on the population (second-level) average. **B**. Plot of peak voxels (voxel within a region of volume change showing largest effect) selected from regions of interest highlighted (**A**), wherein age is plotted on the x-axis, and the absolute Jacobian determinants plotted on the y-axis. Here a value of 1 means the voxel is no different than the average, anything above 1 is relatively

Figure 4.2 (continued) larger, and below 1 is relatively smaller. Ranges are not normalized to enhance comparison at each specific location in space. Trajectories are modeled as third order natural splines to reflect statistical modeling. **C**. t-statistic map of group (POL L vs SAL) by age (third order natural spline) thresholded between 5% FDR (bottom, t=3.59) and 1% FDR (top, t=5.29). **D**. Plots of peak voxels as described in (**B**) with curves modeled as third order natural splines to reflect statistics.

4.5.2 Early MIA-exposure induces behavioural alterations in adolescence

In adolescence, compared to SAL, POL E offspring traveled less in the anxiogenic center zone of the open field relative to total distance of the arena, however this effect did not survive multiple comparisons correction (t=-2.294, p=0.039, q=0.429; **Figure 4.3A**). They buried more marbles suggesting greater stereotypy/anxiety (t=2.937, p=0.003, q=0.033; **Figure 4.3B**). A striking impairment in sensorimotor gating was observed (t=-4.202, $p=4.0\times10^{-7}$, $q=4.0\times10^{-6}$; **Figure 4.3C**), sustained across prepulse tones (i.e., no group-by-prepulse level interaction: t=-0.995, p=0.321; **Figure 4.3D**). Surprisingly, adolescent offspring showed no social impairments **Figure 4.3E**). Behavioural alterations were no longer present in adult POL E offspring apart from a subthreshold impairment in social novelty behaviour (t=-2.369, p=0.0311, q=0.341; **Figure 4.3F**), wherein POL E mice preferred the familiar to the novel social target.

Adolescent POL L offspring did not show any significant behavioural deficits. Some subtle alterations emerged in adulthood, such as in ASST, but did not survive Bonferroni correction. Details in **supplement 4.9.7, supplementary table 4.S4 & supplementary figure 4.S12;** see **supplement 4.9.7.2 & supplementary figure 4.S13** for sex-differences).

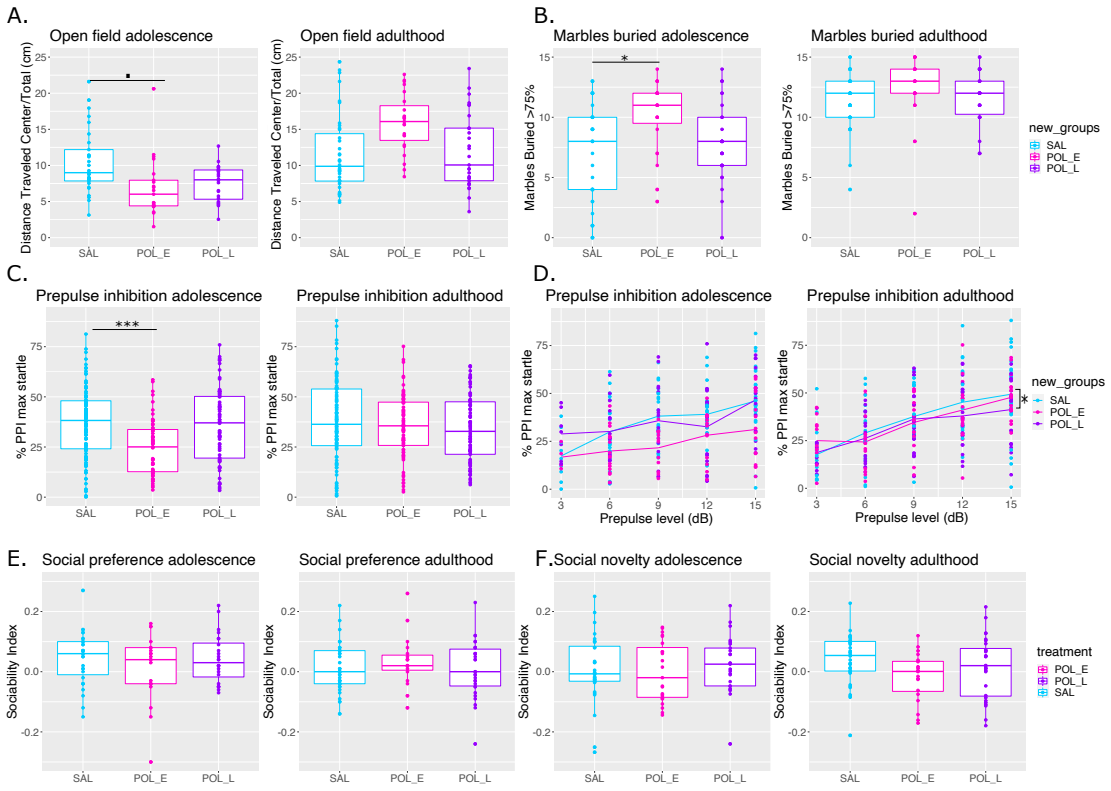


Figure 4.3. Early MIA-exposure induces transient behavioural impairments whereas late MIA-exposure does not affect behaviour. Behavioural results for adolescent (left) and adult (right) offspring from the three treatment groups: SAL (cyan), POL E (magenta), POL L (purple). For all boxplots the midline represents the median of the data, the box represents the interquartile range, with whiskers denoting the full range of the data. **A.** In adolescence (left), POL E offspring travel less in the center zone (relative to the total distance traveled) at a subthreshold level (t=-2.294, p=0.039 not significant following Bonferroni correction). No statistically significant differences observed in adulthood (right). **B**. Significantly more marbles are buried by POL E adolescent offspring (left; t=2.937, p=0.003) than SAL offspring. No group differences were observed in adulthood (right). **C**. POL E offspring show a significant decrease in % prepulse inhibition based on maximum amplitude of startle reaction to startle tone (left; t=-4.202, 4.0×10^{-7}). This deficit is no longer present in adulthood (right). **D**. %PPI is plotted for each group with increasing prepulse tone on the x-axis and %PPI based on maximum amplitude of startle reaction on the y-axis for adolescence (left) and adulthood (right). No significant differences in slopes are observed between groups, however the POL E offspring are impaired at all levels in adolescence. **E**. No significant differences in sociability index for the social preference task (i.e., preference for novel mouse over non-social object) between groups at either adolescence (left) or adulthood (right). **F**. No significant differences in sociability index for social novelty (i.e., preference for novel mouse over familiar mouse) between groups. A subthreshold impairment was observed in POL E adult offspring (t=-2.369, p=0.031). ˙p<0.05; *p<0.0045 (Bonferroni correction threshold); ***p<0.0001

4.5.3. Multivariate analysis of brain-behaviour data links variation in autism- and schizophrenia-related behaviours to volume changes in key brain regions

Based on univariate analyses we identified the adolescent/early adult period as one of greatest deviation from "normative" trajectory (particularly for POL E offspring). Using PLS to examine adolescent whole-brain anatomical alterations and 21 behavioural metrics across 4 tests (PND 38; **Figure 4.1**) we identified two significant LVs; LV1 described a pattern of brain-behaviour covariation (29% covariance explained, p=0.034), whereas LV2 described a brain pattern associated with sex and litter size (19% covariance explained, p=0.002) (**Figure 4.4A**; **supplement 4.9.8 & supplementary figure 4.S14)**. We chose to focus on LV1 where we observed a pattern of attenuated behavioural impairment, i.e., decreased locomotion and anxiety-like behaviour, more social interactions, and less impairment in sensorimotor gating associated with larger volume of the ACC, somatomotor cortex, and striatum. A pattern of greater behavioural impairment, including increased anxiety-like behaviour and locomotion, fewer social interactions, and impaired sensorimotor gating was associated with smaller volume in the dorsal and ventral hippocampus, thalamic nuclei, and cerebellum (vermis, crus I and II) **(Figure 4.4BC**).

Correlations between brain-behaviour weights suggests that POL E offspring load more strongly on this pattern than POL L or SAL, albeit not for all subjects (**Figure 4.4D; supplementary figure 4.S15** shows brain-behaviour correlation plot after outlier removal). Finally, to determine how this pattern changes in adulthood, we applied the adolescent brain-behaviour weights for LV1 to adult brain and behaviour data. We observed a shift along the brain axis but not the behaviour axis, suggesting that, as mice age, changes in brain patterns are disconnected from changes in behavioural patterns (**supplementary figure 4.S16**).

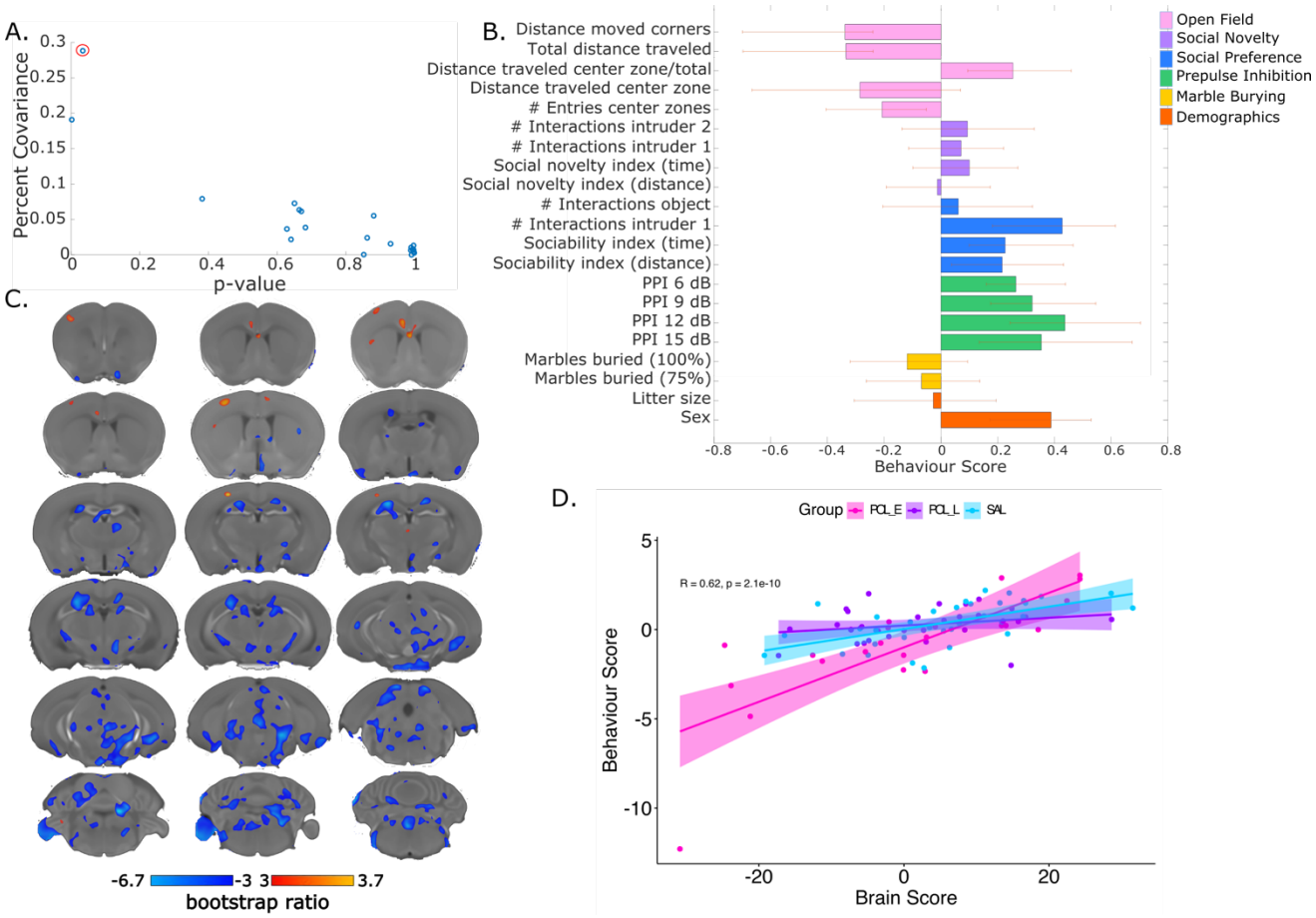


Figure 4.4. Partial least squares (PLS) analysis results for first latent variable (LV1). **A**. Covariance explained (y-axis) and permutation p-values (x-axis) for all 21 LVs in the PLS analysis. LV1 is circled in red (p=0.034, %covariance=29%) and was chosen for subsequent investigation based on the covariance explained and behavioural relevance of results. **B**. Behaviour weight for each behavioural measure included in the analysis showing how much they contribute to the pattern of LV1. Singular value decomposition estimates the size of the bars whereas confidence intervals are estimated by bootstrapping. Bars with error bars that cross the 0 line should not be considered. **C**. Brain loading bootstrap ratios for the LV1 deformation pattern overlaid on the population average, with positive bootstrap ratios in orange-yellow (indicative or larger volume), and negative in blue (indicative of smaller volume). Colored voxels make significant contributions to LV1. **D**. Correlation of individual mouse brain and behaviour score, color coded by treatment group with a trend line per group. One outlier on the behaviour score circled in dark gray. Early poly I:C (POL E) offspring (magenta) express this pattern more strongly than the saline controls (SAL) and late poly I:C (POL L) groups.

4.5.4. Early MIA-exposure induces transcriptional changes in adolescence

Informed by our brain-behaviour maps and knowledge of schizophrenia- and ASD-related pathology (Li et al. 2019; Nicolson et al. 2006; Cheung et al. 2010; Radulescu et al. 2014; Fujiwara et al. 2007; Calabrese et al. 2008; Simms et al. 2009; Schumann et al. 2010), we assessed transcriptional alterations in the ACC, dHIP, and vHIP (**Figure 4.1)**. We observed many differentially expressed genes (DEGs; $p<0.05$) comparing POL E to SAL E in the dHIP (246 down- and 131 upregulated, $q<0.05$), with subtle differences in the vHIP (37 down-, 12 upregulated, $q<0.05$) and ACC (17 down-, 0 upregulated, $q<0.05$) (**Figure 4.5A-C**). Several genes were significantly downregulated across all three ROIs, including *Nfkbia*, a key driver of the pro-inflammatory immune response (Starace et al. 2008), and *Klf2*, *Ddit4*, and *Per1* (**Figure 4.5DE).**

Pathway enrichment analysis of upregulated dHIP genes identified enrichment of fibroblast growth factor signaling (FGF 1, 2, 3), an evolutionarily controlled signaling pathway necessary for embryogenesis and synaptogenesis (Balasubramanian and Zhang 2016). Autistic behaviour and regulation of microRNAs (miR-466i-3p, miR-362-3p, miR-329-3p) were also enriched. Downregulated dHIP genes were enriched for apoptosis, a critical neurodevelopmental process shown to be disrupted in MIA-exposed offspring (Roth and D'Sa 2001; Bergdolt and Dunaevsky 2019) and microRNAs (miR-3097-3p, miR-499-5p, miR-717). Enrichment of NF-kappa-B inhibitor alpha signaling and erythrocyte differentiation, both implicated in the immune system, was also observed (Morera and MacKenzie 2011; Ferreiro and Komives 2010) **(Figure 4.5E; supplementary table 4.S5)**.

In the vHIP, downregulated genes were enriched for IL-17 signaling pathway, necessary to induce ASD-like phenotype in MIA-exposed mice (Choi et al. 2016), apoptosis, NF-kappa-B inhibitor alpha signaling, chronic myeloid leukemia and small cell lung cancer. ACC upregulated DEGs were enriched for white fat cell differentiation and pro-inflammatory IL-1 signaling (Gabay, Lamacchia, and Palmer 2010). Results for pooled ROI are described in **supplement 4.9.9.1.** No striking sex differences were observed (**supplement 4.9.9.2**).

We compared identified DEGs to transcripts previously identified in post-mortem human ASD (prefrontal and temporal cortex (Gandal et al. 2018)) and schizophrenia samples (pan-cortical (Gandal et al. 2018), prefrontal cortex and hippocampus (Lanz et al. 2019)). Although disease gene lists were not significantly enriched in our mice, *FGF2*, *Tbx4*, and *Ccdc92* were upregulated in both the dHIP of our mice, and the hippocampus of individuals with schizophrenia (Lanz et al.

2019). Further, there was some overlap in pooled downregulated genes in the pan-cortical schizophrenia sample, including *Nfrkb* (related to *Nfkbia* which was downregulated in all 3 ROIs), amongst others listed in **supplementary table 4.S6**.

RRHO identified transcriptional synchrony between the dHIP, vHIP, and ACC following prenatal MIA-exposure. We observed overlap between all pairwise comparisons, with the strongest overlap in dHIP and vHIP downregulated genes (4932) **(supplementary figure 4.S17A**). Overlapping upregulated genes across ROIs were enriched for myelin associated processes, oxidative phosphorylation, and mitochondrial function. Overlapping downregulated genes across all ROIs were enriched for RNA processing and transcriptional regulation (see **supplement 4.9.9.2** & **supplementary figure 4.A17B** for sex differences**)**.

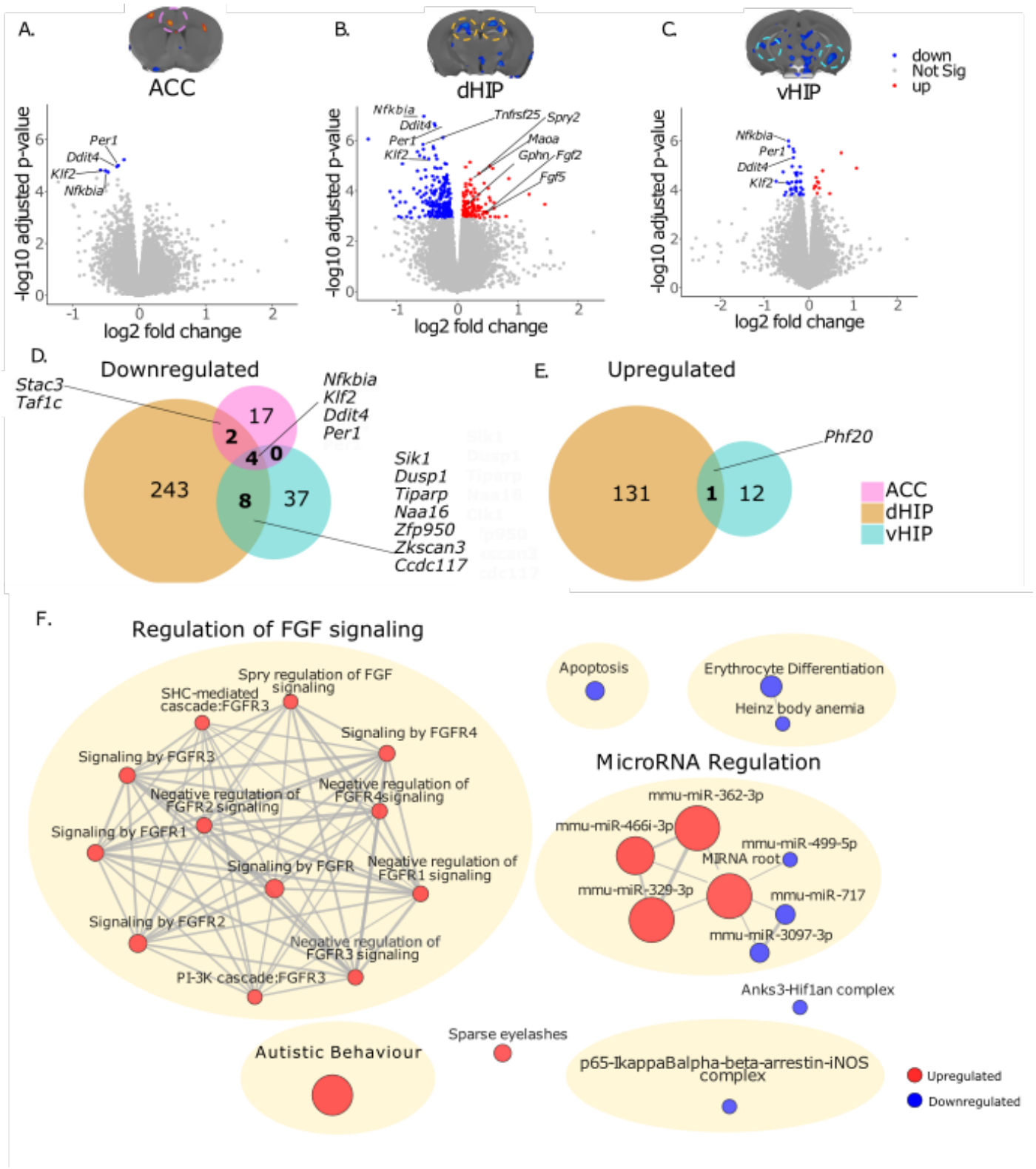


Figure 4.5 (caption on next page).

Figure 4.5 (continued). Transcriptional alteration in the adolescent brain at PND38 following MIA-exposure at GD9. Volcano plots for the ACC (**A**), dHIP (**B**), and vHIP (**C**) with significantly downregulated genes in blue, and upregulated genes in red. Genes that are either down- or upregulated in multiple ROIs (as shown in **D & E**) are highlighted in the volcano plots (on the left side). For the dHIP volcano plot (**B**) gene names on the right-hand side are enriched for FGF signaling, and also identified in human postmortem hippocampal samples from individuals with schizophrenia. Venn diagram showing overlap in downregulated (**D**) and upregulated (**E**) genes per ROI. **F**. Gene enrichment analysis results for the dHIP, with upregulated enrichment in red and downregulated in blue. Upregulated genes were significantly enriched for FGF signaling, as well as autistic behaviours, sparse eyelashes, and microRNA regulation. MicroRNA regulation was also enriched for downregulated genes, as were apoptosis, erythrocyte differentiation, heinz body anemia, and p65-IKβKα-β-arrestin-iNOS-complex.

4.6 Discussion

In this study, we present whole-brain developmental trajectories (using longitudinal neuroimaging) and multi-behavioural phenotyping of mice prenatally exposed to early or late MIA from childhood to adulthood. Integrating data from the period of greatest vulnerability (adolescence) we characterize patterns of brain-behaviour covariation. We investigated transcriptional alterations in key regions (ACC, dHIP, vHIP) in the most affected group (POL E) to characterize the molecular underpinnings of the MIA-induced brain-behaviour patterns. Our findings suggest that prenatal MIA-exposure late in gestation leads to subthreshold deficits, while exposure early in gestation opens a window of vulnerability in adolescence/early adulthood characterized by an accelerated increase in brain volume and emergence of sensorimotor gating and stereotypy/anxiety-like deficits, which normalize in adulthood. These deviations may be driven by transcriptional differences in genes involved in FGF signaling, autistic behaviours, inflammatory pathways, and microRNA regulation particularly in the adolescent dHIP.

4.6.1 The case for longitudinal investigation

Longitudinal neuroimaging approaches are ideal for characterization of brain development and have demonstrated the emergence of neurodevelopmental alterations in disorders such as schizophrenia or ASD prior to disease onset (Piontkewitz, Arad, and Weiner 2012; Wolff, Jacob,

and Elison 2018). Longitudinal studies are sensitive and characterize interindividual differences (Mills and Tamnes 2014), thereby requiring fewer participants than cross-sectional studies to detect subtle differences in brain structure (Gur, Keshavan, and Lawrie 2007; Nieman et al. 2007; Lerch et al. 2012; Mills and Tamnes 2014). Previous studies from our group have demonstrated the utility of a longitudinal design to study ageing, genotype associations, and treatments (Gallino et al. 2019; Rollins et al. 2019; Guma et al. 2018, 2019; Kong et al. 2018), while others provide important insight into early brain development (Szulc et al. 2015) or sex differences (Qiu et al. 2018). Using our longitudinal approach, we identified transient deviations in development due to MIA-exposure which may have been missed by focusing on cross-sectional differences (Guma, Plitman, and Chakravarty 2019).

4.6.2 Early MIA-exposure is associated with greater deviations in neurodevelopmental trajectories

MIA-exposure affected several regions implicated in ASD or schizophrenia including the striatum and hippocampus, and ACC (Lieberman et al. 2018; Varghese et al. 2017; van Erp et al. 2016; Nicolson et al. 2006), as well as the amygdala (Baron-Cohen et al. 2000; van Erp et al. 2016), periaqueductal gray (Koropouli et al. 2020; George, Ameli, and Koob 2019; Freeman et al. 2018) somatosensory cortices (Shin Yim et al. 2017; Balasco, Provenzano, and Bozzi 2019; Teale et al. 2013) and septal nuclei (Butler et al. 2012; Wegiel et al. 2014). The overshoot in adolescence/early-adulthood is reminiscent of the brain overgrowth observed in ASD (Hazlett et al. 2011; Schumann et al. 2010; Donovan and Basson 2017; Bedford et al. 2019). Conversely, the altered hippocampal and ACC morphology is analogous to schizophrenia (Calabrese et al. 2008; Simms et al. 2009). Previous MRI-based studies examining GD9 MIA-exposure have identified microstructural alterations in similar regions such as the ACC, hippocampus, lateral septum, and ventral striatum, albeit later in the lifespan (Qi Li et al. 2009; Li et al. 2015). POL E offspring exhibited behavioural impairments relevant to ASD and schizophrenia, namely sensorimotor gating, stereotypy/repetitive behaviours, and subthreshold anxiety-like behaviours, consistent with previous reports (Meyer et al. 2006). In contrast, we observed transient impairments in adolescence that were resolved in adulthood, whereas others have reported deficits in adulthood (Meyer et al. 2006; Piontkewitz, Arad, and Weiner 2011b).

Exposure late in gestation led to subtler alterations in brain morphology and did not associate with behavioural impairments. Interestingly, the shape of developmental curves was flatter, with regions involved in emotional processing and reward, such as the amygdala and nucleus accumbens showing decreases in adulthood. Parallels may be drawn between this trajectory shape and those observed in MRI studies of schizophrenia and rat MIA-offspring (Piontkewitz, Arad, and Weiner 2011b; Crum et al. 2017). For example, the amygdala has been implicated in previous MIA-studies in humans showing altered volume and connectivity (Graham et al. 2018), as well as in neuropsychiatric disorders (Schumann, Bauman, and Amaral 2011). Previous cross-sectional MRI studies examining the impact of GD17 MIA-exposure in adult offspring report white matter and cerebellar volume decreases and enlarged 4th ventricles (Richetto et al. 2017; Qi Li et al. 2009).

We did not observe any adolescent or adult behavioural deficits in the GD17 exposed offspring. Perhaps GD17-exposure results in subtle alterations below our detection threshold, or in behavioural domains which we did not test. Memory and cognitive deficits have been observed in GD17 MIA-exposed adult offspring (Meyer, Nyffeler, Yee, et al. 2008; Meyer et al. 2006; Bitanihirwe et al. 2010); we observed subthreshold deficits in cognitive performance. Importantly, previous work comparing the effects of GD9 and 17 MIA-exposure also observed diverging effects on offspring development (Meyer et al. 2006; Meyer, Nyffeler, Schwendener, et al. 2008; Li et al. 2009). Taken together, these findings indicate that MIA-induced increases in maternal cytokine levels early in gestation may have more profound effects on offspring development (Giovanoli et al. 2013; Gumusoglu and Stevens 2019; Conway and Brown 2019; Estes and McAllister 2016). Recent work suggests that MIA-exposure may result in subgroups with dissociable behavioural, transcriptional, and immunological profiles, which may further explain heterogeneity in MIA-findings (Mueller et al. 2021).

To our knowledge, there are no longitudinal neuroimaging or behavioural studies to date investigating GD 9 or GD 17 MIA-exposure, nor any cross-sectional investigations in adolescence/early-adulthood (Guma, Plitman, and Chakravarty 2019; Gumusoglu and Stevens 2019). Neuroimaging studies of MIA-exposed nonhuman primates and rabbits in early childhood/adolescence report altered brain development, in line with our findings (Short et al. 2010; Willette et al. 2011; Bauman et al. 2014; Zhang et al. 2018). Some longitudinal neuroimaging studies conducted in GD 15-exposed rats report enlarged lateral ventricles, often

observed in schizophrenia (van Erp et al. 2016), decreased cortical and hippocampal volume and altered microstructure, all emerging in adulthood, not adolescence (Crum et al. 2017; Piontkewitz, Arad, and Weiner 2011b, [a] 2011; Piontkewitz, Assaf, and Weiner 2009). Methodological differences (Kentner et al. 2019) including gestational timing, administration route, dose of immunogen, species, background strain, and animal housing may account for these differences (see (Kentner et al. 2019; Weber-Stadlbauer and Meyer 2019).

Although speculative, it is worth considering what neurodevelopmental processes may be altered by GD 9 or GD 17 MIA-exposure. At GD 9 microglia colonization, neuronal and immune cell migration, neurogenesis, and cortical plate formation are initiated (Selemon and Zecevic 2015; Semple et al. 2013; Barbara Clancy et al. 2007). At GD 17 the organization of cortical and hippocampal layers is underway, as are synaptogenesis, gliogenesis, and apoptosis (Estes and McAllister 2016; Knuesel et al. 2014; B. Clancy, Darlington, and Finlay 2001). Previously, MIA-exposure at GD 9 has been associated with an increased density of activated microglia, which is thought to interfere with synaptic pruning and circuit formation (Le Belle et al. 2014; Tronnes et al. 2016; Manitz et al. 2016). Alterations to myelin-related structure and gene expression have been observed in GD17 MIA-exposed offspring (Richetto et al. 2017).

4.6.3 Identifying brain-behaviour associations

One major limitation when examining neurodevelopmental phenotypes is the tendency to draw associations between these structures and a single behaviour (Schnack 2019). These strategies disregard the network-like architecture of the brain and its relationship to behavioural phenotypes (Petersen and Sporns 2015; Di Martino et al. 2014). In contrast, multivariate strategies, such as PLS, move beyond simplistic associations to better understand brain-behaviour relationships (Xia et al. 2018; Uddin and Karlsgodt 2018; Ball et al. 2020). While often used in human imaging studies (Kirschner et al. 2020; Shafiei et al. 2020), PLS provides a novel, streamlined method to assess cross-sectional brain-behaviour patterns from large cohorts of deeply phenotyped mice. It permits inclusion of multiple measures from the same individual while accounting for their inter-relatedness. Further, it balances hypothesis driven experimental choices (brain regions, behaviours tested, etc.) with data-driven investigation of their relationships. Finally,

the use of multivariate methods may promote cross-species translation by improving methodological homology between human and rodent neuroimaging studies.

4.6.4 Neuroimaging-driven RNA sequencing reveals potential molecular underpinnings of brain-behaviour relationships

We observed significant enrichment of gene ontology terms for FGF signaling pathways, autistic behaviour, and microRNA regulation amongst upregulated dHIP genes. We also saw enrichment of immune pathways such as IL-17 in the vHIP, IL-1 in the ACC, and NFK-B in the d- and vHIP (Choi et al. 2016), providing further evidence for MIA-induced immune system dysregulation (Ratnayake et al. 2013; Smith et al. 2007; Rudolph et al. 2018). FGFs are a family signaling proteins (Diez Del Corral and Morales 2017; Terwisscha van Scheltinga, Bakker, and Kahn 2010) that play a critical role in regulating brain growth and connectivity (Diez Del Corral and Morales 2017; Terwisscha van Scheltinga, Bakker, and Kahn 2010)), cell survival and proliferation, neurogenesis and neuronal repair (Reuss and von Bohlen und Halbach 2003; Guillemot and Zimmer 2011). Preclinical studies suggest that disrupting FGF inhibits cortical growth and gyrification, development of neural progenitors (Matsumoto et al. 2017), hippocampal synaptogenesis and cell maturation (Nandi et al. 2018; Dabrowski et al. 2015), and left-right symmetry, all phenomena observed in schizophrenia and autism (Neugebauer and Yost 2014).

Given their role in regulating cortical size and connectivity, mutations in FGF genes have been proposed to increase vulnerability to ASD (Rubenstein 2010; Vaccarino et al. 2009). Mutations to FGF receptor 2 (Terwisscha van Scheltinga, Bakker, and Kahn 2010; O'Donovan et al. 2009; Jungerius et al. 2008), which was upregulated in the dHIP of our mice, has also been observed and the hippocampi of individuals with schizophrenia (Lanz et al. 2019). In spite of these homologies with schizophrenia given the considerable overlap in genetic and environmental risk factors across neurodevelopmental disorders, links between MIA and specific disorders should be made with caution.

4.6.5 Translational relevance of our model with clinical findings: specific relevance to child/adolescent health

Human studies demonstrate that exposure to elevated maternal IL-6 levels due to lifestyle (i.e., obesity, stress), lead to alterations in neonatal brain networks involved in social, emotional, and cognitive development and toddler executive function (Rudolph et al. 2018; Spann et al. 2018). Increased amygdala volume and functional connectivity to the somatosensory cortex, thalamus, caudate, parahippocampal gyrus have also been observed (Graham et al. 2018). *In utero* exposure to more severe maternal inflammation due to cytomegalovirus, Zika virus, or *Toxoplasma Gondii* results in severe developmental impairments including micro- or hydrocephalus, white matter lesions, mental retardation, and blindness (Guma, Plitman, and Chakravarty 2019). This line of research is even more critical in the current COVID-19 pandemic as the number of MIA-exposed offspring is expected to rise (Martins-Filho et al. 2020; Liu et al. 2020; Zimmer et al. 2020).

In humans, most maternal infections do not lead to neurodevelopmental disorders in offspring; MIA is thought to act as a disease primer, increasing susceptibility to effects of genetic mutations and environmental risk factors (Ayhan, McFarland, and Pletnikov 2016). MIA-exposure increases risk for schizophrenia if there is an existing family history of the disease (Blomström et al. 2016) or is combined with another risk factor, such as maternal anemia (Nielsen, Meyer, and Mortensen 2016). Further, *in utero* MIA-exposure has been found to exacerbate morphological alterations in offspring with schizophrenia (Ellman et al. 2010). Synergizing effects between low dose MIA-exposure and genetic mutations associated with schizophrenia or ASD, (DISC1 (Abazyan et al. 2010; Lipina et al. 2013), NRG1 (Vuillermot et al. 2012), NR4A2, TSC2(Crawley 2007)), or environmental risk factors (adolescent stress (Giovanoli et al. 2013) or exposure to drugs of abuse (Dalton et al. 2012)) have been shown to cause greater deficits than either insult alone (Reisinger et al. 2015; Meyer 2014).

4.6.6 Limitations

The findings in this paper should be considered in light of their limitations. No animal model can fully recapitulate the human condition, therefore not all observations made here may apply to human pathology and brain development (Semple et al. 2013). However, we do observe

interesting parallels between our MIA-exposed mice and psychiatric patients' altered trajectories of brain, behaviour, and transcription. Further, studies investigating MIA-exposure in humans also report that earlier exposure induces more serious downstream effects on offspring (see (Guma, Plitman, and Chakravarty 2019) for review). Although both the neuroimaging and behavioural studies conducted were longitudinal, our multivariate and transcriptional analyses were cross-sectional. Future work should extend these analyses across the lifespan. We did not detect statistically significant sex differences in neuroanatomy or behaviour, however greater neuroanatomical alterations in male POL E (vs POL L) offspring were observed, which may indicate existence of subtle sex-effects (S. Patel et al. 2020; Buka et al. 2001). Surprisingly we did not observe strong social deficits, only subthreshold social novelty deficits in adult early MIA-exposed offspring, which should be interpreted with caution. There are mixed reports of social deficits in MIA-offspring, possibly due to MIA-protocol (Piontkewitz, Arad, and Weiner 2011b; Meyer et al. 2006) however they are central to ASD (Cossío et al. 2017; Choi et al. 2016; Gumusoglu and Stevens 2019).

4.6.7 Conclusions

We comprehensively examined the effects of prenatal MIA-exposure, a risk factor for neuropsychiatric disorders, at two different gestational timepoints, on offspring brain and behaviour development. We applied multivariate analyses to integrate these modalities to investigate underlying transcriptional changes in the group and age at which we detected the greatest changes. Taken together, these findings suggest that prenatal MIA-exposure early in gestation interferes with neurodevelopment more than late gestational exposure. This leads to transient deviations in brain and behaviour development in adolescence and early adulthood, potentially increasing susceptibility to other risk factors, but, which, in the absence of subsequent challenge, normalize later in adulthood. These may be linked to altered transcription of genes involved in FGF signaling and inflammatory pathways.

4.7.1 Acknowledgements

We acknowledge that this manuscript has been submitted as a preprint to BioRxiv (MS ID#: BIORXIV/2020/406454), We would like to thank the Ludmer Center of neuroinformatics and mental health (http://ludmercentre.ca/) at McGill University for their support with the transcriptional analysis. Additionally, we would like to thank Dr. Joseph Rochford for his guidance with the prepulse inhibition testing and analysis, as well as Gülebru Ayranci, PhD for her help with MRI acquisition, and the Douglas Animal Facility staff for their support with animal care. Finally, we would like to thank Dr's Bruno Giros and Salah El Mestikawy for lending us their centrifuge.

4.7.2 Conflict of interest statement

The authors report no conflicts of interest.

4.8 Supplementary Methods

4.8.1 Animals & maternal immune activation protocol

All procedures were approved by McGill University's Animal Care Committee under the guidelines of the Canadian Council on Animal Care. C57BL/6J mice were bred in our facility under a 12-hour light cycle (8am-8pm), with food and water access *ad libitum*. Females and males of breeding age (8-12 weeks) were placed in new cages (1:1 ratio) for up to 2 days until seminal plug is observed. This was considered gestational day (GD) 0. Each female was weighed and moved to a new cage. Animals were weighed again on injection day to confirm pregnancy.

C57BL/6J mice bred in our facility were used throughout the study. Pregnant dams were randomly assigned to one of four treatment groups: (1) poly I:C (P1530-25MG polyinosinic:polycytidylic acid sodium salt TLR ligand tested; Sigma Aldrich) (5 mg/kg, intraperitoneally) at gestational day (GD) 9 (n=5), (2) vehicle (0.9% sterile NaCl solution) at GD9 (n=4), (3) poly I:C at GD 17 (n=6), or (4) vehicle at GD 17 (n=5). GD9 corresponds roughly to the end of the first trimester in human gestation and GD 17 corresponds to the end of the second trimester (Semple et al. 2013; Barbara Clancy et al. 2007; Meyer et al. 2006).

4.8.2 Experimental design

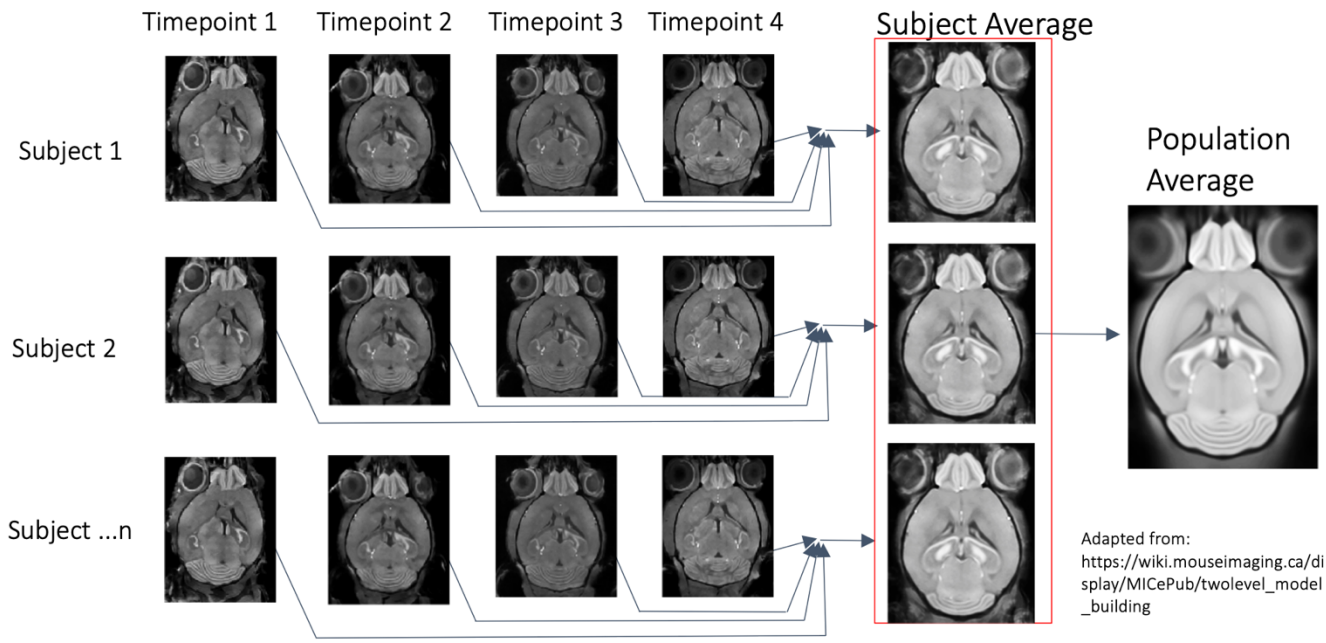
Offspring were weaned and sexed at postnatal day (PND) 21 and housed 2-4 per cage. Up to three offspring of each sex were kept per litter. Offspring exposed to prenatal MIA via viral mimetic poly I:C or vehicle were examined using structural MRI (100 μm^3) at PND 21, 38, 60, and 90 at the Douglas Institute (7 Tesla small animal MRI, Bruker). Assessment of exploratory behaviour (open field test), social behaviour (social preference), stereotypic behaviour (marble burying task), and sensorimotor gating (prepulse inhibition) was performed following scans at PND 38 (adolescence). All behaviours were repeated after the PND 90 scan (adulthood) with the addition of a cognitive flexibility measure (attentional set shifting).

4.8.3 Magnetic resonance imaging acquisition and processing

Acquisition details: Twenty-four hours prior to each MRI scan, mice were injected with $MnCl_2$ (62.5 mg/kg) for contrast enhancement. Anesthesia was induced with 5% isoflurane in oxygen and maintained with 1.5% isoflurane during the scan. Scans were conducted in a 7 Tesla Bruker, with a 30 cm bore magnet with AVANCE electronics. A 3D FLASH (Fast, Low Angle SHot) sequence was used with TE/TR of 4.5ms/20ms.

Preprocessing details: T1-weighted scans were preprocessed by stripping native coordinates, flipping left-right to maintain fidelity, denoising, correcting (Friedel et al. 2014; B. B. Avants et al. 2008) inhomogeneities in the bias field using the N4 algorithm (Tustison et al. 2010), and registering in LSQ6 alignment (i.e. 6 degrees of freedom are allowed for imagine alignment: translations and rotations along x, y, and z dimensions) (Ashburner and Friston 1998; Friedel et al. 2014; Collins et al. 1994). Visual quality control (QC) was performed to exclude any scans that had artifacts or signal drop-off that would prevent accurate registrations; subjects who had only 1 of 4 usable scans were also excluded from further processing (n=27 excluded; https://github.com/CoBrALab/documentation/wiki/Mouse-QC-Manual-(Structural)).

Registration details: Linear LSQ12 registration was applied to the preprocessed LSQ6 images using 12 degrees of freedom are allowed including those in LSQ6 as well as 3 scales and 3 shear parameters (Friedel et al. 2014; Lerch et al. 2008; Kovačević et al. 2004; Avants et al. 2008; Avants et al. 2011); **Supplementary figure 4.S1**). Relative Jacobian determinants explicitly model only the non-linear part of the deformations and remove residual global linear transformations (attributable to differences in total brain size). Absolute Jacobians (without removal of overall linear transformations) were used to better determine what localized changes in volume are attributable to global changes in volume (Chung et al. 2001).



Supplementary figure 4.S1. Schematic of the registration pipeline. Each subject (mouse) was scanned at 4 timepoints throughout development (timepoint 1, 2, 3, 4). The arrows indicate that the 4 scans for each subject were registered to create a subject average. All subject averages were then registered to create s population average for the entire study.

4.8.4 Behavioural testing

4.8.4.1 Open field test

Mice were gently placed in the center of a 45 x 45 cm^2 rectangular light grey arena and allowed to explore for 15 minutes. Behaviour was video recorded. Areas were conceptually partitioned into zones in which distance traveled and time spent were measured: a center zone (40% of the total area), corners, and middle edges. We assessed distance traveled in the center zone relative to total distance traveled using the following model, which was applied to all tests and defined as behavioural model 1: group (SAL as reference) and sex (male as references) as fixed effects, and litter as a random effect to account for possible litter variability.

4.8.4.2 Three chambered social preference and social novelty task

Mice were habituated (10 minutes) under red light to a three-chamber plastic box (26 (l) x 21.6 (w) x 21.6 (h) cm) with divider panels that have open doors, with a wire container (9.5 (h) 7.6 (d) cm) in each of the two extreme chambers. To measure social preference (10 minutes), time spent interacting with a stranger mouse was compared to that with a non-social object using the following social preference index formula 1:

([distance traveled intruder 1 zone] / [distance traveled object zone + distance traveled intruder 1 zone]) - 0.5. (1)

Similarly, to measure social novelty (10 minutes), the non-social object was replaced with another stranger mouse, and a social novelty index was calculated as formula 2:

([distance traveled intruder 2 zone] / [distance traveled intruder 1 zone + distance traveled intruder 2 zone]) - 0.5 (2)

We assessed group differences in social preference and novelty indices using behavioural model 1. Stranger mice were the same strain, sex, and similar age (within 2 weeks) of the test mice and were habituated to the wire containers (20 minutes twice a day) for two days prior to the test.

4.8.4.3 Marble burying task

Mice were placed in a standard home cage (28 (h) x 17 (w) x 12.7 (h) cm) filled with ~7 cm layer of woodchip bedding (new for each mouse) and 15 equidistant standard marbles for 30 minutes, as previously described (Deacon 2006). Marbles were classified as either buried 100% (nothing visible), buried at 75% (only a bit visible) or unburied (<75% buried). For the marble burying task, group differences in the number of marbles buried (75% and 100%) were tested with the behavioural model 1 described in the main text **4.4.3.** (group (SAL as reference) and sex (male as reference) as fixed effects, and litter as a random effect to account for possible variability due to litter) using a Poisson distribution.

4.8.4.4 Prepulse inhibition

Prepulse inhibition (PPI) to acoustic startle was measured using commercially available startle chambers (San Diego Instruments, San Diego, CA) consisting of a Plexiglass chamber (8 cm diameter, 16 cm long) mounted on a Plexiglass base with a sound-attenuating chamber, and a

speaker located in the ceiling of the chamber (24 cm above the animal) to provide the background noise (70 dB) and the acoustic stimuli. A piezoelectric accelerometer fixed to the animal enclosure frame was used to detect and transduce motion resulting from the animal's startle response. A microcomputer using a commercial software package by SR-LAB was used to control pulse parameters, and digitize (0-4095), rectify, and record the stabilimeter readings. Animals were placed in the Plexiglass restrainers, and after 5 minutes of acclimatization. Mice underwent a total of 50 trials (5-30 s intertrial duration).

Startle magnitude to a 50 ms 120 dB stimulus, in absence of prepulse, was measured in the first 8 and final 7 trials. For the middle 35 trials, the startle tone was either presented alone, or preceded by a 30ms prepulse stimulus ranging from 3-15 dB above background noise (73-85 dB) and varying randomly between trials in 3 dB increments (5 trials per prepulse stimulus). A measure of maximum and average startle response was derived from the 100 1 ms readings taken starting from the beginning of the startle stimulus onset. Percent PPI from each prepulse intensity formula 3:

> (averaged over trials) was calculated using the formula: [(startle response - prepulse response) / startle response] x 100 (3)

Differences in maximum startle amplitude were investigated overall (behavioural model 1) and by prepulse intensity with a group-by-trial interaction added to behaviour model 1.

4.8.4.5 Attentional set shifting task

Food deprivation: four days following the PPI in adulthood, food deprivation was commenced for the attentional set shifting task (ASST). On the first day, the animal's body weight was measured at *ad libitum* conditions, and food was removed from all containers. Weight was maintained above 85% of initial body weight for each mouse by feeding 1 and 2 grams of food per mouse per day. One ceramic bowl was introduced per cage in which some pieces of Honey Nut Cheerios (General Mills, Canada) were placed as well as their regular chow in order to habituate mice to the bowls, and to reward food.

Apparatus: testing chambers used for the ASST task consisted of opaque Plexiglass boxes (45 x 24 x 18 cm) with white plastic walls (15 x 11.5 cm) to separate the two choosing compartments. Two ceramic bowls (6 cm diameter) containing several digging mediums and odors

were placed within each choice chamber. A separate plexiglass box was placed in front of the testing chamber to serve as a waiting area between trials. For each trial, mice were gently placed in the testing chamber with access to the two choice chambers.

Habituation: after one day of food deprivation, mice were habituated to the testing chamber with two 20-minute sessions per day for four days. Mice were trained to find small pieces of Honey Nut Cheerio cereal (General Mills, Canada) by digging in two bowls containing bedding material. The amount of bedding medium covering the food reward was progressively increased on each day, until it was fully covered by the third day. Animals were required to dig efficiently (8 correct responses in a row), defined as vigorous displacement of medium with upper paws reaching the hidden reward in the training bowls, prior to starting ASST. Touching the rim or smelling the bowls at the surface of the medium was not considered digging.

ASST Paradigm: the ASST comprised a successive series of discriminations in which mice had to choose between two bowls filled with different combinations of odors and digging mediums to find a hidden food reward. These were trained within 5 consecutive days and included simple discrimination (SD), compound discrimination (CD), intra-dimensional shift (ID), intradimensional shift reversal (IDR), and extradimensional shift (ED) (**Supplementary table 4.S1)**. Each stage was considered complete when mice reached a learning criterion of six consecutive correct responses. If a mouse stopped responding for three consecutive trials, the session was stopped, and training was resumed the following day (Colacicco et al. 2002). In the SD, one dimension (odor) was presented; mice had to discriminate between nutmeg (baited) and basil (non-baited). The next stage, CD, relied on the same previously trained odor discrimination (nutmeg/baited, basil/non-baited), however a new stimulus was introduced (medium: crystals and vase filler). For the ID, new combinations of odors (curry/baited, garlic/non-baited) and digging mediums (pompoms and shredded paper) were presented wherein mice were still required to discriminate according to the trained dimension: odor. For the IDR, the baited odor was switched from that of the previous day. Finally, for the ED, new pairs of stimuli were be introduced, but now the previously irrelevant stimulus dimension (medium) was relevant and predicted the baited bowl (wooden beads/baited and flower petals), whereas odor, the previously relevant stimulus now had to be ignored (paprika and ginger). The first four trials of the SD were exploratory in which

mice were allowed to dig in the non-baited bowl and correct their choice (an error was still recorded); after this, mice were not allowed to correct their response.

Supplementary table 4.S1. Attentional Set Shifting Task (ASST) details.

	Dimension		**Exemplar Combination**		**Exemplar Compound Stimuli**	
ASST Stage	**Relevant**	**Irrelevant**	**Correct**	**Incorrect**	**Correct (baited)**	**Incorrect (non-baited)**
SD	Odor	Medium	O1, M1	O2, M1	Nutmeg/ Bedding	Basil/Bedding
CD	Odor	Medium	O1, M2, M3	O2, M2, M3	Nutmeg/ Vase Filler; Nutmeg/ Crystals	Basil/ Vase Filler; Basil/ Crystals
ID	Odor	Medium	O3, M4, M5	O4, M4, M5	Curry/ Shredded Paper; Curry/ Pompoms	Garlic/ Shredded Paper; Garlic/ Pom poms
IDR	Odor	Medium	O4, M4, M5	O3, M4, M5	Garlic/ Shredded Paper; Garlic/ Pom poms	Curry/ Shredded Paper; Curry/ Pompoms
ED	Medium	Odor	M6, O5, O6	M7, O5, O6	Wooden beads/ Ginger; Wooden beads/ Paprika	Plastic flower petals/ Ginger; Plastic flower petals/ Paprika

SD= simple discrimination, **CD=** compound discrimination, **ID=** Intradimensional shift, **IDR=** Intradimensional shift reversal, **ED=** Extra-dimensional shift, **O=** odour, **M=** medium.

4.8.5 Perfusions

One week following the last behavioural test (ASST), mice were anaesthetized with a pentobarbital overdose, and fixed by transcardiac perfusion with 4% paraformaldehyde (PFA) in phosphate buffered saline solution. Brains were collected and immersed in PFA overnight at 4 ℃

for 24 hours, after which they were transferred to long-term storage solution of phosphate buffered solution with 0.02% Sodium Azide.

4.8.6 Assessment of maternal cytokines levels

In a separate group of dams, poly I:C or saline was injected as described above (3 GD 9-POL, 3 GD 17-POL, 2 GD 9-SAL, 2 GD 17-SAL). Three hours following injection, dams were sacrificed by decapitation without euthanasia, and trunk blood was collected in a 1.5mL Eppendorf tube. The blood was allowed to coagulate at room temperature for 30 minutes, and then centrifuged for 10 minutes at 4 ℃, with 2000 revolutions per minute. Serum was collected and stored at -80 ℃ until ready for analysis. Serum samples were shipped to the University of Maryland Core Cytokine Facility (http://www.cytokines.com/) for multiplex ELISA to measure levels of IL-6, TNF-α, IL-1β, IL-10 to the immunostimulatory potential of our poly I:C. We chose to use a separate group of dams to ensure we could collect enough blood for analysis, and so as not to introduce an additional stressful experience for the dam, thereby potentially confounding the neurodevelopmental trajectory of offspring. Detection ranges were as follows IL-6 (1.95-8000 pg/ml), TNF-α (0.85-3500 pg/ml), IL-1β (3.75-15000 pg/ml), IL-10 (5-20000 pg/ml).

4.8.7 Statistical analysis

4.8.7.1 Model comparisons

Models were compared with increasing complexity using the minc Log Likelihood Ratio (LLR) function. This was computed for every voxel in the brain, therefore, for each compared model, we ensured that even if the model was significantly better based on LLR, it also covered a great proportion of the brain. We first compared natural spline fits of increasing complexity to model our age including a first order (model 1), second order (model 2), or third order (model 3) fit (interacting with our group term). Next, inclusion of a random slope for age was also assessed (however the model did not converge, model 4). Finally, we investigated the effects of a 3-way interaction of age-by-group-by-sex (model 5).

Model 1: $Y_{subject,j} = \beta_0 + \beta_1 sexF_{subject,j} + \beta_2 groupPOL\_E_{subject,j} + \beta_3 groupPOL\_L_{subject,j} + \beta_4 age(ns,1)1_{subject,j} + \beta_5 ns(age,1)1{:}group\_POL\_E_{subject,j} + \beta_6 ns(age,1)1{:}group\_POL\_L_{subject,j} + \mathbf{b_1} subject + \mathbf{b_2} litter + \epsilon_{subject,j}$

Model 2: $Y_{subject,j} = \beta_0 + \beta_1 sexF_{subject,j} + \beta_2 groupPOL\_E_{subject,j} + \beta_3 groupPOL\_L_{subject,j} + \beta_4 age(ns,2)1_{subject,j} + \beta_5 age(ns,2)2_{subject,j} + \beta_6 ns(age,2)1{:}groupPOL\_E_{subject,j} + \beta_7 ns(age,2)1{:}groupPOL\_L_{subject,j} + \beta_8 ns(age,2)2{:}groupPOL\_E_{subject,j} + \beta_9 ns(age,2)2{:}groupPOL\_L_{subject,j} + \mathbf{b_1} subject + \mathbf{b_2} litter + \epsilon_{subject,j}$

Model 3: $Y_{subject,j} = \beta_0 + \beta_1 sexF_{subject,j} + \beta_2 groupPOL\_E_{subject,j} + \beta_3 groupPOL\_L_{subject,j} + \beta_4 age(ns,3)1_{subject,j} + \beta_5 age(ns3)2_{subject,j} + \beta_6 age(ns3)3_{subject,j} + \beta_7 ns(age,3)1{:}groupPOL\_E_{subject,j} + \beta_8 ns(age,3)1{:}groupPOL\_L_{subject,j} + \beta_9 ns(age,3)2{:}groupPOL\_E_{subject,j} + \beta_{10} ns(age,3)2{:}groupPOL\_L_{subject,j} + \beta_{11} ns(age,3)3{:}groupPOL\_E_{subject,j} + \beta_{12} ns(age,3)3{:}groupPOL\_L_{subject,j} + \mathbf{b_1} subject + \mathbf{b_2} litter + \epsilon_{subject,j}$

Model 4: $Y_{subject,j} = \beta_0 + \beta_1 sexF_{subject,j} + \beta_2 groupPOL\_E_{subject,j} + \beta_3 groupPOL\_L_{subject,j} + \beta_4 age(ns,3)1_{subject,j} + \beta_5 age(ns,3)2_{subject,j} + \beta_6 age(ns,3)3_{subject,j} + \beta_7 ns(age,3)1{:}groupPOL\_E_{subject,j} + \beta_8 ns(age,3)1{:}groupPOL\_L_{subject,j} + \beta_9 ns(age,3)2{:}groupPOL\_E_{subject,j} + \beta_{10} ns(age,3)2{:}groupPOL\_L_{subject,j} + \beta_{11} ns(age,3)3{:}groupPOL\_E_{subject,j} + \beta_{12} ns(age,3)3{:}groupPOL\_L_{subject,j} + \mathbf{b_1} subject + \mathbf{b_2} subject{:}age_{subject,j} + \mathbf{b_3} litter + \epsilon_{subject,j}$

Model 5: $Y_{subject,j} = \beta_0 + \beta_1 sexF_{subject,j} + \beta_2 groupPOL\_E_{subject,j} + \beta_3 groupPOL\_L_{subject,j} + \beta_4 age(ns,3)1_{subject,j} + \beta_5 age(ns,3)2_{subject,j} + \beta_6 age(ns,3)3_{subject,j} + \beta_7 ns(age,3)1{:}groupPOL\_E_{subject,j} + \beta_8 ns(age,3)1{:}groupPOL\_L_{subject,j} + \beta_9 ns(age,3)2{:}groupPOL\_E_{subject,j} + \beta_{10} ns(age,3)2{:}groupPOL\_L_{subject,j} + \beta_{11} ns(age,3)3{:}groupPOL\_E_{subject,j} + \beta_{12} ns(age,3)3{:}groupPOL\_L_{subject,j} + \beta_{13} ns(age,3)1{:}sexF_{subject,j} + \beta_{14} ns(age,3)2{:}sexF_{subject,j} + \beta_{15} ns(age,3)3{:}sexF_{subject,j} + \beta_{16} sexF{:}groupPOL\_E_{subject,j} + \beta_{17} sexF{:}groupPOL\_L_{subject,j} + \beta_{18} ns(age,3)1{:}sexF{:}groupPOL\_E_{subject,j} + \beta_{19} ns(age,3)1{:}sexF{:}groupPOL\_L_{subject,j} + \beta_{20} ns(age,3)2{:}sexF{:}groupPOL\_E_{subject,j} + \beta_{21} ns(age,3)2{:}sexF{:}groupPOL\_L_{subject,j} + \beta_{22} ns(age,3)3{:}sexF{:}groupPOL\_E_{subject,j} + \beta_{23} ns(age,3)3{:}sexF{:}groupPOL\_L_{subject,j} + \mathbf{b_1} subject + \mathbf{b_2} litter + \epsilon_{subject,j}$

Y= outcome measures (i.e. blurred absolute Jacobian determinants); β_i= fixed effect coefficient; β_0 = equation intercept; **b** = random predictor; ϵ = random error; **j** = repeated measure per subject; **:** = interaction; POL E = early poly I:C group relative to SAL as reference; POL L = late poly I:C group relative to SAL as reference; SexF = female sex relative to male as reference

4.8.7.2 Comparison of SAL E and SAL L

We investigated whether there were differences in neuroanatomical development in our two control groups (SAL E and SAL L). Using a brain mask, we ran a whole-brain voxel-wise linear mixed-effects model on the subject-level (first level) absolute Jacobian determinant files for each subject over all 4 timepoints. We assessed an age, modeled as a third order natural spline, by injection timing interaction, with sex as a covariate (fixed effects), and used mouse id and litter as random intercepts. A False Discovery Rate (FDR) correction was applied to correct for multiple testing.

4.8.7.3 Partial least squares analysis

Inputs: PLS cannot handle missing data. Thus, in order to maximize our ability to detect brain-behaviour patterns, we performed data imputation on the behavioural data so that any mouse with a scan passing QC could have a full set of behavioural metrics to use (22 mice were removed). We did this using the singular value decomposition function SVD.miss in R (Fuentes, Guttorp, and Sampson 2006). Briefly, this completes a data matrix using iterative singular value decomposition, replacing the missing values by linear regression of the columns, and making use of the column averages. The input brain matrix included whole-brain voxel-wise DBM measures (87 by 508169 voxel matrix) from the PND 38 scan. The imputed input behavioural data included behavioural metrics for all tests performed immediately following that PND 38 scan (78 x 20 matrix including: OFT: velocity in center, total distance, distance moved in center; SOPT: frequency of entries in object circle, and intruder 1 circle, ratio of time spent in intruder 1 vs. object circle, ratio of distance moved in intruder 1 and object boxes; SONT: same as SOPT; PPI: PP6 max, PP9 max, PP12 max, PP15max; marbles buried 100%, marbles buried 75%). As described in the main text, sex and litter size were also included in the behaviour matrix as 'demographic' measures. The imaging and behavioural data were organized into two matrices X (imaging), and Y (behaviour), with subjects as rows and variables in columns. MATLAB version 2019/b was used to run the PLS analysis.

Permutation testing: was used to assess the statistical significance of each LV wherein the rows (subjects) of the brain data matrix were randomly shuffled to 1) nullify dependencies between brain and behaviour (n=1000 repetitions) and 2) generate a null distribution of possible brain-behaviour correlations. SVD was applied to these "null" correlations, generating a distribution of

singular values under the null hypothesis. The probability that a permuted singular value exceeds the original, non-permuted singular value allows us to generate the p-value (Zeighami et al. 2019; Patel et al. 2020). A threshold of $p<0.05$ was used (95% or greater chance that the singular value of the non-permuted data exceeds that of a permuted singular value).

Bootstrap resampling: was applied to assess the contribution of individual brain and behaviour variables to each LV. Subjects (rows for both X and Y matrices) were randomly sampled and replaced (n=1000) to generate a set of resampled correlation matrices to which SVD was applied to generate a sampling distribution for each weight of the singular vectors. The ratio of each singular vector weight and its bootstrap-estimated standard error were used to calculate a "bootstrap ratio" for each voxel. Voxels that make large contributions to certain patterns can therefore be identified by large bootstrap ratios.

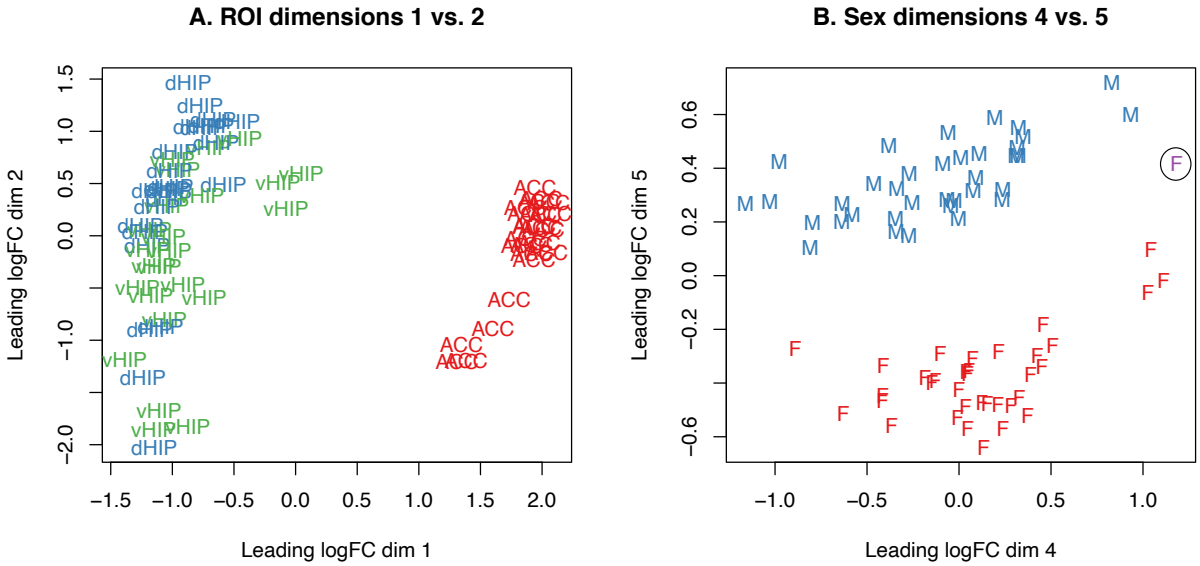
4.8.8 Transcriptional analysis

4.8.8.1 Sample collection and pre-processing

A separate cohort of mice (PND38; POL E n=6M/6F, 5 litters, SAL E n=6M/6F, 6 litters) were euthanized in their home cage, brains were rapidly extracted and placed in chilled PBS and sliced (1 mm thick sections); tissue was punched from the ACC (bregma +0.14 mm), dHIP (bregma -2.80 mm), and vHIP (bregma -3.08 mm) and flash frozen on dry ice (death to freezing all samples < 3 minutes). RNA extraction was performed using ReliaPrep Tissue RNA Miniprep system (Promega). RNA quantity and integrity was analyzed using Nanodrop (Thermo Fisher) and Bioanalyzer (Agilent). The samples were randomized during library preparation. Sequencing was performed on Illumina NovaSeq 6000 at the McGill University Génome Québec Innovation Centre for 72 samples, 3 regions in adolescent POL E or SAL E mice (PND38; 6males/6females per group).

Pre-alignment quality control was performed using FastQC (https://hbctraining.github.io/Intro-to-rnaseq-hpc-O2/lessons/02_assessing_quality.html). Reads were filtered for a minimum Phred score of 30 and a minimum read length of 20 as well as trimmed off the first base pair from the 5' end. Alignment was performed using the STAR aligner (Version 2.7.3a_2020-01-23) (Dobin et al. 2013) on the mouse genome build (mm10)/(GRCm38.p6) (GCA_000001635.8) downloaded from Ensembl Non-specific filtering removed genes with zero

counts and lowly expressed genes that did not meet the requirement of a minimum of one count per million (cpm) in at least six samples. Only genes annotated as protein coding according to ensembl's biomart Mus.musculus package (https://bioconductor.org/packages/release/data/annotation/html/Mus.musculus.html) were retained (15048 genes). Genes were subjected to a trimmed mean of M-values normalization method (Robinson and Oshlack 2010). Normalized data were inspected for outlier samples using unsupervised hierarchical clustering of subjects by multidimensional scaling (MDS) and principal component analysis (PCA) to identify potential outliers greater than two standard deviations from these averages. Outlier detection was also performed using high dimensional extension of Cook's influence measure (Cook 1986), which identified no outliers, however one sample (ACC, F, POL E) was removed as it was close to the outlier cut-off and displayed expression signals in Y chromosome genes (indicative of possible contamination; **Supplementary figure 4.S2**).



Supplementary figure 4.S2. Multidimensional scaling (MDS) plots of log-counts per million (CPM) Values. **A.** Over dimensions 1 and 2 with samples coloured and labeled by region of interest. **B.** Over dimensions 4 and 5 with samples coloured and labeled by sex. Distances on the plot correspond to the leading fold-change, which is the average (root-mean-square) log2-fold-change for the 500 genes most divergent between each pair of samples by default. Magenta circle denotes the sample that was excluded from analysis (**ACC**=anterior cingulate cortex; **dHIP**=dorsal hippocampus; **vHIP**=ventral hippocampus; **M**=male; **F**=female; **ROI**=region of interest).

4.8.8.2 Differential Expression Analysis

DE analysis was performed according to the limma-voom pipeline (Ritchie et al. 2015), using a generalized linear model (GLM), log-normal distribution and a nominal significance threshold multiple testing adjusted p-values of $p<0.05$. Correction for multiple comparisons was performed using FDR (Benjamini and Hochberg 1995). The voomWithQualityWeights() method (Liu et al., 2015) was used to assign additional weight to more variable samples, allowing for more statistical power and accounting for heteroscedastic nature of the data. Sex and ROIs were used as confounding variables for the GLM model as well as blocking on the individual level using limma's duplicateCorrelation() method (Smyth et al., 2005), to account for the 3 ROIs being taken from the same specimen. T-statistics, moderated F-statistic, and log-odds of differential expression were estimated by limma's ebayes() method using the robust parameter to account for individual gene outliers (Phipson et al., 2016).

4.8.8.3 Rank Rank Hypergeometric Overlap Test

We applied a rank rank hypergeometric overlap test (RRHO; recently updated version of the software (Cahill et al., 2018)), which builds on the previous version by accurately detecting overlap of the change in genes in both the same and opposite directions in the same dataset, to measure the concordance of differential gene expression patterns between POL E and SAL E data in a multitude of settings. RRHO is a threshold-free approach to identify concordant and discordant overlaps between expression profiles and to measure the degree and significance of said overlap (Plaisier et al., 2010). Full differential expression lists were ranked by the -log10(p-value) multiplied by the sign of the fold change from the DE analysis. We assessed concordance between the sexes (male (M) vs. female (F)) for all brain regions combined as well as within each individual brain region. RRHO maps compute the normal approximation of difference in log odds ratio and standard error of overlap between conditions POL vs. SAL (control) for each pixel. This z-score is then converted to a p-value and corrected for multiple comparisons across pixels (Benjamini and Yekutieli, 2001).

4.8.8.4 Pathway enrichment analysis

In order to gain further insight from the DE and RNA-Seq results, we performed pathway enrichment analysis. Firstly, we used the g:Profiler (Raudvere et al., 2019) R client to identify pathways whose genes were significantly enriched or overrepresented in a gene list of interest

compared to a background gene list. Resulting pathways were selected using an FDR adjusted p value threshold (Q value) < 0.05 and ranked by normalized enrichment score. Finally, to organize and visualize pathway analyses results, we used the EnrichmentMap (Merico et al., 2010) and Autoannotate (Kucera et al., 2016) packages made available within the Cytoscape platform (Shannon et al., 2003).

4.8.8.5 Gene overlap analysis

To compared our resulting differentially expressed gene (DEG) lists to published data on human diseases, notably schizophrenia DEGs (Lanz et al. 2019) and schizophrenia and autism spectrum disorder (ASD) pancortical DEGs (Gandal et al. 2018), we used the GeneOverlap package (Li Shen and Mount Sinai (2019), R package version 1.20.0.) to probe enrichment of disease gene lists in our DEG lists by Fisher's exact test.

4.9 Supplementary Results

4.9.1 Poly I:C injection does increase pro-inflammatory cytokines

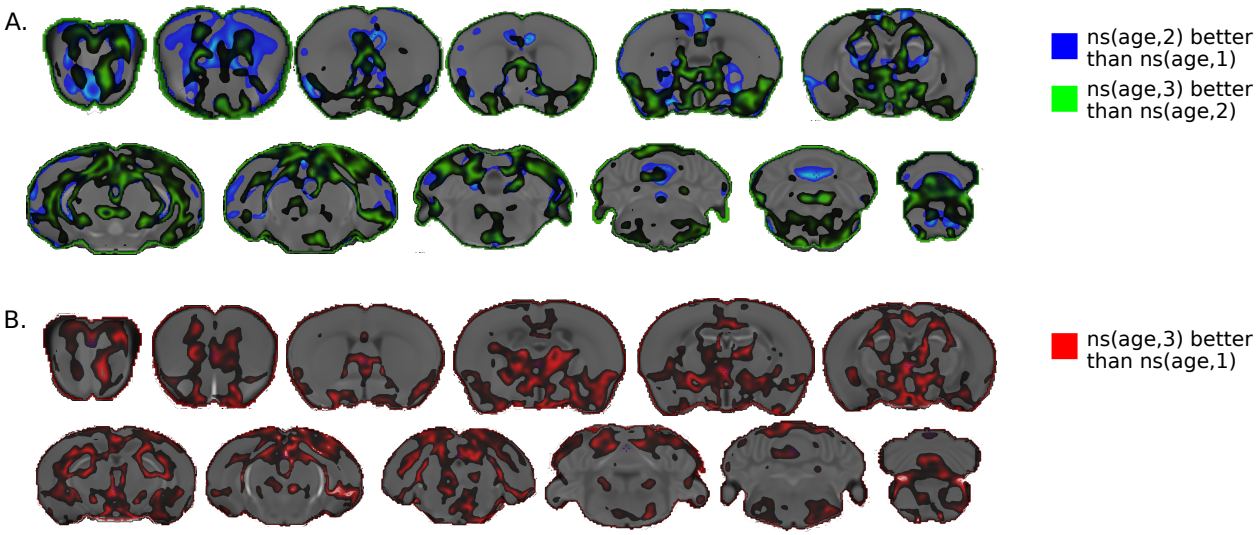
We observed an increase in levels of pro-inflammatory cytokines IL-6 and IL-1β, but not TNF-α, in a separate cohort of pregnant dams 3-hours post poly I:C injection on GD 9 relative to saline control on GD 9. Exposure to poly I:C on GD 17 increased levels of pro-inflammatory cytokines IL-6 and TNF-α, but not IL-1β relative to GD 17 saline controls. There were no differences in levels of anti-inflammatory cytokine IL-10 for any of the 4 groups (**Supplementary Table 4.S2)**.

Supplementary Table 4.S2. Maternal serum cytokine levels for our 4 treatment groups, mean [range]

	IL-1β (pg/ml)	**IL-6 (pg/ml)**	**IL-10 (pg/ml)**	**TNF-α (pg/ml)**
SAL E (n=2)	5.00 [3.65-6.34]	32.95 [30.58-35.32]	33.83 [11.43-56.22]	23.39 [20.72-26.05]
SAL L (n=2)	6.610 [6.34-6.88]	23.27 [22.22-24.31]	21.52 [8.30-34.73]	6.84 [5.99-7.69]
POL E (n=3)	11.56 [9.80-12.95]	3578.84 [3624.87-3740.81]	44.05 [36.93-49.70]	8.20 [7.91-8.35]
POL L (n=3)	5.45 [5.00-6.34]	1298.69 [54.36-3173.25]	16.73 [13.67-19.58]	20.35 [14.21-31.18]

4.9.2 Model comparisons

Using the minc log likelihood function (LLR) in R, we found that model 3 (third order natural spline of age) was better than models 1 (first order) and 2 (second order) (LLR=13.702, q=0.01; **Supplementary figure 4.S3)**. The addition of sex as an interaction term did not result in a better fitting model (model 5), and the addition of a random slope for age was too complex of a model to fit, as it did not converge (model 4). Thus, we chose model 3 for our analyses.

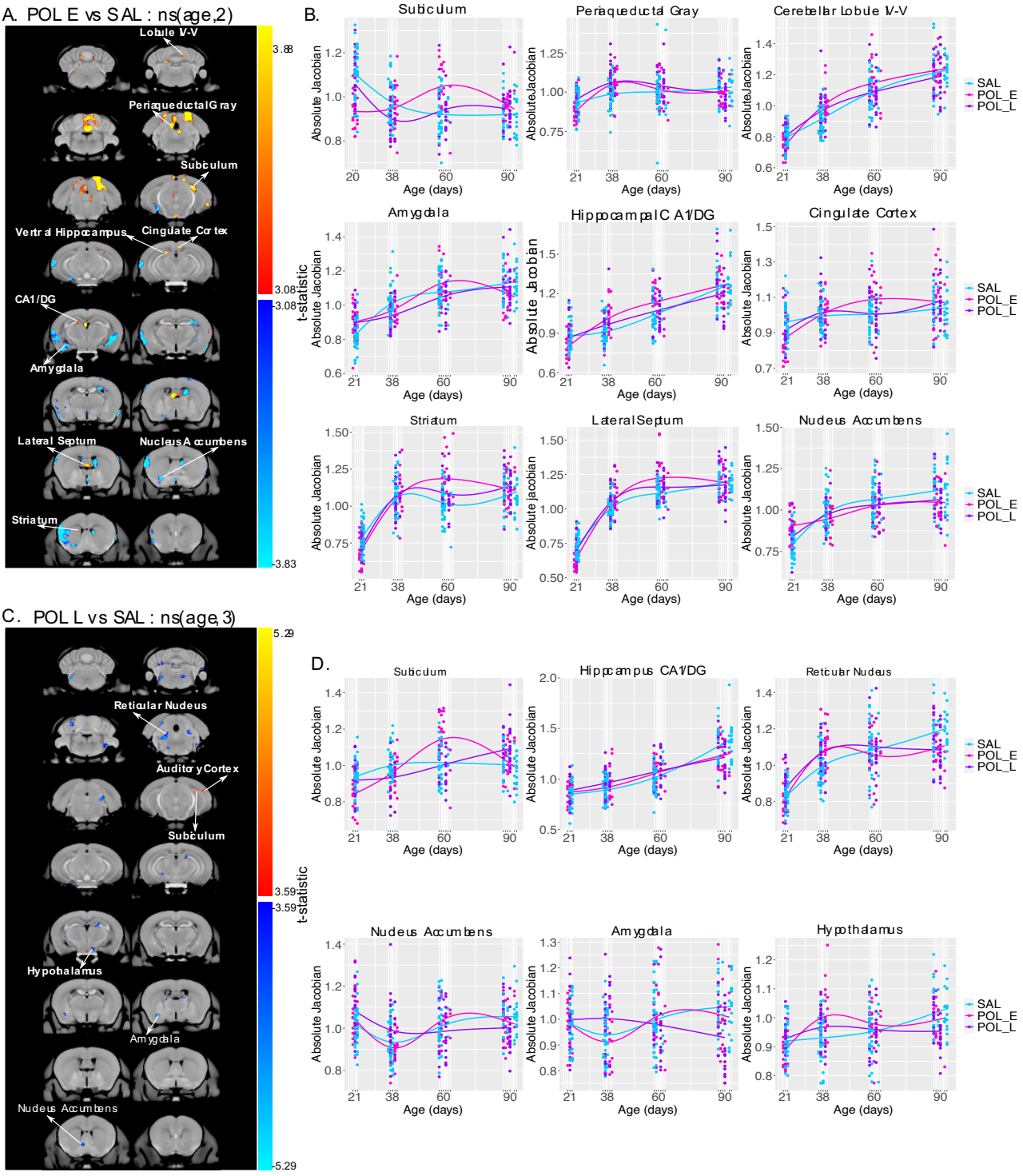


Supplementary figure 4.S3. Comparison of 3 linear mixed-effects models with different age fits. **A.** Regions in blue were better fit by modeling age as a second order natural spline (ns(age,2)) relative to a first order natural spline fit for age (ns(age,1)). Whereas regions in green were better modeled by a third order natural spline fit for age (ns(age,3)) relative to the second order age fit (ns(age,2)) according to the log-likelihood ratio test ($q<0.05$). **B**. Regions in red were better modeled by a third order natural spline fit (ns(age,3)) relative to a first order age fit (ns(age,1)) ($q<0.05$), supporting our choice of a third order natural spline fit for our age term. Maps are overlaid on the population average from the study.

4.9.3 Longitudinal neuroanatomical changes due to early and late MIA-exposure for all age fits: first, second, and third order natural splines

We present a more detailed results figure for regions affected by early MIA-exposure for the second order age term, and late MIA-exposure for the third order age term, both highlighted in

the main text. Additionally, we provide a table to summarize the effects based on peak voxels of the regions highlighted here (**Supplementary figure 4.S4 & Supplementary Table 4.S3**).



Supplementary figure 4.S4 (caption on next page).

Supplementary figure 4.S4 (continued). Differences in developmental trajectories for early poly I:C group (POL E) vs saline controls (SAL) & the late poly I:C group (POL L) vs SAL (thresholded at 5% False discovery rate (FDR)) **A**. t-statistic map of group (POL E vs SAL) by age (second order natural spline) thresholded between 5% FDR (bottom, t=3.08) and 1% FDR (top, t=3.83) overlaid on the population (second-level) average. **B**. Plot of peak voxels (voxel within a region of volume change showing largest effect) selected from regions of interest highlighted (**A**), wherein age is plotted on the x-axis, and the absolute Jacobian determinants plotted on the y-axis. Here a value of 1 means the voxel is no different than the average, anything above 1 is relatively larger, and below 1 is relatively smaller. Ranges are not normalized to enhance comparison at each specific location in space. Trajectories are modeled as third order natural splines to reflect statistical modeling. **C**. t-statistic map of group (POL L vs SAL) by age (third order natural spline) thresholded between 5% FDR (bottom, t=3.59) and 1% FDR (top, t=5.29). **D**. Plots of peak voxels as described in (**B**) with curves modeled as third order natural splines to reflect statistics.

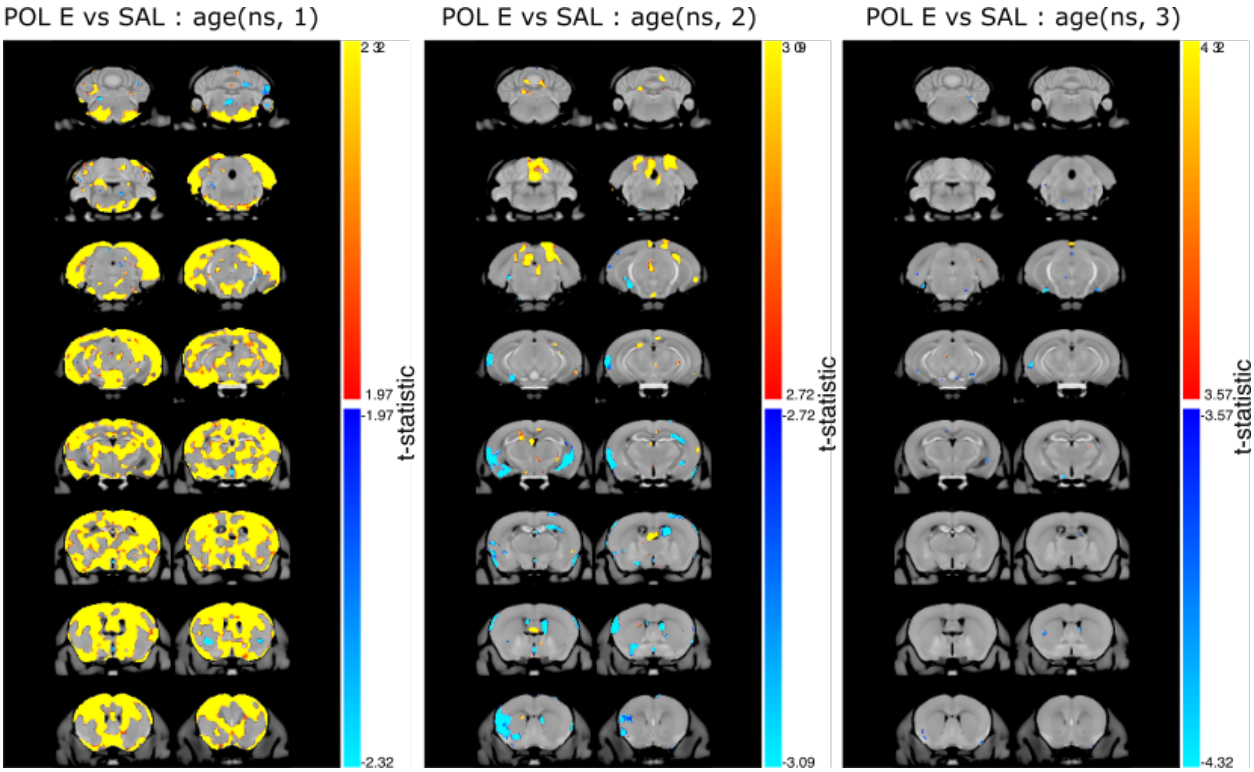
Supplementary Table 4.S3. Summary of statistical results for peak voxels in key regions of interest based on the key regions highlighted in results figure 2 and supplementary figure S4 main statistical model:

Model 3: $Y_{subject,j} = \beta_0 + \beta_1 sexF_{subject,j} + \beta_2 groupPOL\_E_{subject,j} + \beta_3 groupPOL\_L_{subject,j} + \beta_4 age(ns,3)1_{subject,j} + \beta_5 age(ns3)2_{subject,j} + \beta_6 age(ns3)3_{subject,j} + \beta_7 ns(age,3)1{:}groupPOL\_E_{subject,j} + \beta_8 ns(age,3)1{:}groupPOL\_L_{subject,j} + \beta_9 ns(age,3)2{:}groupPOL\_E_{subject,j} + \beta_{10} ns(age,3)2{:}groupPOL\_L_{subject,j} + \beta_{11} ns(age,3)3{:}groupPOL\_E_{subject,j} + \beta_{12} ns(age,3)3{:}groupPOL\_L_{subject,j} + \mathbf{b_1} subject + \mathbf{b_2} litter + \epsilon_{subject,j}$

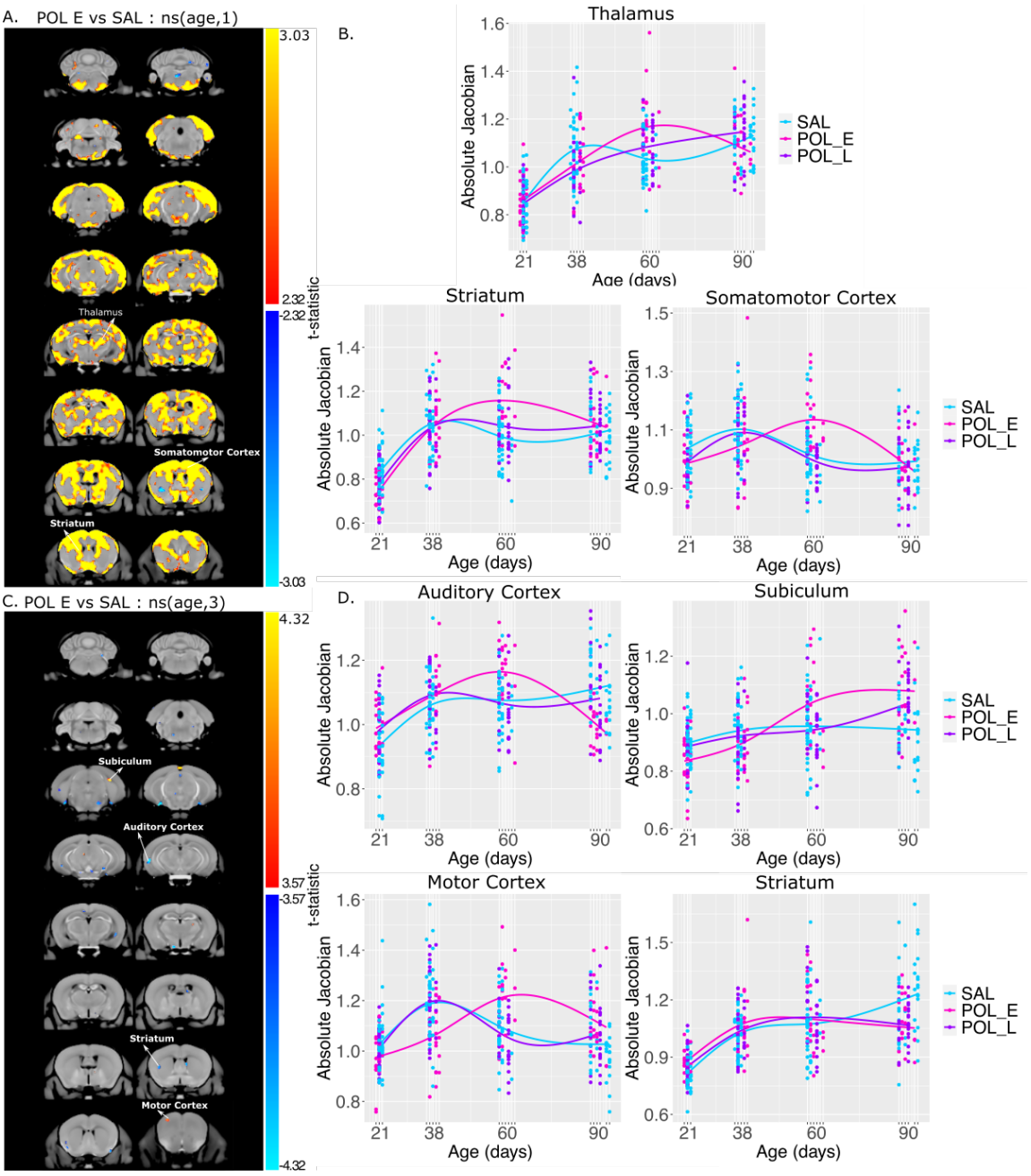
	POL E vs SAL : age ($\beta_9 ns(age,3)2{:}groupPOL\_E_{subject,j}$)		
Region	**Coordinates (x,y,z)**	**t-value**	**q-value**
Subiculum	Voxel: 88, 64, 59 World: 2.4, -1.7, 1.7	5.871	$7.517x10^{-5}$
Periaqueductal Gray	Voxel: 58, 55, 53 World: -0.6, -3.0, 1.0	4.980	0.00071
Cerebellar Lobule IV-V	Voxel: 71, 42, 56 World: 0.8, -4.0 ,1.4	4.032	0.0063
Amygdala	Voxel: 36, 82, 27 World: -2.7, 0.0, -1.5	-3.874	0.0091
Hippocampal CA1/DG	Voxel: 50, 79, 62 World: -1.3, -0.1, 1.8	3.561	0.0186
Cingulate Cortex	Voxel: 67, 84, 67 World: 0.4, 0.2, 2.5	3.110	0.0484
Striatum	Voxel: 51, 118, 52 World: -1.2, 3.5, 1.0	3.273	0.0349
Lateral Septum	Voxel: 59, 100, 53 World: -0.3,1.7,0.9	5.610	0.00015
Nucleus Accumbens	Voxel: 43, 112, 32 World: -2.0, 3.0, -1.0	-5.092	0.00057
	POL L vs SAL : age ($\beta_{12} ns(age,3)3{:}groupPOL\_L_{subject,j}$)		
	Coordinates (x,y,z)	**t-value**	**q-value**
Subiculum	Voxel: 92, 66, 59 World: 2.9, -1.6, 1.7	4.680	0.0138
Hippocampus CA1/DG	Voxel: 77, 80, 60 World: 1.4, -0.3, 1.8	-4.552	0.0154
Reticular nucleus	Voxel: 49, 54, 37 World: -1.4, -2.8, -0.5	-4.782	0.0130
Nucleus accumbens	Voxel: 56, 115, 37 World: -0.8, 3.2, -0.6	-4.546	0.0154
Amygdala	Voxel: 37, 98, 27 World: -2.6, 1.6, -1.5	4.748	0.0132
Hypothalamus	Voxel: 73, 82, 25 World: 1.0, 0.0, -1.7	-3.942	0.0292

POL E= GD 9-exposed poly I:C group; **POL L**=GD 17-exposed poly I:C group

The interaction between age modeled as a third order natural spline and group was significant for POL E offspring relative to SAL (t= 4.323, <5%FDR) in small subregions of the right and left subiculum, auditory, motor, and posterior cingulate cortex. This was reflective of an overgrowth in the early adult period in the POL E offspring. Finally, the first order natural spline of age by group interaction was also significant in many regions for POL E vs. SAL offspring (t=3.035, <1%FDR), indicative of a steeper increase in volume over time for POL E offspring relative to the SAL offspring. These regions are mainly cortical, including the motor and somatosensory cortices, visual, and auditory, as well as the bilateral striatum, thalamus, hypothalamus with some subregions of the dorsal and ventral hippocampus as well (**Supplementary figure 4.S5-6)**.



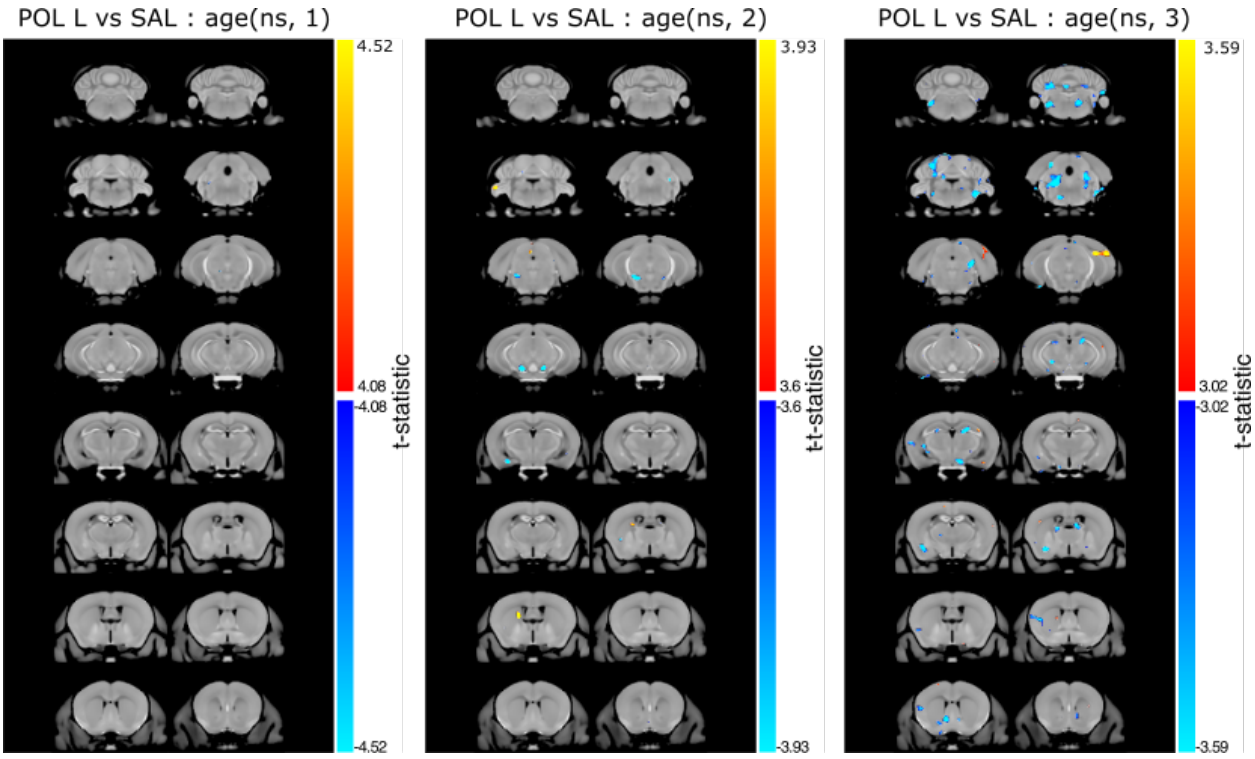
Supplementary figure 4.S5. Summary of results for POL E vs SAL. T-statistic maps for POL E vs SAL first order natural spline of age (left), second order natural spline of age (middle), and third order natural spline of age (right) overlaid on population average (t-statistics thresholded between 10% and 5% FDR).



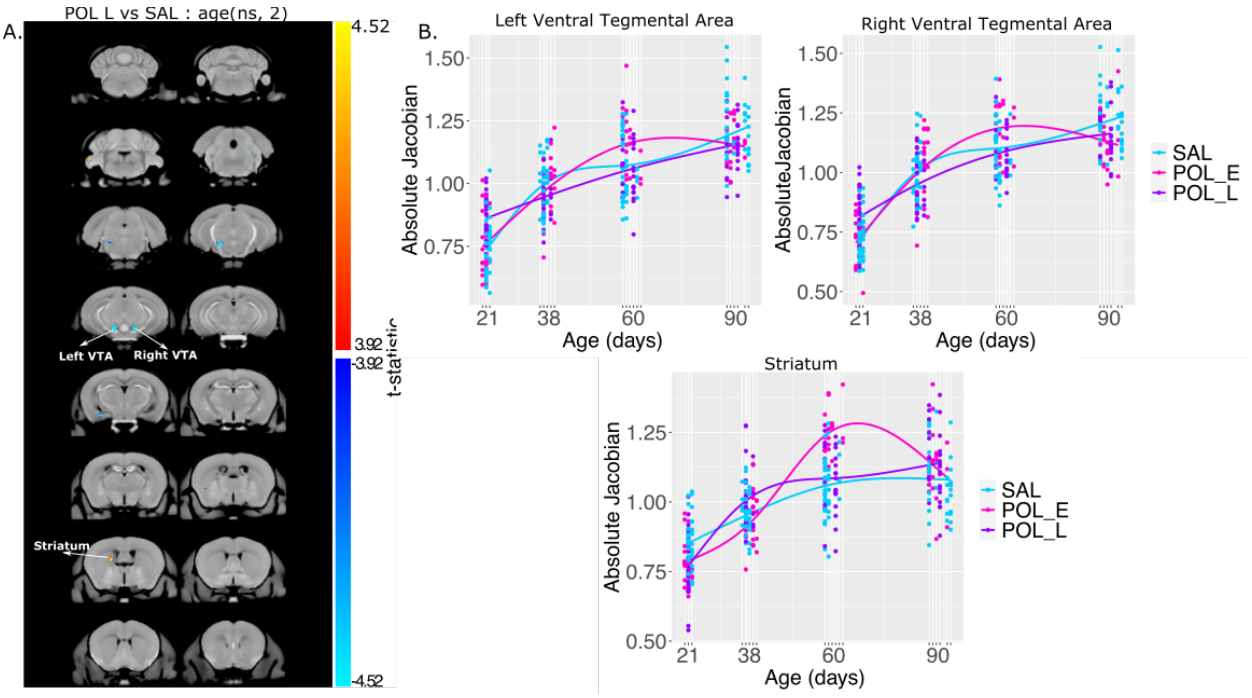
Supplementary figure 4.S6. POL E vs SAL first order and third order natural splines of age plots. **A**. t-statistic map of group (POL E vs SAL) by age (first order natural spline) (t=3.035, <1%FDR). **B**. Plot of peak voxels selected from regions of interest highlighted (**A**), wherein age is plotted on the x-axis, and the absolute Jacobian determinants plotted on the y-axis. Here a value of 1 means the voxel is no different than the average, anything above one is relatively larger, and blow 1 is relatively smaller. Ranges are not normalized to enhance comparison at each specific location in space. Lines are modeled as third order natural splines to reflect statistics. **C**. t-statistic map of group (POL E vs SAL) by age (third order natural spline) (t= 4.323, <5%FDR). **D**. Plots of peak voxels as described in (**B**) with curves modeled as third order natural splines to reflect statistics.

4.9.4 Longitudinal comparison of POL L vs SAL for first and second order natural splines of age

The interaction between the second order spline of age and POL L relative to SAL was significant (t=4.523, <1%FDR) in the bilateral ventral tegmental area, the striatum, and tenia tecta. In these regions, the POL L offspring displayed a flatter curve relative to SAL (and POL E). Finally, the interaction between POL L and the first order spline of age was statistically significant (t=4.519, <5%FDR) only in a few negligible voxels (**Supplementary figure 4.S7-8)**.



Supplementary figure 4.S7. Summary of results for POL L vs SAL. T-statistic maps for POL L vs SAL first order spline of age (left), second order spline of age (middle), and third order spline of age (right) overlaid on population average (t-statistics thresholded between 5% and 10% FDR).

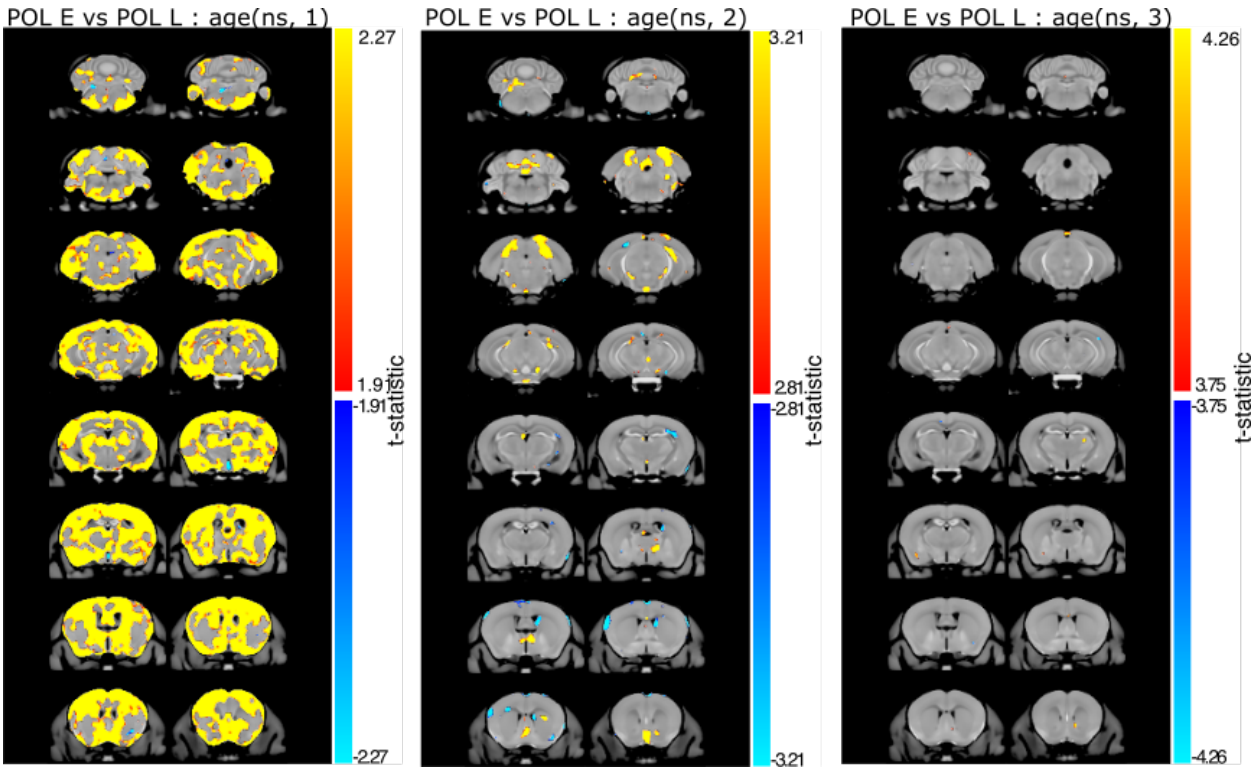


Supplementary figure 4.S8. POL L vs SAL for second order natural spline of age plots. **A**. t-statistic map of group (POL L vs SAL) by age (second order natural spline) (t=4.523, <1%FDR). **B**. Plot of peak voxels selected from regions of interest highlighted (**A**), wherein age is plotted on the x-axis, and the absolute Jacobian determinants plotted on the y-axis. Lines are modeled as third order natural splines to reflect statistics.

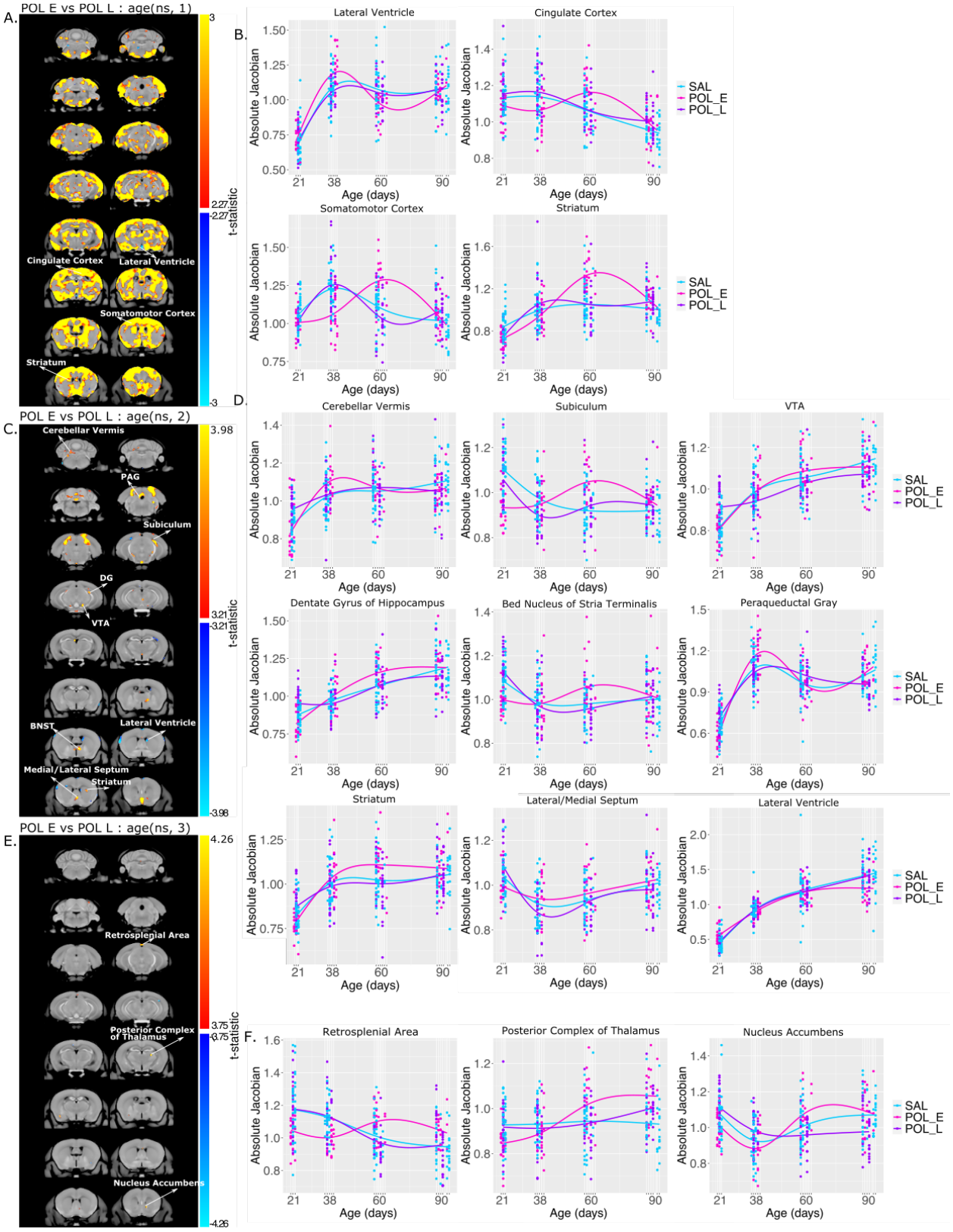
4.9.5 Longitudinal comparison of POL E vs POL L

We re-ran our statistical analysis with the POL L group as the reference group rather than SAL in order to better compare differences between the two POL exposed groups. We observed a significant group (POL E vs. POL L) by age (third order natural spline) interaction (t=4.268, <10%FDR) in a few voxels in the amygdala, nucleus accumbens, thalamus, hippocampus. There was also a significant group (POL E vs POL L) by age (second order natural spline; t=3.977, <1%FDR) in very similar regions to what was observed for the POL E vs SAL comparison, including the striatum, lateral septum, CA1 and dentate gyrus of the hippocampus, subiculum, thalamus, periaqueductal gray, and cerebellum; additionally, differences in the VTA/substantia nigra, bed nucleus of stria terminalis were also observed (**Supplementary figure 4.S9).** These regions displayed a similar trajectory as described in the POL E vs SAL comparison whereby the POL E group had a smaller volume at PND21, followed by an overshoot in the P38-60 period, and

a normalization at PND 90. Finally, there was also a significant effect for the interaction with the first order natural spline of age in the majority of the cortex, as well as the striatum, and many thalamic regions, indicative of a steeper growth in the POL E relative to the POL L group (t=3.001, <1%FDR) (**Supplementary figure 4.S10)**.



Supplementary figure 4.S9. Summary of results for POL E vs POL L. T-statistic maps for POL E vs POL L first order natural spline of age (left), second order natural spline of age (middle), and third order natural spline of age (right) overlaid on population average (t-statistics thresholded between 5% and 10% FDR).

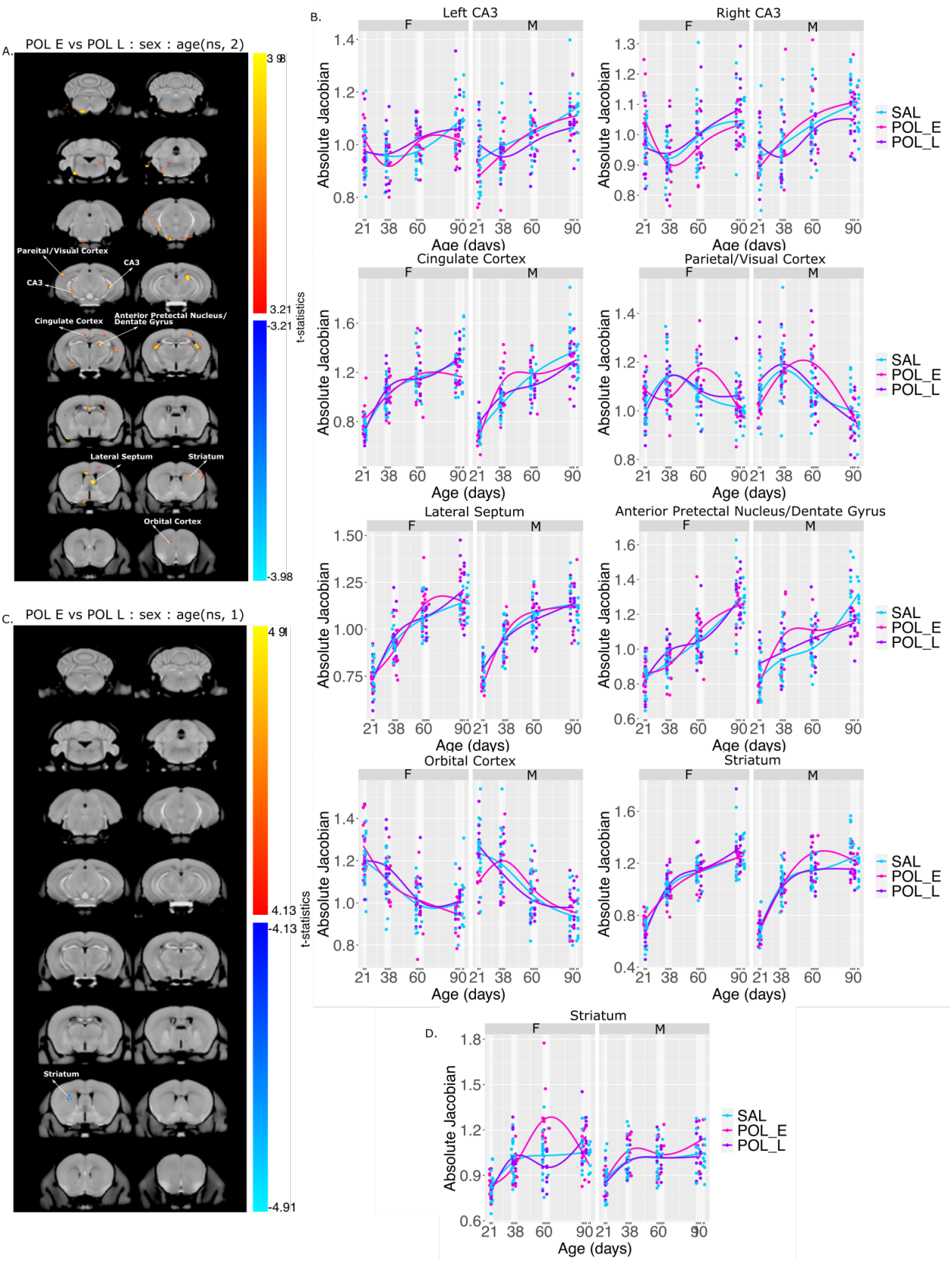


Supplementary figure 4.S10 (caption on next page).

Supplementary figure 4.S10 (continued). POL E vs POL L for all the natural spline fits of age: first order, second order, and third order. **A**. t-statistic map of group (POL E vs POL L) by age (first order natural spline) (t=3.001, <1%FDR). **B**. Plot of peak voxels selected from regions of interest highlighted (**A**), wherein age is plotted on the x-axis, and the absolute Jacobian determinants plotted on the y-axis. Lines are modeled as third order natural splines to reflect statistics. **C.** t-statistic map of group (POL E vs POL L) by age (second order natural spline) thresholded (t=3.977, <1%FDR). **D**. Plots of peak voxels as described in (**B**) with curves modeled as third order natural splines to reflect statistics. **E**. t-statistic map of group (POL E vs POL L) by age (third order natural spline) (t=4.268, <10%FDR). **F**. Plots of peak voxels as described in (**B**) with curves modeled as third order natural splines to reflect statistics.

4.9.6 Longitudinal sex differences

Our longitudinal analysis revealed a no significant sex by group by age interactions with SAL as the reference group. However, with POL L as the reference group, there was a significant POL E by sex by age interaction for the second order natural spline of age fit (t=3.980, <5%FDR). In regions such as the dorsal striatum, lateral septum, orbital cortex, dentate gyrus, hippocampal CA3, cingulate cortex, and parieto-visual cortex, male offspring seemed to have a more pronounced volume peak in the adolescent/early adult period relative to POL L offspring, whereas the difference between female offspring was much more subtle. Similarly, the interaction for first order natural spline of age was also significant, albeit at a more lenient threshold (t=4.907, <10%FDR), in the left striatum, wherein the female offspring had a more pronounced volume increase over time than males, particularly in the early adult period.



Supplementary figure 4.S11 (caption on next page).

Supplementary figure 4.S11 (continued). Sex differences in brain development between POL E and POL L offspring. A. T-statistic map of group (POL E vs. POL L) by age (second order natural spline) by sex interaction thresholded between 5% FDR (t=3.980). B. Plot of peak voxels selected from regions of interest highlighted (A), wherein age is plotted on the x-axis, and the absolute Jacobian determinants plotted on the y-axis. C. t-statistic map of group (POL E vs POL L) by age (first order natural spline) thresholded at 10% FDR (bottom, t=4.91). D. Plots of peak voxels as described in (B) with curves modeled as third order natural splines to reflect statistics.

4.9.7 Behavioural findings

4.9.7.1 Main effects

4.9.7.1.1 Social preference and social novelty

Prenatal MIA exposure did not affect significantly affect preference for a novel mouse over inanimate object for either adolescent POL E (t=-1.109, p=0.289) or POL L (t=-0.209, p=0.839) offspring or adult POL E (t=1.818, p=0.093) or POL L offspring (t=0.380, p=0.7123). Similarly, there were no significant group differences for the social novelty portion of the task for adolescent POL E (t=-0.330, p=0.742) or POL L (t=0.447, p=0.656) offspring. In adulthood, there was a significant impairment in social novelty behaviour for POL E offspring (t=-2.369, p=0.0311) wherein they spent less time exploring the novel intruder relative to the familiar one. Adult POL L offspring did not differ significantly from SAL offspring (t=-1.257, p=0.233).

4.9.7.1.2 Prepulse inhibition

Adolescent POL E offspring were found to have significant impairment in sensorimotor gating, with significantly decreased percent prepulse inhibition relative to SAL (t=-4.335, p=0.0000005). No difference was observed for POL L offspring relative to SAL (t=-0.373, p=0.709). In adulthood, no differences were observed for POL E (t=-0.283, p=0.7837) or POL L (t=-0.549, p=0.5983) offspring.

We also investigated whether or not there was an interaction between increasing prepulse level and group. We did not observe any significant differences in trajectory in adolescence for POL E (t=-0.995, p=0.3209) or POL L (t=-0.887, p=0.376361) offspring in adolescence. We did observe a significant interaction for POL L offspring in adulthood (t=-2.211, p=0.0281) but not for POL E offspring (t=-1.401, p=0.163). There was a significant interaction between prepulse

level and POL L group (t=-2.211, p=0.0281) indicative of POL L deficits emerging only at louder prepulse tones (12 and 15 dB). Finally, on the ASST, adult POL L offspring displayed impaired learning and flexibility as they required significantly more trials than SAL to reach learning criterion on the intradimensional shift portion of the task (t=2.486, p=0.0129) (summarized in **Supplementary table 4.S4)**.

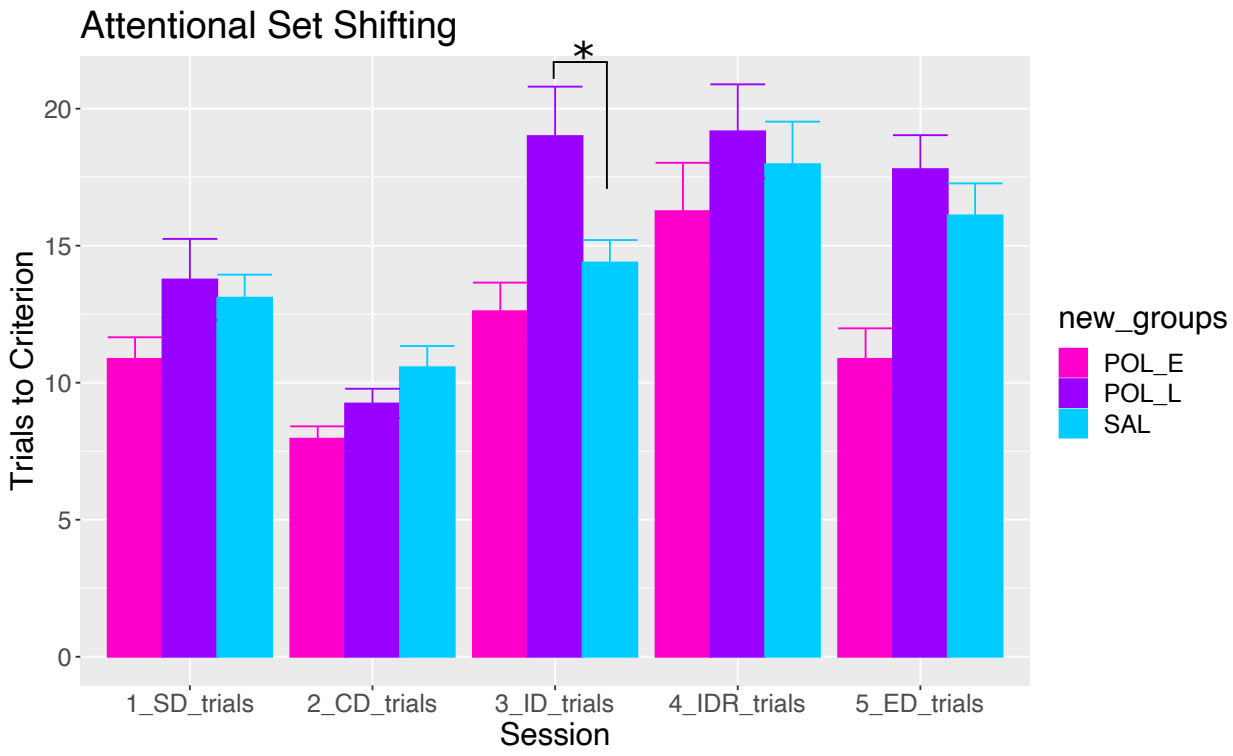
Supplementary table 4.S4. Summary of all behavioural results for POL E and POL L offspring relative to SAL controls. T-values, p-values (uncorrected) and q-values (corrected) are bolded if they survive Bonferroni correction (q-value=p<0.0045).

	Adolescence		**Adulthood**	
	POL E	**POL L**	**POL E**	**POL L**
OFT	t=-2.294, p=0.039, q=0.429	t=-1.716, p=0.108, q=1.000	t=1.116, p=0.281, q=3.091	t=-0.069, p=0.946, q=1.000
Marble Burying	**t=2.937, p=0.003, q=0.033**	t=0.901, p=0.368, q=1.000	t=1.055, p=0.291, q=3.201	t= 0.117, p=0.907, q=1.000
SOPT	t=-1.109, p=0.289, q=1.000	t=-0.209, p=0.839, q=1.000	t=1.818, p=0.093, q=1.000	t=0.380, p=0.712, q=1.000
SONT	t=-0.330, p=0.742, q=1.000	t=0.447, p=0.656, q=1.000	t=-2.369, p=0.031, q=0.341	t=-1.257, p=0.233, q=1.000
PPI	Overall: **t=-4.202, p=0.0000004, q=0.000004** By PP tone: t=-0.995, p=0.321, q=1.000	Overall: t=-0.373, p=0.709, q=1.000 By PP tone: t=-0.887, p=0.376, q=1.000	Overall: t=-0.283, p=0.784, q=1.000 By PP tone: t=-1.401, p=0.163, q=1.000	Overall: t=-0.549, p=0.598, q=1.000 By PP tone: t=-2.211, p=0.0281, q=0.310
ASST	NA	NA	CD: t=-0.823, p=0.411, q=1.000 ID: t=0.522, p=0.602, q=1.000 IDR: t=0.918, p=0.359, q=1.000 ED: t=-1.879, p=0.060, q=0.660	CD: t=1.853, p=0.064, q=0.704 ID: t=2.486, p=0.0129, q=0.142 IDR: t=0.158, p=0.874, q=1.000 ED: t=0.535, p=0.593, q=1.000

POL E= GD9-exposed poly I:C group; **POL L**=GD17-exposed poly I:C group; **OFT**= open field test; **SOPT=** social preference task; **SONT**= social novelty task; **PPI**= prepulse inhibition; **ASST**= attentional set shifting; **SD=** simple discrimination, **CD=** compound discrimination, **ID=** Intradimensional shift, **IDR=** Intradimensional shift reversal, **ED=** Extra-dimensional shift.

4.9.7.1.3 Attentional set shifting task

Adult POL L offspring required more trials than SAL to reach learning criterion on the IDR portion (t=2.486, p=0.0129) suggesting some impairment in learning; they also required slightly more trials on the CD portion of the task ((t=-1.853 0.064). POL E offspring required fewer trials than SAL in the ED portion of the task (t=-1.879, p=0.0602), perhaps indicative of greater flexibility or poorer learning of the previous day's task (**Supplementary figure 4.S12)**.



Supplementary figure 4.S12. Attentional set shifting task results. Bar graph showing trails to reach learning criterion (6 correct trials in a row) for each portion of the task: **SD=** simple discrimination, **CD=** compound discrimination, **ID=** Intradimensional shift, **IDR=** Intradimensional shift reversal, **ED=** Extra-dimensional shift. Only subtle differences emerged, with POL L requiring more trials in the ID to reach learning criterion (t=2.486, p=0.0129). *p<0.05.

4.9.7.2 Behavioural sex differences

No sex differences were observed in either the open field task or the marble burying task (**Supplementary figure 4.S13)**.

4.9.7.2.1 Social preference and social novelty

Examination of sex differences in social preference did not result in a sex-by-group interaction for adolescent mice, however there was a trend level effect for the main effect of POL E treatment (t=-1.893, p=0.0671), wherein POL E offspring, particularly males, had a lower sociability index. In adulthood, we observed a trend level sex-by-group interaction for POL E offspring (t=-1.829, p=0.0712) wherein POL E males actually had a higher sociability index relative to SAL with no difference for females. The POL E main effect was also significant in this model (t=2.627, p=0.0124). No sex effects were observed in adolescent offspring for social novelty behaviour. The same was true for adult behaviour, however the POL E main effect was significant (t=-2.051p=0.0471).

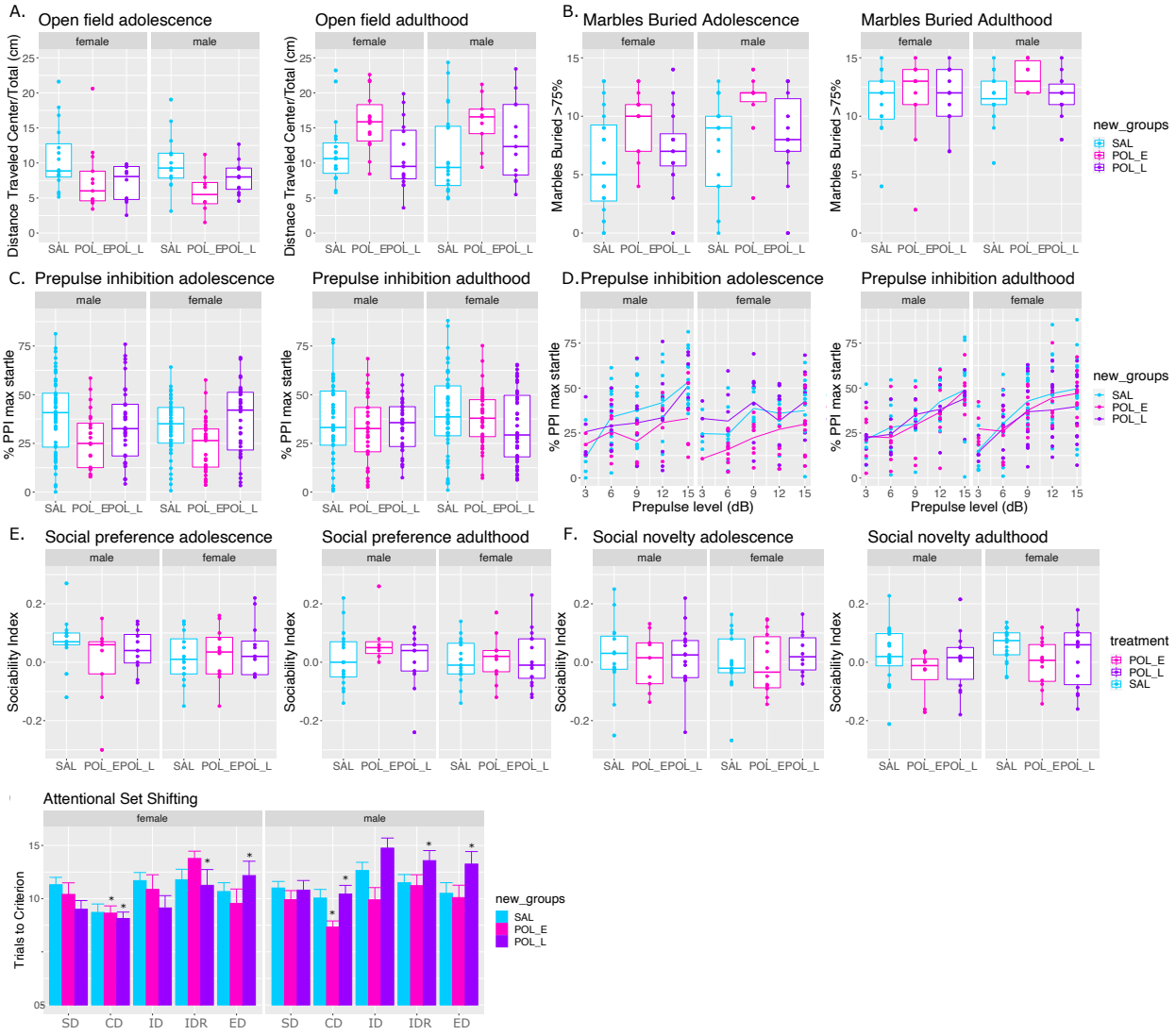
4.9.7.2.2 Sensorimotor gating

We also explored sex differences and observed no significant sex-by-group by prepulse level interactions, however this model revealed a number of trend level group-by-prepulse level interactions for POL E offspring at PP 15 (t=-1.830, p=0.0691), and for POL L offspring at PP 12 (t=-1.784, p=0.0764). Sex differences were also investigated with increasing prepulse level. There were no sex-by-group-by-level interactions in adolescence, however the group-by-level interaction for POL E (t=-1.755, p=0.0809) and the sex-by-level interaction (t=-1.957, p=0.0520) both trended towards significance, indicative of the fact that females, and POL E offspring had lower percent PPI. No effects were observed in adulthood.

4.9.7.2.3 Attentional set shifting task

Exploration of sex differences revealed a number of sex-by-group-by-trial interactions as follows. For the CD portion of the task the three-way interaction was significant both for POL E (t=2.156, p=0.0311) and POL L (t=2.138, p=0.0325) groups vs. SAL wherein POL E females required more trials than males to reach learning criterion and POL L females required fewer trials than males. Further, the three-way interaction was also significant for the POL L group relative to

SAL for the IDR (t=2.690, p=0.0071) and ED (t=4.140 p=3.47e-05) portions of the task wherein POL L females required more trials to reach learning criterion than males (relative to SAL controls).

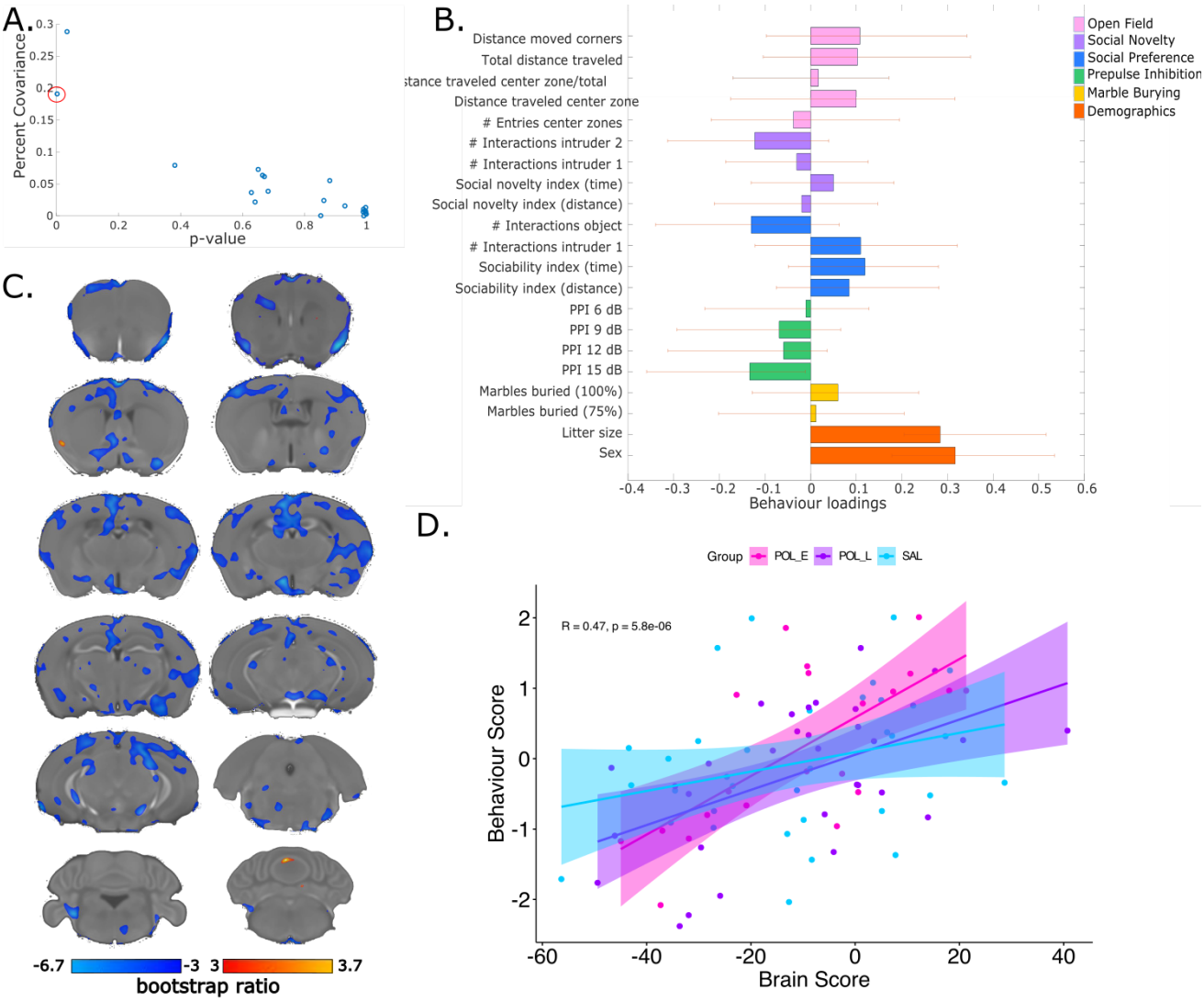


Supplementary figure 4.S13 (caption continued on next page). Behavioural results split by sex. Results for adolescent (left) and adult (right) offspring from our three groups: SAL (cyan), POL E (magenta), POL L (purple), with male results on the left, panels, and female results on the right. For all boxplots the midline represents the median of the data, the box represents the first and third quartiles, and the vertical line and dots represent the end range of the data. **A.** Open field data for distance traveled in the center zone relative to total distance traveled. In adolescence (left), results. No statistically significant differences observed in adulthood (right). **B**. Significantly more marbles buried by POL E adolescent offspring (left; and no differences in adulthood (right). **C**. No

Supplementary figure 4.S13 (continued). significant differences in PPI overall, or **D.** over increasing prepulse tone. **E**. No significant differences in sociability index for the social preference task (i.e., preference for novel mouse over non-social object) between groups at either adolescence (left) or adulthood (right). **F**. No significant differences in sociability index for social novelty (i.e., preference for novel mouse over familiar mouse) between groups. **F**. In the ASST task, a significant For the CD portion of the task the three-way interaction was observed in CD for POL E ($t=2.156$, $p=0.0311$) and POL L ($t=2.138$, $p=0.0325$) groups vs. SAL, and for the POL L group relative to SAL in IDR ($t=2.690$, $p=0.0071$) and ED ($t=4.140$ $p=3.47e-05$). ˙ $p<0.05$; *$p<0.0045$ (Bonferroni correction threshold); ***$p<0.0001$

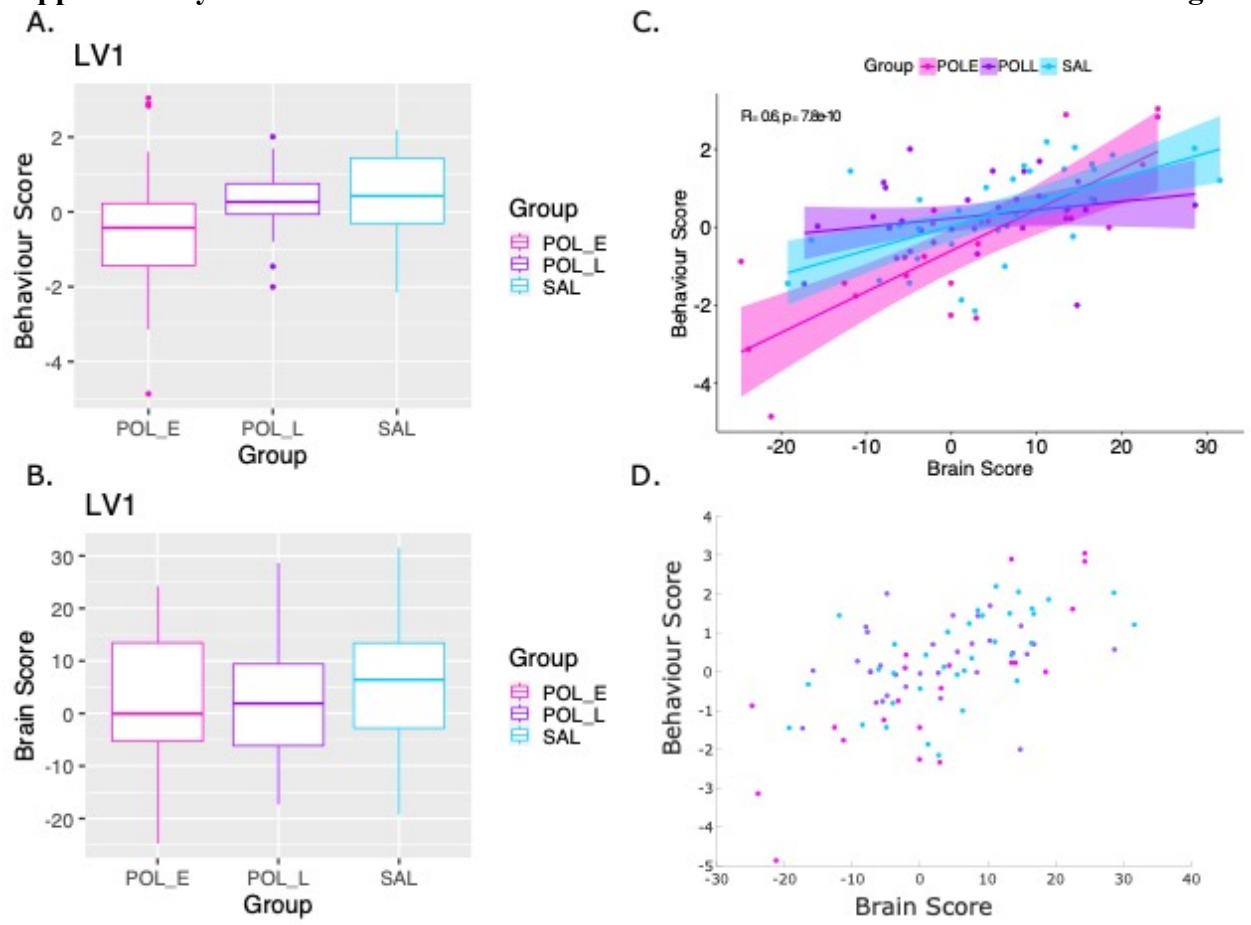
4.9.8 Multivariate analysis of brain-behaviour data

The second latent variable (LV2: 19% covariance explained, $p=0.002$) identified a pattern of brain-behaviour driven by sex and litter size. Female sex and belonging to a large litter was associated with smaller volume of regions in blue, such as motor, somatosensory, cingulate, auditory, and visual cortices, nucleus accumbens, amygdala, hippocampus (**Supplementary figure 4.S14)**. Further details for the brain and behaviours loadings from the first LV, described in section **4.5.3 & Figure 4.4** are presented in **supplementary figure 4.S15,** while the exploration of how brain-behaviour scores calculated in adolescence translate to the adult timepoint is described in **supplementary figure 4.S16.**

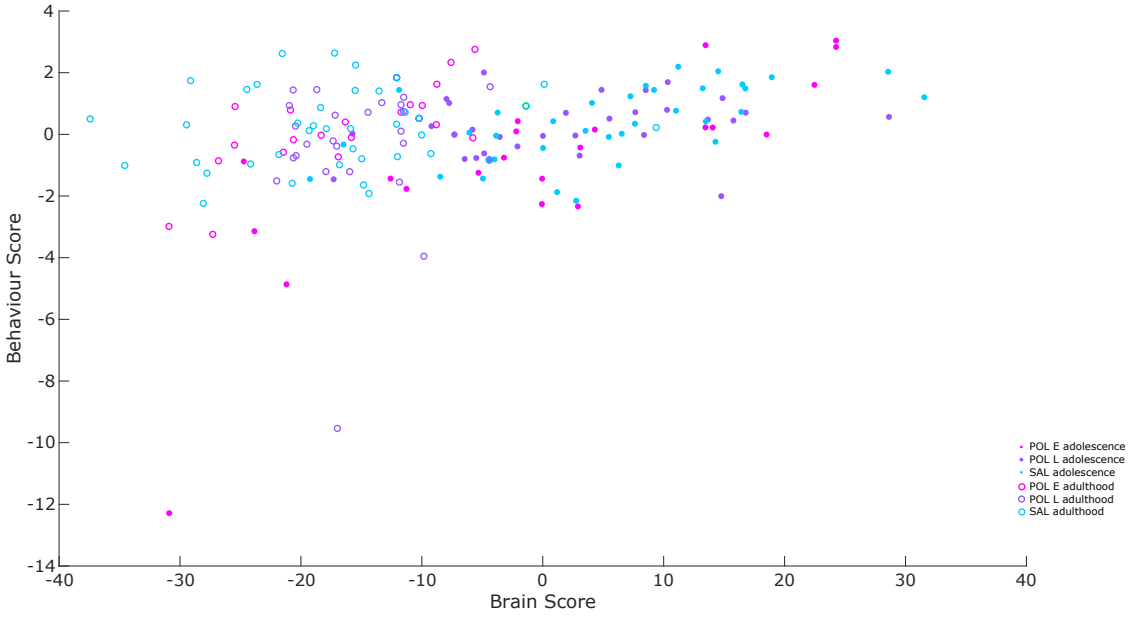


Supplementary figure 4.S14. PLS results for LV2. **A**. Covariance explained (y-axis) and permutation p-values (x-axis) for all 21 LVs in the PLS analysis. LV2 is circled in red (19% covariance explained, p=0.002). **B**. Behaviour score pattern for each behavioural measure included in the analysis. Singular value decomposition estimates the size of the bars, and confidence intervals are calculated by bootstrapping. **C**. Brain pattern bootstrap rations overlaid on the population average, with positive bootstrap ratios in orange-yellow, and negative in blue. **D**. Correlation of individual mouse brain and behaviour score color coded by treatment group.

Supplementary figure



4.S15. Breakdown of brain and behaviour scores by group. Boxplot for LV1 behaviour score **(A)** and brain score **(B)** for each of the three groups. **C.** Correlation between brain-behaviour weights in adolescence with a correlation line per group, highlighting that the POL E group has the strongest brain-behaviour correlation for the LV1 pattern. **D.** Correlation of brain-behaviour without one POL E subject that was an outlier in their behaviour score.



Supplementary figure 4.S16. Projection of adolescent brain-behaviour scores into adulthood. Correlation between the brain-behaviour weights for LV1computed on adolescent data (filled in dots) multiplied by brain and behaviour data for the same tests and same mice in adulthood (open dots). SAL (cyan), POL E (magenta), POL L (purple)

4.9.9 Transcriptional results

4.9.9.1 Differential gene expression and pathway enrichment analysis for pooled ROIs (ACC, dHIP, vHIP)

Pooling all ROIs, we identified 962 genes significantly ($q<0.05$) down-regulated and 668 genes upregulated in POL E relative to SAL mice. Pathway enrichment analysis for upregulated DEGs across pooled ROIs identified genes associated with myelin sheath, mitochondrion, negative regulation of FGF1, 2, 3, 4 signaling, TCA cycle, to name a few. Downregulated DEGs pooled across ROIs were enriched for processes such as nucleoplasm, nucleic acid metabolic process, osteoclast differentiation, transcription, IL-1-signaling pathway, and more. These results recapitulate some of the terms observed in the ROI specific analyses, which provide a more nuanced understanding of the transcriptional landscape in the MIA-exposed brains.

4.9.9.2 Sex differences in differential gene expression

When further breaking down the groups to investigate sex effects, more DEGs were identified in male than female mice in all ROIs. For the dHIP, 199 down- and 381 upregulated genes were observed in males, and 0 in females. For the vHIP 2 down- and 2 upregulated genes were observed in males, whereas 31 down- and 11 upregulated genes were observed in females, and for the ACC 28 down- and 4 upregulated genes were observed in males, and 1 down regulated gene in females.

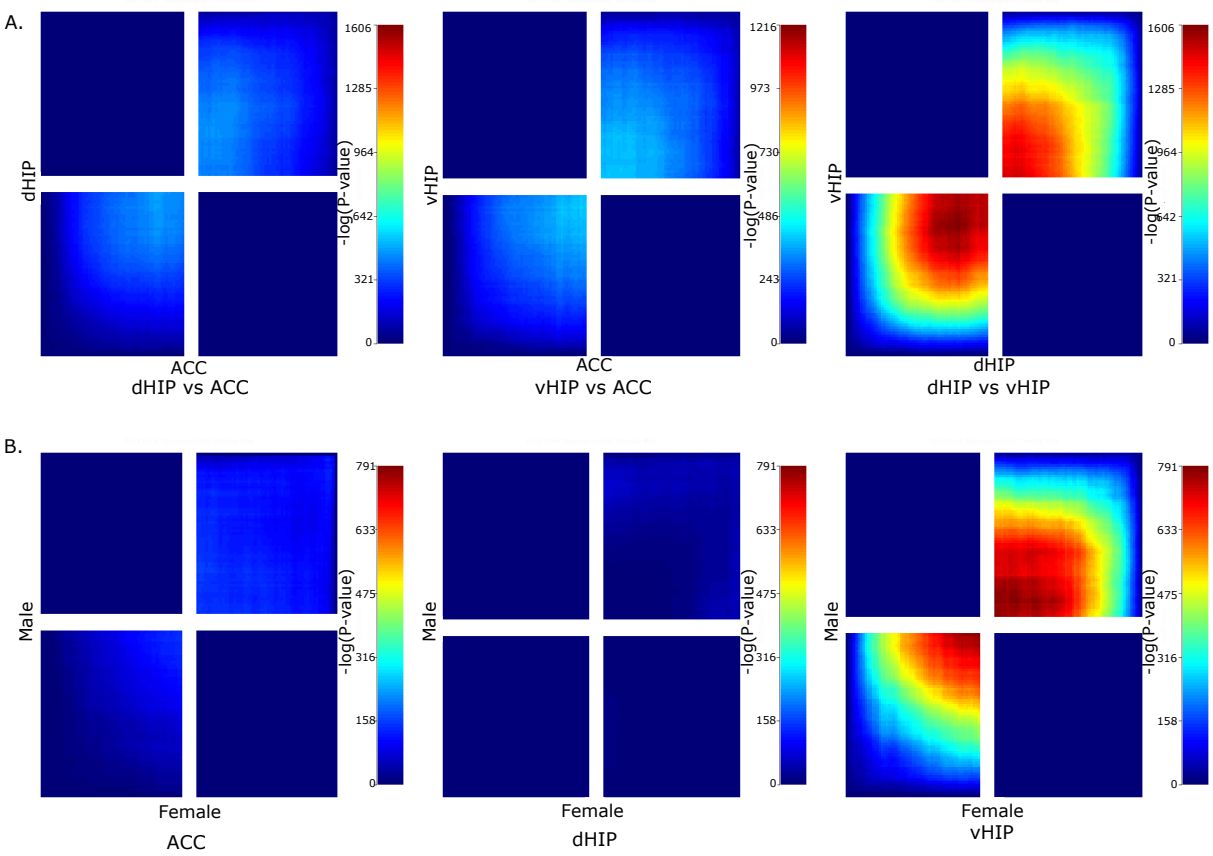
Upregulated genes for males in the ACC were enriched for non-homologous end joining genes, involved in repairing double-strand breaks in DNA, whereas downregulated genes were enriched for various inflammatory markers including TNF signaling, T cell receptor pathways, IL-17 and IL-1 signaling, as well as apoptosis, and oxidative stress. In the male dHIP, upregulated genes were enriched for synapse structure and function, dendrites, and neurotransmitter release, whereas downregulated genes were enriched for HDMs demethylate histones, and various miRNAs (miR-499-5p, miR-208b-3p, miR-208a-3p). Finally, the male vHIP upregulated genes were enriched for metal sequestration by antimicrobial proteins.

For females, upregulated vHIP genes were enriched for IL-1 signaling, as well as sweat gland function. No other ROIs had significant enchantment results.

4.9.9.3 Sex differences in RRHO

The results were very concordant as expected, we observed the vHIP brain region as carrying most of the differential expression overlap concordance signal between males and females (**Supplementary figure 4.S17**). The strongest overlap for males was for coordinately upregulated genes in the dHIP and vHIP (4878), followed by downregulated genes in these two ROIs (4271). There was also robust overlap for ACC and dHIP upregulated genes (4102), and to a lesser extent for concordantly downregulated genes (2365). Overlap between the ACC and vHIP was also observed with 3252 coordinately upregulated and 3127 downregulated genes. Genes coordinately downregulated in the ACC and dHIP, ACC and vHIP, and dHIP and vHIP were enriched for ribosomal function, translation, and mRNA. Coordinately upregulated genes for all pairs of ROIs were enriched for myelination, oligodendrocyte differentiation, synaptic vesicle regulation, and intracellular transport (**Supplementary figure 4.S17A)**.

For females, the strongest overlap was observed for coordinately downregulated genes in the ACC and vHIP (4116), followed by upregulated genes in the ACC and dHIP (3800), upregulated genes in the ACC and vHIP (3615), and the dHIP and vHIP (3186). Surprisingly there was very little overlap in concordantly downregulated genes in the ACC and dHIP (830) and dHIP and vHIP (247). Pathway analysis revealed concordantly upregulated genes in all pairs of ROIs were enriched for mitochondrial function, translation, and ribosomal function, in addition to synaptic function for the dHIP and vHIP upregulated genes. Concordantly downregulated genes were less homogeneous; in the ACC and dHIP overlap, enrichment was observed for protein-protein interactions at synapses; for the ACC and vHIP, enrichment was observed for cilium assembly and organization, extracellular structural matrix, microglia pathogen phagocytosis pathway; finally, for the dHIP and vHIP, enrichment was observed for serotonin and anxiety related events, complement and coagulation cascades, and IL-17 signaling (**Supplementary figure 4.S17B)**.



Supplementary figure 4.S17. A. RRHO heatmaps for the dHIP vs ACC (left), vHIP vs ACC (middle) and vHIP vs dHIP (right). For heat maps, the top left quadrant indicates the overlap in genes up-regulated in the first region and downregulated in the second region. The top right quadrant indicates overlap in genes downregulated in both regions. The bottom left quadrant indicates overlap in genes upregulated in both regions. The bottom right quadrant indicates overlap in genes downregulated in the first region and upregulated in the second. **B**. RRHOs heatmaps comparing up- and downregulated genes in males vs. females for each ROI, with ACC (left), dHIP (middle), vHIP (right).

4.9.9.4 Supplementary tables

Summary tables for differential gene expression:

Supplementary table 4.S5. g:Profiler results for pathway enrichment analysis for POL E vs SAL for ACC, dHIP, vHIP, and all pooled ROIS, for both up- and downregulated DEGs

Structure	Direction	p-value	term size	term id	source	term name
ACC	DOWN	0.011	28	WP:WP2872	WP	White fat cell differentiation
ACC	DOWN	0.019	34	WP:WP37	WP	IL-1 Signaling Pathway
dHIP	UP	0.000	23	REAC:R-MMU-5654732	REAC	Negative regulation of FGFR3 signaling
dHIP	UP	0.000	333	MIRNA:mmu-miR-466i-3p	MIRNA	mmu-miR-466i-3p
dHIP	UP	0.000	25	REAC:R-MMU-5654726	REAC	Negative regulation of FGFR1 signaling
dHIP	UP	0.001	26	REAC:R-MMU-5654727	REAC	Negative regulation of FGFR2 signaling
dHIP	UP	0.001	32	REAC:R-MMU-5654741	REAC	Signaling by FGFR3
dHIP	UP	0.002	396	HP:0000729	HP	Autistic behavior
dHIP	UP	0.003	39	REAC:R-MMU-5654736	REAC	Signaling by FGFR1
dHIP	UP	0.011	23	REAC:R-MMU-5654733	REAC	Negative regulation of FGFR4 signaling
dHIP	UP	0.013	476	MIRNA:mmu-miR-362-3p	MIRNA	mmu-miR-362-3p
dHIP	UP	0.013	476	MIRNA:mmu-miR-329-3p	MIRNA	mmu-miR-329-3p
dHIP	UP	0.015	96	KEGG:04217	KEGG	Necroptosis

dHIP	UP	0.016	47	HP:0000653	HP	Sparse eyelashes
dHIP	UP	0.019	57	REAC:R-MMU-5654738	REAC	Signaling by FGFR2
dHIP	UP	0.030	31	REAC:R-MMU-5654743	REAC	Signaling by FGFR4
dHIP	UP	0.033	10	REAC:R-MMU-5654704	REAC	SHC-mediated cascade:FGFR3
dHIP	UP	0.033	16	REAC:R-MMU-1295596	REAC	Spry regulation of FGF signaling
dHIP	UP	0.038	66	REAC:R-MMU-190236	REAC	Signaling by FGFR
dHIP	UP	0.041	6039	MIRNA:000000	MIRNA	MIRNA root
dHIP	UP	0.048	12	REAC:R-MMU-5654710	REAC	PI-3K cascade:FGFR3
dHIP	DOWN	0.017	67	WP:WP1254	WP	Apoptosis
dHIP	DOWN	0.018	79	MIRNA:mmu-miR-3097-3p	MIRNA	mmu-miR-3097-3p
dHIP	DOWN	0.021	13	MIRNA:mmu-miR-499-5p	MIRNA	mmu-miR-499-5p
dHIP	DOWN	0.022	83	MIRNA:mmu-miR-717	MIRNA	mmu-miR-717
dHIP	DOWN	0.031	103	GO:0030218	GO:BP	erythrocyte differentiation
dHIP	DOWN	0.032	2	CORUM:6576	CORUM	Anks3-Hif1an complex
dHIP	DOWN	0.036	3	CORUM:6279	CORUM	p65-IkappaBalpha-beta-arrestin-iNOS complex
dHIP	DOWN	0.039	2	HP:0005511	HP	Heinz body anemia
vHIP	DOWN	0.001	69	KEGG:05220	KEGG	Chronic myeloid leukemia
vHIP	DOWN	0.002	81	KEGG:05222	KEGG	Small cell lung cancer

vHIP	DOWN	0.004	49	KEGG:04657	KEGG	IL-17 signaling pathway
vHIP	DOWN	0.004	3	CORUM:6279	CORUM	p65-IkappaBalpha-beta-arrestin-iNOS complex
vHIP	DOWN	0.004	110	KEGG:04210	KEGG	Apoptosis
vHIP	DOWN	0.010	148	KEGG:05169	KEGG	Epstein-Barr virus infection
vHIP	DOWN	0.036	45	KEGG:05134	KEGG	Legionellosis
Pooled	UP	0.000	201	GO:0043209	GO:CC	myelin sheath
Pooled	UP	0.000	1628	GO:0005739	GO:CC	mitochondrion
Pooled	UP	0.000	27	REAC:R-MMU-5654726	REAC	Negative regulation of FGFR1 signaling
Pooled	UP	0.000	28	REAC:R-MMU-5654727	REAC	Negative regulation of FGFR2 signaling
Pooled	UP	0.000	27	WP:WP434	WP	TCA Cycle
Pooled	UP	0.000	74	WP:WP662	WP	Amino Acid metabolism
Pooled	UP	0.000	25	REAC:R-MMU-5654732	REAC	Negative regulation of FGFR3 signaling
Pooled	UP	0.000	25	REAC:R-MMU-5654733	REAC	Negative regulation of FGFR4 signaling
Pooled	UP	0.000	41	REAC:R-MMU-5654736	REAC	Signaling by FGFR1
Pooled	UP	0.000	60	REAC:R-MMU-5654738	REAC	Signaling by FGFR2
Pooled	UP	0.000	69	REAC:R-MMU-190236	REAC	Signaling by FGFR
Pooled	UP	0.000	34	REAC:R-MMU-5654741	REAC	Signaling by FGFR3

Pooled	UP	0.000	33	REAC:R-MMU-5654743	REAC	Signaling by FGFR4
Pooled	UP	0.000	57	REAC:R-MMU-74752	REAC	Signaling by Insulin receptor
Pooled	UP	0.000	22	KEGG:00020	KEGG	Citrate cycle (TCA cycle)
Pooled	UP	0.000	7	CORUM:52	CORUM	CCT complex (chaperonin containing TCP1 complex)
Pooled	UP	0.000	7	CORUM:51	CORUM	CCT complex (chaperonin containing TCP1 complex)
Pooled	UP	0.000	7	CORUM:132	CORUM	CCT complex (chaperonin containing TCP1 complex)
Pooled	UP	0.000	16	REAC:R-MMU-1295596	REAC	Spry regulation of FGF signaling
Pooled	UP	0.000	51	REAC:R-MMU-917937	REAC	Iron uptake and transport
Pooled	UP	0.001	7	CORUM:3072	CORUM	CCT complex (chaperonin containing TCP1 complex), testis specific
Pooled	UP	0.001	47	REAC:R-MMU-71406	REAC	Pyruvate metabolism and Citric Acid (TCA) cycle
Pooled	UP	0.001	54	REAC:R-MMU-5610785	REAC	GLI3 is processed to GLI3R by the proteasome
Pooled	UP	0.001	73	REAC:R-MMU-195253	REAC	Degradation of beta-catenin by the destruction complex
Pooled	UP	0.001	249	GO:0098798	GO:CC	mitochondrial protein complex
Pooled	UP	0.001	38	WP:WP157	WP	Glycolysis and Gluconeogenesis
Pooled	UP	0.002	31	REAC:R-MMU-109704	REAC	PI3K Cascade
Pooled	UP	0.002	121	REAC:R-MMU-1428517	REAC	The citric acid (TCA) cycle and respiratory electron transport
Pooled	UP	0.002	32	REAC:R-MMU-112399	REAC	IRS-mediated signalling

Pooled	UP	0.002	14	REAC:R-MMU-5654688	REAC	SHC-mediated cascade:FGFR1
Pooled	UP	0.002	227	REAC:R-MMU-5683057	REAC	MAPK family signaling cascades
Pooled	UP	0.003	21	REAC:R-MMU-71403	REAC	Citric acid cycle (TCA cycle)
Pooled	UP	0.003	15	REAC:R-MMU-5654699	REAC	SHC-mediated cascade:FGFR2
Pooled	UP	0.003	363	HP:0009127	HP	Abnormality of the musculature of the limbs
Pooled	UP	0.003	34	REAC:R-MMU-2428924	REAC	IGF1R signaling cascade
Pooled	UP	0.003	34	REAC:R-MMU-2428928	REAC	IRS-related events triggered by IGF1R
Pooled	UP	0.003	34	REAC:R-MMU-2404192	REAC	Signaling by Type 1 Insulin-like Growth Factor 1 Receptor (IGF1R)
Pooled	UP	0.003	419	HP:0003679	HP	Pace of progression
Pooled	UP	0.003	527	REAC:R-MMU-382551	REAC	Transport of small molecules
Pooled	UP	0.003	16	REAC:R-MMU-5654689	REAC	PI-3K cascade:FGFR1
Pooled	UP	0.003	2	CORUM:25	CORUM	9S-cytosolic aryl hydrocarbon (Ah) receptor non-ligand activated complex
Pooled	UP	0.004	12	REAC:R-MMU-5684264	REAC	MAP3K8 (TPL2)-dependent MAPK1/3 activation
Pooled	UP	0.004	213	REAC:R-MMU-9607240	REAC	FLT3 Signaling
Pooled	UP	0.004	17	REAC:R-MMU-5654693	REAC	FRS-mediated FGFR1 signaling

Pooled	UP	0.004	17	REAC:R-MMU-5654695	REAC	PI-3K cascade:FGFR2
Pooled	UP	0.004	36	REAC:R-MMU-74751	REAC	Insulin receptor signalling cascade
Pooled	UP	0.005	28	GO:0006099	GO:BP	tricarboxylic acid cycle
Pooled	UP	0.005	112	KEGG:05012	KEGG	Parkinson disease
Pooled	UP	0.005	18	REAC:R-MMU-5654700	REAC	FRS-mediated FGFR2 signaling
Pooled	UP	0.005	54	REAC:R-MMU-450294	REAC	MAP kinase activation
Pooled	UP	0.005	54	REAC:R-MMU-448424	REAC	Interleukin-17 signaling
Pooled	UP	0.005	54	REAC:R-MMU-5676590	REAC	NIK-->noncanonical NF-kB signaling
Pooled	UP	0.005	54	REAC:R-MMU-5607761	REAC	Dectin-1 mediated noncanonical NF-kB signaling
Pooled	UP	0.005	68	REAC:R-MMU-8852276	REAC	The role of GTSE1 in G2/M progression after G2 checkpoint
Pooled	UP	0.005	96	KEGG:01200	KEGG	Carbon metabolism
Pooled	UP	0.006	1381	REAC:R-MMU-1430728	REAC	Metabolism
Pooled	UP	0.006	2606	GO:0031090	GO:CC	organelle membrane
Pooled	UP	0.006	50	REAC:R-MMU-6781823	REAC	Formation of TC-NER Pre-Incision Complex
Pooled	UP	0.006	155	HP:0003677	HP	Slow progression
Pooled	UP	0.008	15	GO:0033178	GO:CC	proton-transporting two-sector ATPase complex, catalytic domain
Pooled	UP	0.008	438	GO:0007005	GO:BP	mitochondrion organization

Pooled	UP	0.008	483	REAC:R-MMU-199991	REAC	Membrane Trafficking
Pooled	UP	0.009	79	REAC:R-MMU-168138	REAC	Toll Like Receptor 9 (TLR9) Cascade
Pooled	UP	0.009	36	REAC:R-MMU-5696394	REAC	DNA Damage Recognition in GG-NER
Pooled	UP	0.010	60	REAC:R-MMU-8878166	REAC	Transcriptional regulation by RUNX2
Pooled	UP	0.010	59	REAC:R-MMU-1169091	REAC	Activation of NF-kappaB in B cells
Pooled	UP	0.010	205	REAC:R-MMU-5684996	REAC	MAPK1/MAPK3 signaling
Pooled	UP	0.010	151	HP:0002460	HP	Distal muscle weakness
Pooled	UP	0.010	22	REAC:R-MMU-5654696	REAC	Downstream signaling of activated FGFR2
Pooled	UP	0.010	200	REAC:R-MMU-5673001	REAC	RAF/MAP kinase cascade
Pooled	UP	0.010	319	GO:0005759	GO:CC	mitochondrial matrix
Pooled	UP	0.010	975	HP:0031826	HP	Abnormal reflex
Pooled	UP	0.012	23	REAC:R-MMU-5654687	REAC	Downstream signaling of activated FGFR1
Pooled	UP	0.013	8	REAC:R-MMU-390471	REAC	Association of TriC/CCT with target proteins during biosynthesis
Pooled	UP	0.013	21	GO:0101031	GO:CC	chaperone complex
Pooled	UP	0.013	120	REAC:R-MMU-8856828	REAC	Clathrin-mediated endocytosis
Pooled	UP	0.014	97	REAC:R-MMU-5696398	REAC	Nucleotide Excision Repair

Pooled	UP	0.014	683	HP:0001257	HP	Spasticity
Pooled	UP	0.014	341	HP:0004302	HP	Functional motor deficit
Pooled	UP	0.015	97	REAC:R-MMU-5610787	REAC	Hedgehog 'off' state
Pooled	UP	0.015	168	REAC:R-MMU-69275	REAC	G2/M Transition
Pooled	UP	0.017	2	CORUM:3179	CORUM	Grb2-Shp2 complex, FGF stimulated
Pooled	UP	0.017	170	REAC:R-MMU-453274	REAC	Mitotic G2-G2/M phases
Pooled	UP	0.018	105	REAC:R-MMU-9612973	REAC	Autophagy
Pooled	UP	0.019	65	REAC:R-MMU-166058	REAC	MyD88:MAL(TIRAP) cascade initiated on plasma membrane
Pooled	UP	0.019	65	REAC:R-MMU-975871	REAC	MyD88 cascade initiated on plasma membrane
Pooled	UP	0.019	65	REAC:R-MMU-168179	REAC	Toll Like Receptor TLR1:TLR2 Cascade
Pooled	UP	0.019	65	REAC:R-MMU-168188	REAC	Toll Like Receptor TLR6:TLR2 Cascade
Pooled	UP	0.019	65	REAC:R-MMU-168176	REAC	Toll Like Receptor 5 (TLR5) Cascade
Pooled	UP	0.019	65	REAC:R-MMU-168142	REAC	Toll Like Receptor 10 (TLR10) Cascade
Pooled	UP	0.019	65	REAC:R-MMU-181438	REAC	Toll Like Receptor 2 (TLR2) Cascade
Pooled	UP	0.021	66	REAC:R-MMU-168164	REAC	Toll Like Receptor 3 (TLR3) Cascade
Pooled	UP	0.021	49	REAC:R-MMU-8939902	REAC	Regulation of RUNX2 expression and activity

Pooled	UP	0.021	9	HP:0007153	HP	Progressive extrapyramidal movement disorder
Pooled	UP	0.022	1614	HP:0100022	HP	Abnormality of movement
Pooled	UP	0.022	164	WP:WP246	WP	TNF-alpha NF-kB Signaling Pathway
Pooled	UP	0.023	376	HP:0001332	HP	Dystonia
Pooled	UP	0.023	758	HP:0001251	HP	Ataxia
Pooled	UP	0.025	151	HP:0003128	HP	Lactic acidosis
Pooled	UP	0.026	103	KEGG:00190	KEGG	Oxidative phosphorylation
Pooled	UP	0.027	84	REAC:R-MMU-8856825	REAC	Cargo recognition for clathrin-mediated endocytosis
Pooled	UP	0.027	87	REAC:R-MMU-9020702	REAC	Interleukin-1 signaling
Pooled	UP	0.028	69	REAC:R-MMU-5687128	REAC	MAPK6/MAPK4 signaling
Pooled	UP	0.029	59	KEGG:01230	KEGG	Biosynthesis of amino acids
Pooled	UP	0.030	129	GO:1902600	GO:BP	proton transmembrane transport
Pooled	UP	0.030	139	GO:0006457	GO:BP	protein folding
Pooled	UP	0.031	1251	HP:0031797	HP	Clinical course
Pooled	UP	0.031	70	REAC:R-MMU-1168372	REAC	Downstream signaling events of B Cell Receptor (BCR)
Pooled	UP	0.034	79	WP:WP295	WP	Electron Transport Chain
Pooled	UP	0.035	52	REAC:R-MMU-8854050	REAC	FBXL7 down-regulates AURKA during mitotic entry and in early mitosis
Pooled	UP	0.035	6	CORUM:676	CORUM	Metallothionein-3 complex
Pooled	UP	0.036	89	GO:0051082	GO:MF	unfolded protein binding
Pooled	UP	0.037	3	CORUM:4056	CORUM	Axin1-Dvl1-Gsk3b-Frat1 complex

Pooled	UP	0.039	53	REAC:R-MMU-5610780	REAC	Degradation of GLI1 by the proteasome
Pooled	UP	0.039	53	REAC:R-MMU-69541	REAC	Stabilization of p53
Pooled	UP	0.040	10	REAC:R-MMU-190242	REAC	FGFR1 ligand binding and activation
Pooled	UP	0.043	978	CORUM:0000000	CORUM	CORUM root
Pooled	UP	0.043	2	CORUM:395	CORUM	Succinyl-CoA synthetase, ADP-forming
Pooled	UP	0.044	817	HP:0001288	HP	Gait disturbance
Pooled	UP	0.046	74	REAC:R-MMU-975138	REAC	TRAF6 mediated induction of NFkB and MAP kinases upon TLR7/8 or 9 activation
Pooled	UP	0.048	14	REAC:R-MMU-5654710	REAC	PI-3K cascade:FGFR3
Pooled	UP	0.049	3	CORUM:2550	CORUM	Frs2-Grb2-Shp2 complex, FGF stimulated
Pooled	UP	0.050	16	REAC:R-MMU-5675482	REAC	Regulation of necroptotic cell death
Pooled	UP	0.050	11	REAC:R-MMU-5654219	REAC	Phospholipase C-mediated cascade: FGFR1
Pooled	UP	0.050	12	REAC:R-MMU-5654704	REAC	SHC-mediated cascade:FGFR3
Pooled	DOWN	0.000	3110	GO:0005654	GO:CC	nucleoplasm
Pooled	DOWN	0.001	3950	GO:0090304	GO:BP	nucleic acid metabolic process
Pooled	DOWN	0.001	97	KEGG:04380	KEGG	Osteoclast differentiation
Pooled	DOWN	0.002	3160	GO:0018130	GO:BP	heterocycle biosynthetic process
Pooled	DOWN	0.002	3056	GO:0019219	GO:BP	regulation of nucleobase-containing compound metabolic process
Pooled	DOWN	0.003	2722	GO:0097659	GO:BP	nucleic acid-templated transcription

Pooled	DOWN	0.003	2734	GO:0032774	GO:BP	RNA biosynthetic process
Pooled	DOWN	0.003	3103	GO:0034654	GO:BP	nucleobase-containing compound biosynthetic process
Pooled	DOWN	0.004	2677	GO:0006351	GO:BP	transcription, DNA-templated
Pooled	DOWN	0.006	3166	GO:0019438	GO:BP	aromatic compound biosynthetic process
Pooled	DOWN	0.006	2986	GO:2000112	GO:BP	regulation of cellular macromolecule biosynthetic process
Pooled	DOWN	0.006	520	GO:0071407	GO:BP	cellular response to organic cyclic compound
Pooled	DOWN	0.006	848	GO:0014070	GO:BP	response to organic cyclic compound
Pooled	DOWN	0.007	12	WP:WP2140	WP	Serotonin and anxiety-related events
Pooled	DOWN	0.007	2629	GO:1903506	GO:BP	regulation of nucleic acid-templated transcription
Pooled	DOWN	0.007	2632	GO:2001141	GO:BP	regulation of RNA biosynthetic process
Pooled	DOWN	0.008	3250	GO:1901362	GO:BP	organic cyclic compound biosynthetic process
Pooled	DOWN	0.008	3096	GO:0010556	GO:BP	regulation of macromolecule biosynthetic process
Pooled	DOWN	0.009	1184	TF:M00177_1	TF	Factor: CREB; motif: NSTGACGTAANN; match class: 1
Pooled	DOWN	0.010	3	CORUM:6279	CORUM	p65-IkappaBalpha-beta-arrestin-iNOS complex
Pooled	DOWN	0.011	3522	GO:0016070	GO:BP	RNA metabolic process
Pooled	DOWN	0.011	2580	GO:0006355	GO:BP	regulation of transcription, DNA-templated
Pooled	DOWN	0.012	2843	GO:0051252	GO:BP	regulation of RNA metabolic process
Pooled	DOWN	0.014	16	WP:WP2141	WP	Serotonin and anxiety
Pooled	DOWN	0.016	36	WP:WP37	WP	IL-1 Signaling Pathway
Pooled	DOWN	0.017	4343	GO:0006139	GO:BP	nucleobase-containing compound metabolic process
Pooled	DOWN	0.019	4450	GO:0046483	GO:BP	heterocycle metabolic process

Pooled	DOWN	0.019	33	MIRNA:mmu-miR-539-3p	MIRNA	mmu-miR-539-3p
Pooled	DOWN	0.022	34	MIRNA:mmu-miR-381-3p	MIRNA	mmu-miR-381-3p
Pooled	DOWN	0.025	725	GO:0016604	GO:CC	nuclear body
Pooled	DOWN	0.026	110	KEGG:04210	KEGG	Apoptosis
Pooled	DOWN	0.031	57	KEGG:05140	KEGG	Leishmaniasis
Pooled	DOWN	0.034	3215	GO:0031326	GO:BP	regulation of cellular biosynthetic process
Pooled	DOWN	0.040	63	KEGG:04662	KEGG	B cell receptor signaling pathway
Pooled	DOWN	0.046	890	REAC:R-MMU-74160	REAC	Gene expression (Transcription)
Pooled	DOWN	0.047	967	GO:0045944	GO:BP	positive regulation of transcription by RNA polymerase II
Pooled	DOWN	0.047	86	KEGG:04668	KEGG	TNF signaling pathway
Pooled	DOWN	0.048	72	KEGG:05235	KEGG	PD-L1 expression and PD-1 checkpoint pathway in cancer
Pooled	DOWN	0.048	64	KEGG:04658	KEGG	Th1 and Th2 cell differentiation

Supplementary table 4.S6. Gene overlap between published human transcriptional findings for schizophrenia from (Gandal et al. 2018) and downregulated genes pooled across ROIs.

mouse_ensembl_gene_id
ENSMUSG00000032501
ENSMUSG00000034936
ENSMUSG00000047867
ENSMUSG00000050592
ENSMUSG00000032413
ENSMUSG00000039765

ENSMUSG00000028256
ENSMUSG00000024764
ENSMUSG00000050471
ENSMUSG00000053453
ENSMUSG00000026289
ENSMUSG00000057265
ENSMUSG00000042210
ENSMUSG00000048285
ENSMUSG00000020866
ENSMUSG00000032470
ENSMUSG00000031796
ENSMUSG00000047603
ENSMUSG00000022749
ENSMUSG00000057858
ENSMUSG00000033543
ENSMUSG00000062822
ENSMUSG00000036061
ENSMUSG00000058013
ENSMUSG00000057894
ENSMUSG00000055692
ENSMUSG00000078490
ENSMUSG00000042185
ENSMUSG00000036760
ENSMUSG00000000305
ENSMUSG00000020021

References

Abazyan, Bagrat, Jun Nomura, Geetha Kannan, Koko Ishizuka, Kellie L. Tamashiro, Frederick Nucifora, Vladimir Pogorelov, et al. 2010. "Prenatal Interaction of Mutant DISC1 and Immune Activation Produces Adult Psychopathology." *Biological Psychiatry* 68 (12): 1172–81.

Ashburner, John, and Karl Friston. 1998. "High-Dimensional Nonlinear Image Registration." *NeuroImage*. https://doi.org/10.1016/s1053-8119(18)31570-2.

Atladóttir, Hjördis Ó., Poul Thorsen, Lars Østergaard, Diana E. Schendel, Sanne Lemcke, Morsi Abdallah, and Erik T. Parner. 2010. "Maternal Infection Requiring Hospitalization During Pregnancy and Autism Spectrum Disorders." *Journal of Autism and Developmental Disorders*. https://doi.org/10.1007/s10803-010-1006-y.

Avants, B. B., C. L. Epstein, M. Grossman, and J. C. Gee. 2008. "Symmetric Diffeomorphic Image Registration with Cross-Correlation: Evaluating Automated Labeling of Elderly and Neurodegenerative Brain." *Medical Image Analysis* 12 (1): 26–41.

Avants, Brian B., Nicholas J. Tustison, Gang Song, Philip A. Cook, Arno Klein, and James C. Gee. 2011. "A Reproducible Evaluation of ANTs Similarity Metric Performance in Brain Image Registration." *NeuroImage* 54 (3): 2033–44.

Ayhan, Yavuz, Ross McFarland, and Mikhail V. Pletnikov. 2016. "Animal Models of Gene-Environment Interaction in Schizophrenia: A Dimensional Perspective." *Progress in Neurobiology* 136 (January): 1–27.

Balasco, Luigi, Giovanni Provenzano, and Yuri Bozzi. 2019. "Sensory Abnormalities in Autism Spectrum Disorders: A Focus on the Tactile Domain, From Genetic Mouse Models to the Clinic." *Frontiers in Psychiatry / Frontiers Research Foundation* 10: 1016.

Balasubramanian, Revathi, and Xin Zhang. 2016. "Mechanisms of FGF Gradient Formation during Embryogenesis." *Seminars in Cell & Developmental Biology* 53 (May): 94–100.

Ball, G., J. Seidlitz, R. Beare, and M. L. Seal. 2020. "Cortical Remodelling in Childhood Is Associated with Genes Enriched for Neurodevelopmental Disorders." *NeuroImage* 215 (July): 116803.

Baron-Cohen, S., H. A. Ring, E. T. Bullmore, S. Wheelwright, C. Ashwin, and S. C. Williams. 2000. "The Amygdala Theory of Autism." *Neuroscience and Biobehavioral Reviews* 24 (3): 355–64.

Bauman, Melissa D., Ana-Maria Iosif, Stephen E. P. Smith, Catherine Bregere, David G. Amaral, and Paul H. Patterson. 2014. "Activation of the Maternal Immune System during Pregnancy Alters Behavioral Development of Rhesus Monkey Offspring." *Biological Psychiatry* 75 (4): 332–41.

Bedford, Saashi A., Min Tae M. Park, Gabriel A. Devenyi, Stephanie Tullo, Jurgen Germann, Raihaan Patel, Evdokia Anagnostou, et al. 2019. "Large-Scale Analyses of the Relationship between Sex, Age and Intelligence Quotient Heterogeneity and Cortical Morphometry in Autism Spectrum Disorder." *Molecular Psychiatry*, April. https://doi.org/10.1038/s41380-019-0420-6.

Benjamini, Yoav, and Yosef Hochberg. 1995. "Controlling the False Discovery Rate: A Practical and Powerful Approach to Multiple Testing." *Journal of the Royal Statistical Society. Series B, Statistical Methodology* 57 (1): 289–300.

Bergdolt, Lara, and Anna Dunaevsky. 2019. "Brain Changes in a Maternal Immune Activation

Model of Neurodevelopmental Brain Disorders." *Progress in Neurobiology* 175 (April): 1–19.

Bitanihirwe, Byron K. Y., Daria Peleg-Raibstein, Forouhar Mouttet, Joram Feldon, and Urs Meyer. 2010. "Late Prenatal Immune Activation in Mice Leads to Behavioral and Neurochemical Abnormalities Relevant to the Negative Symptoms of Schizophrenia." *Neuropsychopharmacology*. https://doi.org/10.1038/npp.2010.129.

Blomström, Åsa, Håkan Karlsson, Renee Gardner, Lena Jörgensen, Cecilia Magnusson, and Christina Dalman. 2016. "Associations Between Maternal Infection During Pregnancy, Childhood Infections, and the Risk of Subsequent Psychotic Disorder--A Swedish Cohort Study of Nearly 2 Million Individuals." *Schizophrenia Bulletin* 42 (1): 125–33.

Boksa, Patricia. 2010. "Effects of Prenatal Infection on Brain Development and Behavior: A Review of Findings from Animal Models." *Brain, Behavior, and Immunity* 24 (6): 881–97.

Brisch, Ralf, Hans-Gert Bernstein, Henrik Dobrowolny, Dieter Krell, Renate Stauch, Kurt Trübner, Johann Steiner, et al. 2011. "A Morphometric Analysis of the Septal Nuclei in Schizophrenia and Affective Disorders: Reduced Neuronal Density in the Lateral Septal Nucleus in Bipolar Disorder." *European Archives of Psychiatry and Clinical Neuroscience* 261 (1): 47–58.

Brown, Alan S., Melissa D. Begg, Stefan Gravenstein, Catherine A. Schaefer, Richard J. Wyatt, Michaeline Bresnahan, Vicki P. Babulas, and Ezra S. Susser. 2004. "Serologic Evidence of Prenatal Influenza in the Etiology of Schizophrenia." *Archives of General Psychiatry* 61 (8): 774–80.

Brown, Alan S., and Urs Meyer. 2018. "Maternal Immune Activation and Neuropsychiatric Illness: A Translational Research Perspective." *The American Journal of Psychiatry* 175 (11): 1073–83.

Buka, S. L., M. T. Tsuang, E. F. Torrey, M. A. Klebanoff, D. Bernstein, and R. H. Yolken. 2001. "Maternal Infections and Subsequent Psychosis among Offspring." *Archives of General Psychiatry* 58 (11): 1032–37.

Butler, Tracy, Daniel Weisholtz, Nancy Isenberg, Elizabeth Harding, Jane Epstein, Emily Stern, and David Silbersweig. 2012. "Neuroimaging of Frontal-Limbic Dysfunction in Schizophrenia and Epilepsy-Related Psychosis: Toward a Convergent Neurobiology." *Epilepsy & Behavior: E&B* 23 (2): 113–22.

Calabrese, Daniel R., Lei Wang, Michael P. Harms, J. Tilak Ratnanather, Deanna M. Barch, C. Robert Cloninger, Paul A. Thompson, Michael I. Miller, and John G. Csernansky. 2008. "Cingulate Gyrus Neuroanatomy in Schizophrenia Subjects and Their Non-Psychotic Siblings." *Schizophrenia Research* 104 (1-3): 61–70.

Cannon, Tyrone D., Theo G. M. van Erp, Carrie E. Bearden, Rachel Loewy, Paul Thompson, Arthur W. Toga, Matti O. Huttunen, Matcheri S. Keshavan, Larry J. Seidman, and Ming T. Tsuang. 2003. "Early and Late Neurodevelopmental Influences in the Prodrome to Schizophrenia: Contributions of Genes, Environment, and Their Interactions." *Schizophrenia Bulletin* 29 (4): 653–69.

Cheung, Charlton, Kevin Yu, Germaine Fung, Meikei Leung, Clive Wong, Qi Li, Pak Sham, Siew Chua, and Gráinne McAlonan. 2010. "Autistic Disorders and Schizophrenia: Related or Remote? An Anatomical Likelihood Estimation." *PloS One* 5 (8): e12233.

Choi, G. B., Y. S. Yim, H. Wong, S. Kim, H. Kim, S. V. Kim, C. A. Hoeffer, D. R. Littman, and J. R. Huh. 2016. "The Maternal Interleukin-17a Pathway in Mice Promotes Autism-like Phenotypes in Offspring." *Science*. https://doi.org/10.1126/science.aad0314.

Chung, M. K., K. J. Worsley, T. Paus, C. Cherif, D. L. Collins, J. N. Giedd, J. L. Rapoport, and A. C. Evans. 2001. "A Unified Statistical Approach to Deformation-Based Morphometry." *NeuroImage* 14 (3): 595–606.

Clancy, Barbara, Barbara L. Finlay, Richard B. Darlington, and K. J. S. Anand. 2007. "Extrapolating Brain Development from Experimental Species to Humans." *Neurotoxicology* 28 (5): 931–37.

Clancy, B., R. B. Darlington, and B. L. Finlay. 2001. "Translating Developmental Time across Mammalian Species." *Neuroscience* 105 (1): 7–17.

Colacicco, Giovanni, Hans Welzl, Hans-Peter Lipp, and Hanno Würbel. 2002. "Attentional Set-Shifting in Mice: Modification of a Rat Paradigm, and Evidence for Strain-Dependent Variation." *Behavioural Brain Research* 132 (1): 95–102.

Collins, D. Louis, D. Louis Collins, Peter Neelin, Terrence M. Peters, and Alan C. Evans. 1994. "Automatic 3D Intersubject Registration of MR Volumetric Data in Standardized Talairach Space." *Journal of Computer Assisted Tomography*. https://doi.org/10.1097/00004728-199403000-00005.

Conway, Fiona, and Alan S. Brown. 2019. "Maternal Immune Activation and Related Factors in the Risk of Offspring Psychiatric Disorders." *Frontiers in Psychiatry / Frontiers Research Foundation* 10 (June): 430.

Cook, R. Dennis. 1986. "Assessment of Local Influence." *Journal of the Royal Statistical Society. Series B, Statistical Methodology* 48 (2): 133–55.

Cope, Z. A., S. B. Powell, and J. W. Young. 2016. "Modeling Neurodevelopmental Cognitive Deficits in Tasks with Cross-Species Translational Validity: Translating Models of Neurodevelopmental Disorders." *Genes, Brain, and Behavior* 15 (1): 27–44.

Cossío, Lourdes Fernández de, Lourdes Fernández de Cossío, Andrea Guzmán, Suzanne van der Veldt, and Giamal N. Luheshi. 2017. "Prenatal Infection Leads to ASD-like Behavior and Altered Synaptic Pruning in the Mouse Offspring." *Brain, Behavior, and Immunity*. https://doi.org/10.1016/j.bbi.2016.09.028.

Crawley, Jacqueline N. 2007. *What's Wrong With My Mouse?: Behavioral Phenotyping of Transgenic and Knockout Mice*. John Wiley & Sons.

Crum, William R., Stephen J. Sawiak, Winfred Chege, Jonathan D. Cooper, Steven C. R. Williams, and Anthony C. Vernon. 2017. "Evolution of Structural Abnormalities in the Rat Brain Following in Utero Exposure to Maternal Immune Activation: A Longitudinal in Vivo MRI Study." *Brain, Behavior, and Immunity* 63 (July): 50–59.

Dabrowski, Ania, Akiko Terauchi, Cameron Strong, and Hisashi Umemori. 2015. "Distinct Sets of FGF Receptors Sculpt Excitatory and Inhibitory Synaptogenesis." *Development* 142 (10): 1818–30.

Dalton, Victoria S., Mathieu Verdurand, Adam Walker, Deborah M. Hodgson, and Katerina Zavitsanou. 2012. "Synergistic Effect between Maternal Infection and Adolescent Cannabinoid Exposure on Serotonin 5HT1A Receptor Binding in the Hippocampus: Testing the 'Two Hit' Hypothesis for the Development of Schizophrenia." *ISRN Psychiatry*. https://doi.org/10.5402/2012/451865.

Deacon, Robert M. J. 2006. "Digging and Marble Burying in Mice: Simple Methods for in Vivo Identification of Biological Impacts." *Nature Protocols* 1 (1): 122–24.

Dekel, Nava, Yulia Gnainsky, Irit Granot, and Gil Mor. 2010. "Inflammation and Implantation." *American Journal of Reproductive Immunology* 63 (1): 17–21.

Diez Del Corral, Ruth, and Aixa V. Morales. 2017. "The Multiple Roles of FGF Signaling in the

Developing Spinal Cord." *Frontiers in Cell and Developmental Biology* 5 (June): 58.
Di Martino, Adriana, Damien A. Fair, Clare Kelly, Theodore D. Satterthwaite, F. Xavier Castellanos, Moriah E. Thomason, R. Cameron Craddock, et al. 2014. "Unraveling the Miswired Connectome: A Developmental Perspective." *Neuron* 83 (6): 1335–53.
Dobin, Alexander, Carrie A. Davis, Felix Schlesinger, Jorg Drenkow, Chris Zaleski, Sonali Jha, Philippe Batut, Mark Chaisson, and Thomas R. Gingeras. 2013. "STAR: Ultrafast Universal RNA-Seq Aligner." *Bioinformatics* 29 (1): 15–21.
Donovan, Alex P. A., and M. Albert Basson. 2017. "The Neuroanatomy of Autism--a Developmental Perspective." *Journal of Anatomy* 230 (1): 4–15.
Eckart, Carl, and Gale Young. 1936. "The Approximation of One Matrix by Another of Lower Rank." *Psychometrika* 1 (3): 211–18.
Eede, Matthijs C. van, Jan Scholz, M. Mallar Chakravarty, R. Mark Henkelman, and Jason P. Lerch. 2013. "Mapping Registration Sensitivity in MR Mouse Brain Images." *NeuroImage* 82 (November): 226–36.
Ellenbroek, Bart, and Jiun Youn. 2016. "Rodent Models in Neuroscience Research: Is It a Rat Race?" *Disease Models & Mechanisms* 9 (10): 1079–87.
Ellman, Lauren M., Raymond F. Deicken, Sophia Vinogradov, William S. Kremen, John H. Poole, David M. Kern, Wei Yann Tsai, Catherine A. Schaefer, and Alan S. Brown. 2010. "Structural Brain Alterations in Schizophrenia Following Fetal Exposure to the Inflammatory Cytokine Interleukin-8." *Schizophrenia Research* 121 (1-3): 46–54.
Erp, T. G. M. van, D. P. Hibar, J. M. Rasmussen, D. C. Glahn, G. D. Pearlson, O. A. Andreassen, I. Agartz, et al. 2016. "Subcortical Brain Volume Abnormalities in 2028 Individuals with Schizophrenia and 2540 Healthy Controls via the ENIGMA Consortium." *Molecular Psychiatry* 21 (4): 547–53.
Estes, Myka L., and A. Kimberley McAllister. 2016. "Maternal Immune Activation: Implications for Neuropsychiatric Disorders." *Science* 353 (6301): 772–77.
Ferreiro, Diego U., and Elizabeth A. Komives. 2010. "Molecular Mechanisms of System Control of NF-kappaB Signaling by IkappaBalpha." *Biochemistry* 49 (8): 1560–67.
Freeman, Sara M., Michelle C. Palumbo, Rebecca H. Lawrence, Aaron L. Smith, Mark M. Goodman, and Karen L. Bales. 2018. "Effect of Age and Autism Spectrum Disorder on Oxytocin Receptor Density in the Human Basal Forebrain and Midbrain." *Translational Psychiatry* 8 (1): 257.
Friedel, Miriam, Matthijs C. van Eede, Jon Pipitone, M. Mallar Chakravarty, and Jason P. Lerch. 2014. "Pydpiper: A Flexible Toolkit for Constructing Novel Registration Pipelines." *Frontiers in Neuroinformatics* 8 (July): 67.
Fuentes, Montserrat, Peter Guttorp, and Paul Sampson. 2006. "Using Transforms to Analyze Space-Time Processes." *C&H/CRC Monographs on Statistics & Applied Probability*. https://doi.org/10.1201/9781420011050.ch3.
Fujiwara, Hironobu, Kazuyuki Hirao, Chihiro Namiki, Makiko Yamada, Mitsuaki Shimizu, Hidenao Fukuyama, Takuji Hayashi, and Toshiya Murai. 2007. "Anterior Cingulate Pathology and Social Cognition in Schizophrenia: A Study of Gray Matter, White Matter and Sulcal Morphometry." *NeuroImage* 36 (4): 1236–45.
Gabay, Cem, Céline Lamacchia, and Gaby Palmer. 2010. "IL-1 Pathways in Inflammation and Human Diseases." *Nature Reviews. Rheumatology* 6 (4): 232–41.
Gallino, Daniel, Gabriel A. Devenyi, Jürgen Germann, Elisa Guma, Chloe Anastassiadis, and M. Mallar Chakravarty. 2019. "Longitudinal Assessment of the Neuroanatomical

Consequences of Deep Brain Stimulation: Application of Fornical DBS in an Alzheimer's Mouse Model." *Brain Research* 1715 (July): 213–23.

Gandal, Michael J., Jillian R. Haney, Neelroop N. Parikshak, Virpi Leppa, Gokul Ramaswami, Chris Hartl, Andrew J. Schork, et al. 2018. "Shared Molecular Neuropathology across Major Psychiatric Disorders Parallels Polygenic Overlap." *Science* 359 (6376): 693–97.

George, David T., Rezvan Ameli, and George F. Koob. 2019. "Periaqueductal Gray Sheds Light on Dark Areas of Psychopathology." *Trends in Neurosciences* 42 (5): 349–60.

Giovanoli, S., H. Engler, A. Engler, J. Richetto, M. Voget, R. Willi, C. Winter, et al. 2013. "Stress in Puberty Unmasks Latent Neuropathological Consequences of Prenatal Immune Activation in Mice." *Science*. https://doi.org/10.1126/science.1228261.

Graham, Alice M., Jerod M. Rasmussen, Marc D. Rudolph, Christine M. Heim, John H. Gilmore, Martin Styner, Steven G. Potkin, et al. 2018. "Maternal Systemic Interleukin-6 During Pregnancy Is Associated With Newborn Amygdala Phenotypes and Subsequent Behavior at 2 Years of Age." *Biological Psychiatry* 83 (2): 109–19.

Guillemot, François, and Céline Zimmer. 2011. "From Cradle to Grave: The Multiple Roles of Fibroblast Growth Factors in Neural Development." *Neuron* 71 (4): 574–88.

Guma, Elisa, Eric Plitman, and M. Mallar Chakravarty. 2019. "The Role of Maternal Immune Activation in Altering the Neurodevelopmental Trajectories of Offspring: A Translational Review of Neuroimaging Studies with Implications for Autism Spectrum Disorder and Schizophrenia." *Neuroscience and Biobehavioral Reviews*. https://www.sciencedirect.com/science/article/pii/S0149763419302088.

Guma, Elisa, Jill Rocchetti, Gabriel A. Devenyi, Arnaud Tanti, Axel Mathieu, Jason P. Lerch, Guillaume Elgbeili, et al. 2018. "Regional Brain Volume Changes Following Chronic Antipsychotic Administration Are Mediated by the Dopamine D2 Receptor." *NeuroImage* 176 (April): 226–38.

Guma, Elisa, Jill Rocchetti, Gabriel A. Devenyi, Arnaud Tanti, Axel P. Mathieu, Jason P. Lerch, Guillaume Elgbeili, et al. 2019. "Role of D3 Dopamine Receptors in Modulating Neuroanatomical Changes in Response to Antipsychotic Administration." *Scientific Reports* 9 (1): 7850.

Gumusoglu, Serena B., and Hanna E. Stevens. 2019. "Maternal Inflammation and Neurodevelopmental Programming: A Review of Preclinical Outcomes and Implications for Translational Psychiatry." *Biological Psychiatry* 85 (2): 107–21.

Gur, Raquel E., Matcheri S. Keshavan, and Stephen M. Lawrie. 2007. "Deconstructing Psychosis with Human Brain Imaging." *Schizophrenia Bulletin* 33 (4): 921–31.

Haddad, Faraj L., Salonee V. Patel, and Susanne Schmid. 2020. "Maternal Immune Activation by Poly I:C as a Preclinical Model for Neurodevelopmental Disorders: A Focus on Autism and Schizophrenia." *Neuroscience and Biobehavioral Reviews* 113 (June): 546–67.

Hazlett, Heather Cody, Michele D. Poe, Guido Gerig, Martin Styner, Chad Chappell, Rachel Gimpel Smith, Clement Vachet, and Joseph Piven. 2011. "Early Brain Overgrowth in Autism Associated with an Increase in Cortical Surface Area before Age 2 Years." *Archives of General Psychiatry* 68 (5): 467–76.

Jungerius, B. J., M. L. C. Hoogendoorn, S. C. Bakker, R. Van't Slot, A. F. Bardoel, R. A. Ophoff, C. Wijmenga, R. S. Kahn, and R. J. Sinke. 2008. "An Association Screen of Myelin-Related Genes Implicates the Chromosome 22q11 PIK4CA Gene in Schizophrenia." *Molecular Psychiatry* 13 (11): 1060–68.

Kentner, Amanda C., Staci D. Bilbo, Alan S. Brown, Elaine Y. Hsiao, A. Kimberley McAllister,

Urs Meyer, Brad D. Pearce, Mikhail V. Pletnikov, Robert H. Yolken, and Melissa D. Bauman. 2019. "Maternal Immune Activation: Reporting Guidelines to Improve the Rigor, Reproducibility, and Transparency of the Model." *Neuropsychopharmacology: Official Publication of the American College of Neuropsychopharmacology* 44 (2): 245–58.

Kirschner, Matthias, Golia Shafiei, Ross D. Markello, Carolina Makowski, Alexandra Talpalaru, Benazir Hodzic-Santor, Gabriel A. Devenyi, et al. 2020. "Latent Clinical-Anatomical Dimensions of Schizophrenia." *Schizophrenia Bulletin*, August. https://doi.org/10.1093/schbul/sbaa097.

Knuesel, Irene, Laurie Chicha, Markus Britschgi, Scott A. Schobel, Michael Bodmer, Jessica A. Hellings, Stephen Toovey, and Eric P. Prinssen. 2014. "Maternal Immune Activation and Abnormal Brain Development across CNS Disorders." *Nature Reviews. Neurology* 10 (11): 643–60.

Kong, Vincent, Gabriel A. Devenyi, Daniel Gallino, Gülebru Ayranci, Jürgen Germann, Colleen Rollins, and M. Mallar Chakravarty. 2018. "Early-in-Life Neuroanatomical and Behavioural Trajectories in a Triple Transgenic Model of Alzheimer's Disease." *Brain Structure & Function*, June. https://doi.org/10.1007/s00429-018-1691-4.

Koropouli, Eleftheria, Nikos Melanitis, Vasileios I. Dimitriou, Asimina Grigoriou, Efstratios Karavasilis, Konstantina S. Nikita, Elias Tzavellas, and Thomas Paparrigopoulos. 2020. "New-Onset Psychosis Associated With a Lesion Localized in the Rostral Tectum: Insights Into Pathway-Specific Connectivity Disrupted in Psychosis." *Schizophrenia Bulletin*, February. https://doi.org/10.1093/schbul/sbaa018.

Kovačević, N., J. T. Henderson, E. Chan, N. Lifshitz, J. Bishop, A. C. Evans, R. M. Henkelman, and X. J. Chen. 2004. "A Three-Dimensional MRI Atlas of the Mouse Brain with Estimates of the Average and Variability." *Cerebral Cortex* 15 (5): 639–45.

Kreitz, Silke, Alice Zambon, Marianne Ronovsky, Lubos Budinsky, Thomas H. Helbich, Spyros Sideromenos, Claudiu Ivan, et al. 2020. "Maternal Immune Activation during Pregnancy Impacts on Brain Structure and Function in the Adult Offspring." *Brain, Behavior, and Immunity* 83 (January): 56–67.

Lanz, Thomas A., Veronica Reinhart, Mark J. Sheehan, Stacey J. Sukoff Rizzo, Susan E. Bove, Larry C. James, Dmitri Volfson, David A. Lewis, and Robin J. Kleiman. 2019. "Postmortem Transcriptional Profiling Reveals Widespread Increase in Inflammation in Schizophrenia: A Comparison of Prefrontal Cortex, Striatum, and Hippocampus among Matched Tetrads of Controls with Subjects Diagnosed with Schizophrenia, Bipolar or Major Depressive Disorder." *Translational Psychiatry* 9 (1): 151.

Le Belle, Janel E., Jantzen Sperry, Amy Ngo, Yasmin Ghochani, Dan R. Laks, Manuel López-Aranda, Alcino J. Silva, and Harley I. Kornblum. 2014. "Maternal Inflammation Contributes to Brain Overgrowth and Autism-Associated Behaviors through Altered Redox Signaling in Stem and Progenitor Cells." *Stem Cell Reports* 3 (5): 725–34.

Lerch, Jason P., Jeffrey B. Carroll, Shoshana Spring, Lisa N. Bertram, Claudia Schwab, Michael R. Hayden, and R. Mark Henkelman. 2008. "Automated Deformation Analysis in the YAC128 Huntington Disease Mouse Model." *NeuroImage* 39 (1): 32–39.

Lerch, Jason P., Lisa Gazdzinski, Jürgen Germann, John G. Sled, R. Mark Henkelman, and Brian J. Nieman. 2012. "Wanted Dead or Alive? The Tradeoff between in-Vivo versus Ex-Vivo MR Brain Imaging in the Mouse." *Frontiers in Neuroinformatics* 6 (March): 6.

Lieberman, J. A., R. R. Girgis, G. Brucato, H. Moore, F. Provenzano, L. Kegeles, D. Javitt, et al. 2018. "Hippocampal Dysfunction in the Pathophysiology of Schizophrenia: A Selective

Review and Hypothesis for Early Detection and Intervention." *Molecular Psychiatry* 23 (8): 1764–72.

Lipina, Tatiana V., Clement Zai, Daniela Hlousek, John C. Roder, and Albert H. C. Wong. 2013. "Maternal Immune Activation during Gestation Interacts with Disc1 Point Mutation to Exacerbate Schizophrenia-Related Behaviors in Mice." *The Journal of Neuroscience: The Official Journal of the Society for Neuroscience* 33 (18): 7654–66.

Li, Qi, Charlton Cheung, Ran Wei, Edward S. Hui, Joram Feldon, Urs Meyer, Sookja Chung, et al. 2009. "Prenatal Immune Challenge Is an Environmental Risk Factor for Brain and Behavior Change Relevant to Schizophrenia: Evidence from MRI in a Mouse Model." *PloS One* 4 (7): e6354.

Li, Q., Y. O. Leung, I. Zhou, L. C. Ho, W. Kong, P. Basil, R. Wei, et al. 2015. "Dietary Supplementation with N-3 Fatty Acids from Weaning Limits Brain Biochemistry and Behavioural Changes Elicited by Prenatal Exposure to Maternal Inflammation in the Mouse Model." *Translational Psychiatry* 5 (September): e641.

Liu, Hong, Li-Ling Wang, Si-Jia Zhao, Joanne Kwak-Kim, Gil Mor, and Ai-Hua Liao. 2020. "Why Are Pregnant Women Susceptible to COVID-19? An Immunological Viewpoint." *Journal of Reproductive Immunology* 139 (June): 103122.

Li, Yue, Minjie Shen, Michael E. Stockton, and Xinyu Zhao. 2019. "Hippocampal Deficits in Neurodevelopmental Disorders." *Neurobiology of Learning and Memory* 165 (November): 106945.

Manitz, Marie Pierre, Jennifer Plümper, Seray Demir, Maike Ahrens, Manuela Eßlinger, Simone Wachholz, Martin Eisenacher, Georg Juckel, and Astrid Friebe. 2016. "Flow Cytometric Characterization of Microglia in the Offspring of PolyI:C Treated Mice." *Brain Research*. https://doi.org/10.1016/j.brainres.2016.02.004.

Martins-Filho, Paulo Ricardo, Diego Moura Tanajura, Hudson P. Santos Jr, and Victor Santana Santos. 2020. "COVID-19 during Pregnancy: Potential Risk for Neurodevelopmental Disorders in Neonates?" *European Journal of Obstetrics, Gynecology, and Reproductive Biology* 250 (July): 255–56.

Matsumoto, Naoyuki, Yohei Shinmyo, Yoshie Ichikawa, and Hiroshi Kawasaki. 2017. "Gyrification of the Cerebral Cortex Requires FGF Signaling in the Mammalian Brain." *eLife* 6 (November). https://doi.org/10.7554/eLife.29285.

McIntosh, Anthony Randal, and Nancy J. Lobaugh. 2004. "Partial Least Squares Analysis of Neuroimaging Data: Applications and Advances." *NeuroImage* 23 Suppl 1: S250–63.

McIntosh, Anthony R., and Bratislav Mišić. 2013. "Multivariate Statistical Analyses for Neuroimaging Data." *Annual Review of Psychology* 64: 499–525.

Meyer, U., P. J. Murray, A. Urwyler, B. K. Yee, M. Schedlowski, and J. Feldon. 2008. "Adult Behavioral and Pharmacological Dysfunctions Following Disruption of the Fetal Brain Balance between pro-Inflammatory and IL-10-Mediated Anti-Inflammatory Signaling." *Molecular Psychiatry*. https://doi.org/10.1038/sj.mp.4002042.

Meyer, Urs. 2014. "Prenatal poly(i:C) Exposure and Other Developmental Immune Activation Models in Rodent Systems." *Biological Psychiatry* 75 (4): 307–15.

Meyer, Urs, Joram Feldon, and Benjamin K. Yee. 2009. "A Review of the Fetal Brain Cytokine Imbalance Hypothesis of Schizophrenia." *Schizophrenia Bulletin* 35 (5): 959–72.

Meyer, Urs, Myriel Nyffeler, Andrea Engler, Adrian Urwyler, Manfred Schedlowski, Irene Knuesel, Benjamin K. Yee, and Joram Feldon. 2006. "The Time of Prenatal Immune Challenge Determines the Specificity of Inflammation-Mediated Brain and Behavioral

Pathology." *The Journal of Neuroscience: The Official Journal of the Society for Neuroscience* 26 (18): 4752–62.

Meyer, Urs, Myriel Nyffeler, Severin Schwendener, Irene Knuesel, Benjamin K. Yee, and Joram Feldon. 2008. "Relative Prenatal and Postnatal Maternal Contributions to Schizophrenia-Related Neurochemical Dysfunction after in Utero Immune Challenge." *Neuropsychopharmacology: Official Publication of the American College of Neuropsychopharmacology* 33 (2): 441–56.

Meyer, Urs, Myriel Nyffeler, Benjamin K. Yee, Irene Knuesel, and Joram Feldon. 2008. "Adult Brain and Behavioral Pathological Markers of Prenatal Immune Challenge during Early/middle and Late Fetal Development in Mice." *Brain, Behavior, and Immunity* 22 (4): 469–86.

Mills, Kathryn L., and Christian K. Tamnes. 2014. "Methods and Considerations for Longitudinal Structural Brain Imaging Analysis across Development." *Developmental Cognitive Neuroscience* 9 (July): 172–90.

Morera, Davinia, and Simon A. MacKenzie. 2011. "Is There a Direct Role for Erythrocytes in the Immune Response?" *Veterinary Research* 42 (July): 89.

Mor, Gil, and Ingrid Cardenas. 2010. "The Immune System in Pregnancy: A Unique Complexity." *American Journal of Reproductive Immunology* 63 (6): 425–33.

Mueller, Flavia S., Joseph Scarborough, Sina M. Schalbetter, Juliet Richetto, Eugene Kim, Amalie Couch, Yohan Yee, et al. 2021. "Behavioral, Neuroanatomical, and Molecular Correlates of Resilience and Susceptibility to Maternal Immune Activation." *Molecular Psychiatry* 26 (2): 396–410.

Nandi, Sayan, Karina Alviña, Pablo J. Lituma, Pablo E. Castillo, and Jean M. Hébert. 2018. "Neurotrophin and FGF Signaling Adapter Proteins, FRS2 and FRS3, Regulate Dentate Granule Cell Maturation and Excitatory Synaptogenesis." *Neuroscience* 369 (January): 192–201.

Neugebauer, Judith M., and H. Joseph Yost. 2014. "FGF Signaling Is Required for Brain Left-Right Asymmetry and Brain Midline Formation." *Developmental Biology* 386 (1): 123–34.

Nicolson, Rob, Timothy J. DeVito, Christine N. Vidal, Yihong Sui, Kiralee M. Hayashi, Dick J. Drost, Peter C. Williamson, Nagalingam Rajakumar, Arthur W. Toga, and Paul M. Thompson. 2006. "Detection and Mapping of Hippocampal Abnormalities in Autism." *Psychiatry Research* 148 (1): 11–21.

Nielsen, Philip R., Urs Meyer, and Preben B. Mortensen. 2016. "Individual and Combined Effects of Maternal Anemia and Prenatal Infection on Risk for Schizophrenia in Offspring." *Schizophrenia Research* 172 (1-3): 35–40.

Nieman, Brian J., Jonathan Bishop, Jun Dazai, Nicholas A. Bock, Jason P. Lerch, Akiva Feintuch, X. Josette Chen, John G. Sled, and R. Mark Henkelman. 2007. "MR Technology for Biological Studies in Mice." *NMR in Biomedicine* 20 (3): 291–303.

O'Donovan, M. C., N. Norton, H. Williams, T. Peirce, V. Moskvina, I. Nikolov, M. Hamshere, et al. 2009. "Analysis of 10 Independent Samples Provides Evidence for Association between Schizophrenia and a SNP Flanking Fibroblast Growth Factor Receptor 2." *Molecular Psychiatry* 14 (1): 30–36.

Pantelis, Christos, Murat Yücel, Emre Bora, Alex Fornito, Renée Testa, Warrick J. Brewer, Dennis Velakoulis, and Stephen J. Wood. 2009. "Neurobiological Markers of Illness Onset in Psychosis and Schizophrenia: The Search for a Moving Target." *Neuropsychology Review* 19 (3): 385–98.

Patel, Raihaan, Christopher J. Steele, Anthony G. X. Chen, Sejal Patel, Gabriel A. Devenyi, Jürgen Germann, Christine L. Tardif, and M. Mallar Chakravarty. 2020. "Investigating Microstructural Variation in the Human Hippocampus Using Non-Negative Matrix Factorization." *NeuroImage* 207 (February): 116348.

Patel, Shrujna, Russell C. Dale, Destanie Rose, Brianna Heath, Christine W. Nordahl, Sally Rogers, Adam J. Guastella, and Paul Ashwood. 2020. "Maternal Immune Conditions Are Increased in Males with Autism Spectrum Disorders and Are Associated with Behavioural and Emotional but Not Cognitive Co-Morbidity." *Translational Psychiatry* 10 (1): 286.

Petersen, Steven E., and Olaf Sporns. 2015. "Brain Networks and Cognitive Architectures." *Neuron* 88 (1): 207–19.

Piontkewitz, Yael, Michal Arad, and Ina Weiner. 2011a. "Risperidone Administered during Asymptomatic Period of Adolescence Prevents the Emergence of Brain Structural Pathology and Behavioral Abnormalities in an Animal Model of Schizophrenia." *Schizophrenia Bulletin* 37 (6): 1257–69.

Piontkewitz, Yael, Michal Arad, and Ina Weiner. 2011b. "Abnormal Trajectories of Neurodevelopment and Behavior Following in Utero Insult in the Rat." *Biological Psychiatry* 70 (9): 842–51.

Piontkewitz, Yael, Michal Arad, and Ina Weiner. 2012. "Tracing the Development of Psychosis and Its Prevention: What Can Be Learned from Animal Models." *Neuropharmacology* 62 (3): 1273–89.

Piontkewitz, Yael, Yaniv Assaf, and Ina Weiner. 2009. "Clozapine Administration in Adolescence Prevents Postpubertal Emergence of Brain Structural Pathology in an Animal Model of Schizophrenia." *Biological Psychiatry* 66 (11): 1038–46.

Plaisier, Seema B., Richard Taschereau, Justin A. Wong, and Thomas G. Graeber. 2010. "Rank–rank Hypergeometric Overlap: Identification of Statistically Significant Overlap between Gene-Expression Signatures." *Nucleic Acids Research* 38 (17): e169–e169.

Qiu, Lily R., Darren J. Fernandes, Kamila U. Szulc-Lerch, Jun Dazai, Brian J. Nieman, Daniel H. Turnbull, Jane A. Foster, Mark R. Palmert, and Jason P. Lerch. 2018. "Mouse MRI Shows Brain Areas Relatively Larger in Males Emerge before Those Larger in Females." *Nature Communications* 9 (1): 2615.

Radulescu, Eugenia, Balaji Ganeshan, Sukhwinder S. Shergill, Nick Medford, Chris Chatwin, Rupert C. D. Young, and Hugo D. Critchley. 2014. "Grey-Matter Texture Abnormalities and Reduced Hippocampal Volume Are Distinguishing Features of Schizophrenia." *Psychiatry Research* 223 (3): 179–86.

Rapoport, J. L., A. M. Addington, S. Frangou, and M. R. C. Psych. 2005. "The Neurodevelopmental Model of Schizophrenia: Update 2005." *Molecular Psychiatry* 10 (5): 434–49.

Ratnayake, Udani, Tracey Quinn, David W. Walker, and Hayley Dickinson. 2013. "Cytokines and the Neurodevelopmental Basis of Mental Illness." *Frontiers in Neuroscience* 7 (October): 180.

Reimand, Jüri, Ruth Isserlin, Veronique Voisin, Mike Kucera, Christian Tannus-Lopes, Asha Rostamianfar, Lina Wadi, et al. 2019. "Pathway Enrichment Analysis and Visualization of Omics Data Using g:Profiler, GSEA, Cytoscape and EnrichmentMap." *Nature Protocols*. https://doi.org/10.1038/s41596-018-0103-9.

Reimand, Jüri, Meelis Kull, Hedi Peterson, Jaanus Hansen, and Jaak Vilo. 2007. "g:Profiler—a Web-Based Toolset for Functional Profiling of Gene Lists from Large-Scale Experiments."

Nucleic Acids Research. https://doi.org/10.1093/nar/gkm226.
Reisinger, Sonali, Deeba Khan, Eryan Kong, Angelika Berger, Arnold Pollak, and Daniela D. Pollak. 2015. "The Poly(I:C)-Induced Maternal Immune Activation Model in Preclinical Neuropsychiatric Drug Discovery." *Pharmacology & Therapeutics* 149 (May): 213–26.
Reuss, Bernhard, and Oliver von Bohlen und Halbach. 2003. "Fibroblast Growth Factors and Their Receptors in the Central Nervous System." *Cell and Tissue Research*. https://doi.org/10.1007/s00441-003-0756-7.
Richetto, Juliet, Robert Chesters, Annamaria Cattaneo, Marie A. Labouesse, Ana Maria Carrillo Gutierrez, Tobias C. Wood, Alessia Luoni, Urs Meyer, Anthony Vernon, and Marco A. Riva. 2017. "Genome-Wide Transcriptional Profiling and Structural Magnetic Resonance Imaging in the Maternal Immune Activation Model of Neurodevelopmental Disorders." *Cerebral Cortex* 27 (6): 3397–3413.
Ritchie, Matthew E., Belinda Phipson, Di Wu, Yifang Hu, Charity W. Law, Wei Shi, and Gordon K. Smyth. 2015. "Limma Powers Differential Expression Analyses for RNA-Sequencing and Microarray Studies." *Nucleic Acids Research* 43 (7): e47.
Robinson, Mark D., and Alicia Oshlack. 2010. "A Scaling Normalization Method for Differential Expression Analysis of RNA-Seq Data." *Genome Biology* 11 (3): R25.
Rollins, Colleen P. E., Daniel Gallino, Vincent Kong, Gülebru Ayranci, Gabriel A. Devenyi, Jürgen Germann, and M. Mallar Chakravarty. 2019. "Contributions of a High-Fat Diet to Alzheimer's Disease-Related Decline: A Longitudinal Behavioural and Structural Neuroimaging Study in Mouse Models." *NeuroImage: Clinical* 21 (January): 101606.
Roth, K. A., and C. D'Sa. 2001. "Apoptosis and Brain Development." *Mental Retardation and Developmental Disabilities Research Reviews* 7 (4): 261–66.
Rubenstein, John L. R. 2010. "Three Hypotheses for Developmental Defects That May Underlie Some Forms of Autism Spectrum Disorder." *Current Opinion in Neurology* 23 (2): 118–23.
Rudolph, Marc D., Alice M. Graham, Eric Feczko, Oscar Miranda-Dominguez, Jerod M. Rasmussen, Rahel Nardos, Sonja Entringer, Pathik D. Wadhwa, Claudia Buss, and Damien A. Fair. 2018. "Maternal IL-6 during Pregnancy Can Be Estimated from Newborn Brain Connectivity and Predicts Future Working Memory in Offspring." *Nature Neuroscience* 21 (5): 765–72.
Schnack, Hugo. 2019. "Assessing Reproducibility in Association Studies." *eLife*. https://doi.org/10.7554/eLife.46757.
Schumann, Cynthia M., Melissa D. Bauman, and David G. Amaral. 2011. "Abnormal Structure or Function of the Amygdala Is a Common Component of Neurodevelopmental Disorders." *Neuropsychologia* 49 (4): 745–59.
Schumann, Cynthia M., Cinnamon S. Bloss, Cynthia Carter Barnes, Graham M. Wideman, Ruth A. Carper, Natacha Akshoomoff, Karen Pierce, et al. 2010. "Longitudinal Magnetic Resonance Imaging Study of Cortical Development through Early Childhood in Autism." *The Journal of Neuroscience: The Official Journal of the Society for Neuroscience* 30 (12): 4419–27.
Selemon, L. D., and N. Zecevic. 2015. "Schizophrenia: A Tale of Two Critical Periods for Prefrontal Cortical Development." *Translational Psychiatry* 5 (August): e623.
Semple, Bridgette D., Klas Blomgren, Kayleen Gimlin, Donna M. Ferriero, and Linda J. Noble-Haeusslein. 2013. "Brain Development in Rodents and Humans: Identifying Benchmarks of Maturation and Vulnerability to Injury across Species." *Progress in Neurobiology*. https://doi.org/10.1016/j.pneurobio.2013.04.001.

Shafiei, Golia, Ross D. Markello, Carolina Makowski, Alexandra Talpalaru, Matthias Kirschner, Gabriel A. Devenyi, Elisa Guma, et al. 2020. "Spatial Patterning of Tissue Volume Loss in Schizophrenia Reflects Brain Network Architecture." *Biological Psychiatry* 87 (8): 727–35.

Shaw, Philip, Nitin Gogtay, and Judith Rapoport. 2010. "Childhood Psychiatric Disorders as Anomalies in Neurodevelopmental Trajectories." *Human Brain Mapping* 31 (6): 917–25.

Shin Yim, Yeong, Ashley Park, Janet Berrios, Mathieu Lafourcade, Leila M. Pascual, Natalie Soares, Joo Yeon Kim, et al. 2017. "Reversing Behavioural Abnormalities in Mice Exposed to Maternal Inflammation." *Nature* 549 (7673): 482–87.

Short, Sarah J., Gabriele R. Lubach, Alexander I. Karasin, Christopher W. Olsen, Martin Styner, Rebecca C. Knickmeyer, John H. Gilmore, and Christopher L. Coe. 2010. "Maternal Influenza Infection during Pregnancy Impacts Postnatal Brain Development in the Rhesus Monkey." *Biological Psychiatry* 67 (10): 965–73.

Simms, Marissa Leigh, Thomas L. Kemper, Clare M. Timbie, Margaret L. Bauman, and Gene J. Blatt. 2009. "The Anterior Cingulate Cortex in Autism: Heterogeneity of Qualitative and Quantitative Cytoarchitectonic Features Suggests Possible Subgroups." *Acta Neuropathologica* 118 (5): 673–84.

Smith, Stephen E. P., Jennifer Li, Krassimira Garbett, Karoly Mirnics, and Paul H. Patterson. 2007. "Maternal Immune Activation Alters Fetal Brain Development through Interleukin-6." *The Journal of Neuroscience: The Official Journal of the Society for Neuroscience* 27 (40): 10695–702.

Spann, Marisa N., Catherine Monk, Dustin Scheinost, and Bradley S. Peterson. 2018. "Maternal Immune Activation During the Third Trimester Is Associated with Neonatal Functional Connectivity of the Salience Network and Fetal to Toddler Behavior." *The Journal of Neuroscience: The Official Journal of the Society for Neuroscience* 38 (11): 2877–86.

Starace, Donatella, Roberta Galli, Alessio Paone, Paola De Cesaris, Antonio Filippini, Elio Ziparo, and Anna Riccioli. 2008. "Toll-like Receptor 3 Activation Induces Antiviral Immune Responses in Mouse Sertoli Cells." *Biology of Reproduction* 79 (4): 766–75.

Szulc, Kamila U., Jason P. Lerch, Brian J. Nieman, Benjamin B. Bartelle, Miriam Friedel, Giselle A. Suero-Abreu, Charles Watson, Alexandra L. Joyner, and Daniel H. Turnbull. 2015. "4D MEMRI Atlas of Neonatal FVB/N Mouse Brain Development." *NeuroImage* 118 (September): 49–62.

Teale, Peter, Bryce Pasko, Dan Collins, Donald Rojas, and Martin Reite. 2013. "Somatosensory Timing Deficits in Schizophrenia." *Psychiatry Research* 212 (1): 73–78.

Terwisscha van Scheltinga, Afke F., Steven C. Bakker, and René S. Kahn. 2010. "Fibroblast Growth Factors in Schizophrenia." *Schizophrenia Bulletin* 36 (6): 1157–66.

Thomason, Moriah E. 2020. "Development of Brain Networks In Utero: Relevance for Common Neural Disorders." *Biological Psychiatry* 88 (1): 40–50.

Tronnes, Ashlie A., Jenna Koschnitzky, Ray Daza, Jane Hitti, Jan Marino Ramirez, and Robert Hevner. 2016. "Effects of Lipopolysaccharide and Progesterone Exposures on Embryonic Cerebral Cortex Development in Mice." *Reproductive Sciences* 23 (6): 771–78.

Tustison, Nicholas J., Brian B. Avants, Philip A. Cook, and James C. Gee. 2010. "N4ITK: Improved N3 Bias Correction with Robust B-Spline Approximation." *2010 IEEE International Symposium on Biomedical Imaging: From Nano to Macro*. https://doi.org/10.1109/isbi.2010.5490078.

Uddin, Lucina Q., and Katherine H. Karlsgodt. 2018. "Future Directions for Examination of Brain Networks in Neurodevelopmental Disorders." *Journal of Clinical Child and*

Adolescent Psychology: The Official Journal for the Society of Clinical Child and Adolescent Psychology, American Psychological Association, Division 53 47 (3): 483–97.

Vaccarino, Flora M., Elena L. Grigorenko, Karen Müller Smith, and Hanna E. Stevens. 2009. "Regulation of Cerebral Cortical Size and Neuron Number by Fibroblast Growth Factors: Implications for Autism." *Journal of Autism and Developmental Disorders* 39 (3): 511–20.

Varghese, Merina, Neha Keshav, Sarah Jacot-Descombes, Tahia Warda, Bridget Wicinski, Dara L. Dickstein, Hala Harony-Nicolas, et al. 2017. "Autism Spectrum Disorder: Neuropathology and Animal Models." *Acta Neuropathologica* 134 (4): 537–66.

Vousden, Dulcie A., Elizabeth Cox, Rylan Allemang-Grand, Christine Laliberté, Lily R. Qiu, Zsuzsa Lindenmaier, Brian J. Nieman, and Jason P. Lerch. 2018. "Continuous Manganese Delivery via Osmotic Pumps for Manganese-Enhanced Mouse MRI Does Not Impair Spatial Learning but Leads to Skin Ulceration." *NeuroImage* 173 (June): 411–20.

Vuillermot, Stéphanie, Eliza Joodmardi, Thomas Perlmann, Sven Ove Ögren, Joram Feldon, and Urs Meyer. 2012. "Prenatal Immune Activation Interacts with Genetic Nurr1 Deficiency in the Development of Attentional Impairments." *The Journal of Neuroscience: The Official Journal of the Society for Neuroscience* 32 (2): 436–51.

Weber-Stadlbauer, Ulrike, and Urs Meyer. 2019. "Challenges and Opportunities of a-Priori and a-Posteriori Variability in Maternal Immune Activation Models." *Current Opinion in Behavioral Sciences* 28 (August): 119–28.

Wegiel, Jerzy, Michael Flory, Izabela Kuchna, Krzysztof Nowicki, Shuang Yong Ma, Humi Imaki, Jarek Wegiel, et al. 2014. "Brain-Region-Specific Alterations of the Trajectories of Neuronal Volume Growth throughout the Lifespan in Autism." *Acta Neuropathologica Communications* 2 (March): 28.

Willette, Auriel A., Gabriele R. Lubach, Rebecca C. Knickmeyer, Sarah J. Short, Martin Styner, John H. Gilmore, and Christopher L. Coe. 2011. "Brain Enlargement and Increased Behavioral and Cytokine Reactivity in Infant Monkeys Following Acute Prenatal Endotoxemia." *Behavioural Brain Research* 219 (1): 108–15.

Wolff, Jason J., Suma Jacob, and Jed T. Elison. 2018. "The Journey to Autism: Insights from Neuroimaging Studies of Infants and Toddlers." *Development and Psychopathology* 30 (2): 479–95.

Wormser, G. P., and R. W. Tolan. 2006. "Infectious Diseases of the Fetus and Newborn Infant, 6th Edition Edited by Jack S. Remington, Jerome O. Klein, Christopher B. Wilson, and Carol J. Baker Philadelphia: Elsevier Saunders, 2006. 1328 Pp., Illustrated. $229.00 (cloth)." *Clinical Infectious Diseases*. https://doi.org/10.1086/501023.

Xia, Cedric Huchuan, Zongming Ma, Rastko Ciric, Shi Gu, Richard F. Betzel, Antonia N. Kaczkurkin, Monica E. Calkins, et al. 2018. "Linked Dimensions of Psychopathology and Connectivity in Functional Brain Networks." *Nature Communications* 9 (1): 3003.

Zeighami, Yashar, Seyed-Mohammad Fereshtehnejad, Mahsa Dadar, D. Louis Collins, Ronald B. Postuma, Bratislav Mišić, and Alain Dagher. 2019. "A Clinical-Anatomical Signature of Parkinson's Disease Identified with Partial Least Squares and Magnetic Resonance Imaging." *NeuroImage* 190 (April): 69–78.

Zhang, Zhi, Amar Jyoti, Bindu Balakrishnan, Monica Williams, Sarabdeep Singh, Diane C. Chugani, and Sujatha Kannan. 2018. "Trajectory of Inflammatory and Microglial Activation Markers in the Postnatal Rabbit Brain Following Intrauterine Endotoxin Exposure." *Neurobiology of Disease* 111 (March): 153–62.

Zimmer, Anna, Alix Youngblood, Adam Adnane, Brian J. Miller, and David R. Goldsmith.

2020. “Prenatal Exposure to Viral Infection and Neuropsychiatric Disorders in Offspring: A Review of the Literature and Recommendations for the COVID-19 Pandemic.” *Brain, Behavior, and Immunity*, November. https://doi.org/10.1016/j.bbi.2020.10.024.

CHAPTER 5: Early and late exposure to prenatal maternal immune activation alters fetal and neonatal mouse neurodevelopment

5.1 Preface

The previous chapter presented a multi-modal characterization of developmental trajectories from childhood to adulthood in offspring exposed to MIA either early or late in gestation. In that chapter, **Chapter 4,** we observed that the developmental trajectories of exposed offspring, particularly those exposed to early gestational MIA, exhibited deviations already detectable at PND 21, wherein their brain volume was smaller than the other two groups in significantly affected regions. In addition to this observation, many of the genes dysregulated by prenatal MIA-exposure in the adolescent dorsal hippocampus were enriched for molecular pathways and processes critical to the earliest stages of brain development. Furthermore, a number of human neuroimaging studies had recently been published, in which alterations in the resting state functional connectivity networks were identified in babies exposed to higher levels of pro-inflammatory cytokines *in utero* as were executive function deficits in those toddlers (Rudolph et al. 2018; Spann et al. 2018). As highlighted in **Chapter 3**, there is a dearth of rodent studies investigating the effects of MIA-exposure on development in this early life phase using MRI.

The observations made in **Chapter 4** and the recently published human work motivated the work presented in this Chapter, which aims to investigate the effects of early or late gestational exposure to MIA on embryo and neonatal brain development. No study to date has taken a similar approach in rodents, to more cleanly dissect the relationship between MIA-exposure at specific gestational stages and offspring brain morphology in the embryonic and neonatal period. This is a necessary step in furthering our understanding of how MIA-exposure might initiate deviations in brain development. In addition to investigating the effects of MIA in understated offspring ages, the work presented here builds upon methodological advances made in **Chapter 4**. It leverages whole-brain neuroimaging techniques and aims to integrate them with either cellular imaging techniques (electron microscopy) in the case of the embryo, or with behavioural assays (ultrasonic vocalization task) in the case of the neonate. By employing a multi-modal approach, we are able

to associate striking brain volume alterations in the embryo, with putative sex-dependent cellular changes, while in the neonate brain, we are able to uncover subtle neuroanatomical and behavioural changes. This work highlights the importance of investigating offspring outcomes proximal to risk factor exposure and integrating across different modalities.

CHAPTER 5

Early and late exposure to prenatal maternal immune activation alters fetal and neonatal mouse neurodevelopment

Elisa Guma [1,2], Maude Bordeleau [3,4], Emily Snook [1,5], Gabriel Desrosiers-Grégoire [1,2], Shoshana Spring [6], Jason P. Lerch [6,7,8], Brian Nieman [6,7], Gabriel A. Devenyi [1,3], Marie-Eve Tremblay [4,9,10], M. Mallar Chakravarty [1,2,3,11]

[1] Computational Brain Anatomy Laboratory, Cerebral Imaging Center, Douglas Mental Health University Institute, Montreal, Quebec, Canada
[2] Integrated Program in Neuroscience, McGill University, Montreal, Quebec, Canada
[3] Department of Psychiatry, McGill University, Montreal Quebec, Canada
[4] Axe Neurosciences, Centre de Recherche du CHU de Québec-Université Laval, Québec, QC, Canada.
[5] Department of Medicine, University of Toronto, Toronto, Ontario, Canada
[6] Mouse Imaging Centre, The Hospital for Sick Children, Toronto, Ontario, Canada.
[7] Department of Neurosciences and Mental Health, The Hospital for Sick Children, Toronto, Ontario, M5G1X8, Canada.
[8] Department of Medical Biophysics, University of Toronto, Toronto, Ontario, Canada
[9] Département de Médecine Moléculaire, Faculté de Médecine, Université Laval, Québec, QC, Canada
[10] Department of Biochemistry and Molecular Biology, The University of British Columbia, Vancouver, BC, Canada
[11] Department of Biological and Biomedical Engineering, McGill University, Montreal, Quebec, Canada

5.2 Abstract

Exposure to maternal immune activation (MIA) *in utero* is a risk factor for neurodevelopmental and psychiatric disorders. MIA-induced deficits in adolescent and adult offspring have been well characterized, however, less is known about the effects of MIA-exposure on embryo and neonate development. We perform high-resolution *ex vivo* magnetic resonance imaging (MRI) to investigate the effects of early (gestational day [GD] 9) and late (GD 17) MIA-exposure and embryo (GD 18) and neonate (postnatal day 8) brain structure. We identify striking neuroanatomical changes in the embryo brain, particularly in the late exposed offspring. We further examined hippocampal neuroanatomy using electron microscopy and identified an increase in dark neuron and glia density in late exposed female embryos and a decrease in males. Neonate brain anatomy appeared largely unaffected, apart from some subtle alterations. To better characterize neurodevelopment, neonate communicative abilities were assayed with the ultrasonic vocalizations task, wherein early exposed offspring displayed decreased communicative ability. Overall, our findings integrate imaging techniques across different scales to identify differential impact of MIA-timing on neurodevelopment in the embryo brain. We integrate MRI and behavioural assays to demonstrate that these changes are no longer present in the neonate brain, while subtle behavioural deficits emerge.

5.3 Introduction

In utero exposure to maternal immune activation (MIA) is an environmental risk factor for neurodevelopmental and psychiatric disorders in exposed offspring (Brown et al. 2001; Selten et al. 1997). Indeed, MIA-exposure in animal models has been shown to induce neuroanatomical and behavioural changes relevant to many neurodevelopmental disorders (Canetta and Brown 2012). MIA leads to an increase in maternal pro-inflammatory cytokines and chemokines which are thought to interfere with fetal brain development by disturbing its delicate ecosystem, potentially by activating microglia, the resident immune cells of the brain (Choi et al. 2016; Gumusoglu and Stevens 2019; Solek et al. 2018; Boksa 2010; Thion, Ginhoux, and Garel 2018). If these changes occur during developmentally sensitive windows, they have the potential to alter neurodevelopmental trajectories, thereby increasing risk for neuropsychiatric disorders later in the

lifespan (Reisinger et al. 2015). Identifying these sensitive windows may be critical to our understanding of the effects of MIA-exposure. Previous work from our group has shown that the gestational timing of MIA-exposure has a differential impact on offspring neuro- and behavioural development and is related to greater variation following early MIA-exposure (gestational day [GD] 9) (Guma et al. 2021). These differences may be attributable to variation in maternal immune responsiveness and fetal brain development across gestation (Guma, Plitman, and Chakravarty 2019).

Although there is significant evidence that MIA-exposure *in utero* alters brain development trajectories in both human (Ellman et al. 2010) and animal models (Crum et al. 2017; Piontkewitz, Arad, and Weiner 2011), it is unclear how proximally to the MIA-exposure these changes can be detected. MIA-induced outcomes have been well characterized in adult offspring (Guma, Plitman, and Chakravarty 2019). However, to better understand the initiation and progression of MIA-induced pathology it is critical to study the impact of MIA-exposure on brain development in gestation and early life. Human neuroimaging studies have identified alterations in functional and structural connectivity in the infant brain following exposure to chronic, low-grade inflammation (as measured by interleukin [IL]-6 levels and/or C-reactive protein [CRP] in the maternal plasma) (Graham et al. 2018; Rudolph et al. 2018; Spann et al. 2018). Even though some observations in early phases of life exist, there is less information regarding how MIA-exposure impacts morphogenesis of the fetus. The work that does exist suggests that MIA-exposure induces acute upregulation of genes involved in immune signaling, hypoxia, and angiogenesis in the fetal brain (Canales et al. 2021). Further, alterations in proliferation, neuronal and glial specification, cortical lamination (Canales et al. 2021; Lombardo et al. 2018), global mRNA translation and altered nascent proteome synthesis had been reported (Kalish et al. 2021).

These exciting findings suggest that effects of MIA-exposure may be detectable in the fetal and neonatal period across mouse and human studies, however, it is unclear whether the transcriptional and histological variation observed in rodents translates to large-scale neuroanatomical changes such as those reported in the human studies, detected by whole-brain imaging approaches. Furthermore, although gestational timing has been shown to have differential effects in adolescent and adult offspring (Meyer et al. 2006; Guma et al. 2021) it is unclear how it affects neurodevelopment in this early life phase.

Alongside our previous work in which brain development was characterized from adolescence to adulthood, here we aim to develop a chronology of how the timing of MIA exposure may impact brain development *in utero* through to the neonatal period. We leverage structural magnetic resonance imaging (MRI), an inherently 3-dimensional imaging technique applicable for mouse phenotyping both *in vivo* and *ex vivo* (Wu and Zhang 2016). This technique allows for a comparable assay across species, providing a potential avenue for establishing cross-species homology (Barron et al. 2021). We examine the effects of *in utero* exposure to early (GD 9) or late (GD 17) MIA with the viral mimetic, polyinosinic:polycytidylic acid (poly I:C), on embryo (GD 18) and neonate (postnatal day [PND] 8) brain morphology using high-resolution *ex vivo* whole-brain magnetic resonance imaging (MRI). To better understand the cellular underpinnings of volumetric changes identified by MRI, we leveraged high-resolution electron microscopy (EM) to examine a cell class identified as a putative marker for neuroinflammation, cellular stress, and disease in the brain parenchyma (Nahirney and Tremblay 2021; Bisht et al. 2016; Henry et al. 2018) as a possible driver of volumetric changes observed: dark glial and neuron cell density. We investigate this primarily in the late exposed embryos given our observations of most drastic change in this group. We also used *ex vivo* MRI and an assay of neonate communicative ability using the ultrasonic vocalization task, to phenotype neonatal mice at postnatal day (PND) 8. Our results demonstrate neuroanatomical alterations in the GD 18 embryo brain following MIA, with differential effects due to timing in many regions, including the dentate gyrus of the hippocampus. Here, we observed a differential effect of GD 17 MIA-exposure on the presence of dark cells in male and female offspring. Conversely, limited alterations were observed in neonate neuroanatomy due to either GD 9 or 17 exposure, while subtle decreases in communication was observed in the GD 9 exposed offspring.

5.4 Materials and methods

5.4.1 Animals, prenatal immune activation, and sample preparation

C57BL/6J female and male mice of breeding age (8-12 weeks old) were subject to timed mating procedures (described in **Supplement 5.8.1**) to generate pregnant dams. For both embryo and neonatal sample collections, pregnant dams were randomly assigned to one of four treatment

groups (**Figure 5.1** for experimental design): (1) poly I:C (P1530-25MG polyinosinic:polycytidylic acid sodium salt TLR ligand tested; Sigma Aldrich) (5mg/kg, intraperitoneally) at gestational day (GD) 9 (POL E; 7 embryo dams, 6 neonate dams), (2) 0.9% sterile NaCl solution at GD 9 (SAL E; 6 embryo dams, 5 neonate dams), (3) poly I:C at GD 17 (POL L; 7 embryo dams, 6 neonate dams), or (4) saline at GD 17 (SAL L; 4 embryo dams, 4 neonate dams).

Embryo Sample Preparation for MRI: On GD 18, pregnant dams were euthanized, embryos were extracted and postfixed in 4% paraformaldehyde (PFA) with 2% Gadolinium (MRI contrast agent; Bracco Imaging S.p.A) in PBS for 1 week. A piece of the yolk sac was collected for genotyping for each embryo in order to identify the sex of the mouse via presence of the SRY gene (performed by Transnetyx, Memphis, TN). Collections were performed in two separate batches (with two different poly I:C batches from the same supplier outlined in **Supplement 5.8.1** and **Table 1**).

Neonatal Sample Preparation for MRI: On postnatal day (PND) 8, ~1 hour following behavioural testing (see **section 5.2.3**) neonates were perfused and in 4% PFA with 2% Gadolinium in PBS. Embryo and neonate samples were transferred to a 0.02% Sodium Azide 1x PBS solution for long-term storage until scanning (see **Supplement 5.8.2.** for more detail; sample numbers in **Table 5.1**). Immunostimulatory potential of poly I:C was confirmed in separate dams, all collected with the first batch of poly I:C (**Supplement 5.8.1, 5.9.1, Supplementary table 5.S1).**

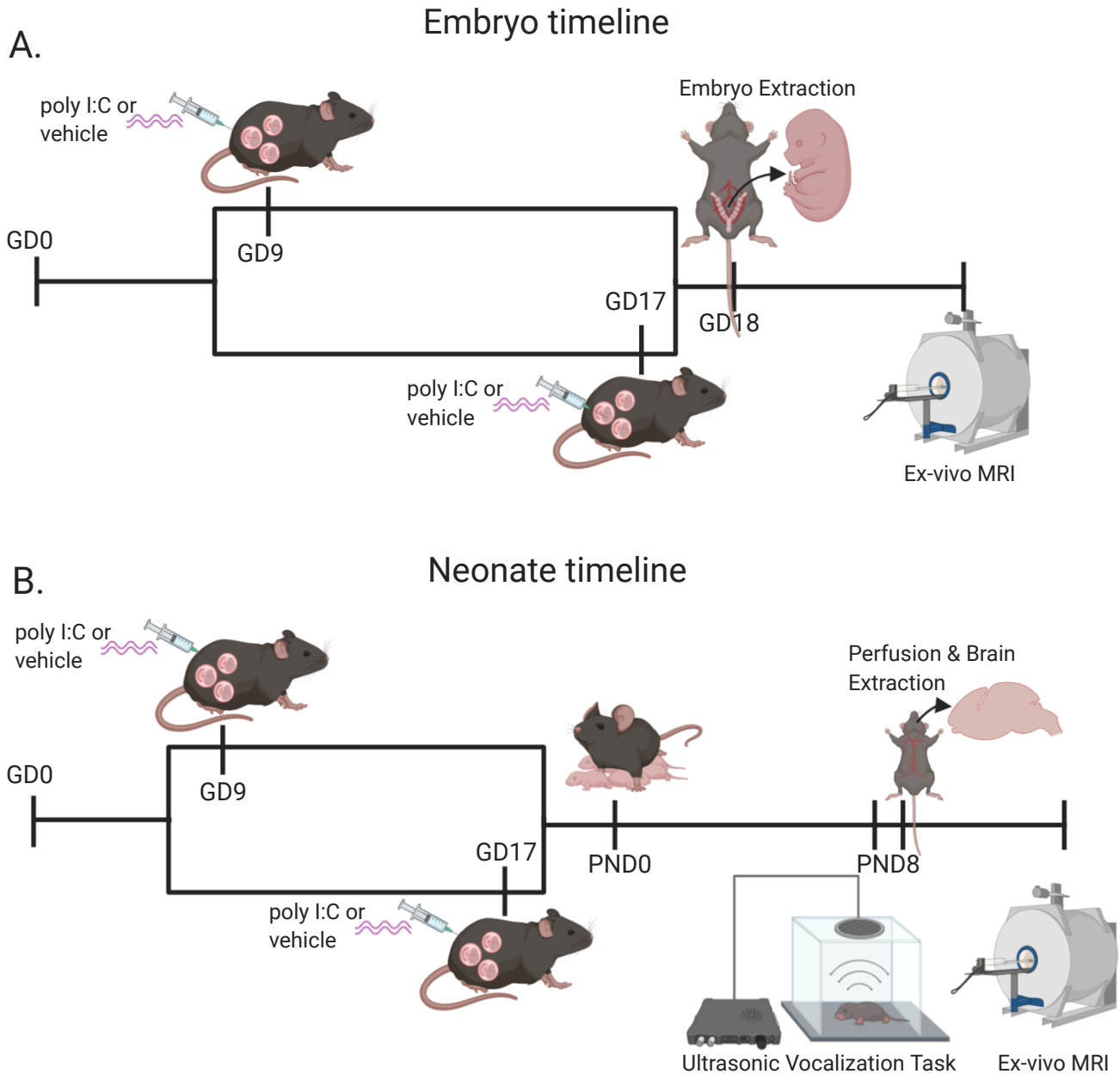


Figure 5.1. Experimental timeline. **A.** Pregnant dams were injected (i.p.) with poly I:C (5mg/kg) or vehicle (0.9% sterile NaCl) solution on gestational day (GD) 9 or 17. On GD 18, pregnant dams were euthanized, and embryos were extracted, and prepared for high resolution *ex-vivo* MRI. **B.** Pregnant dams were injected on either GD 9 or 17 with either poly I:C or vehicle (as in A). On postnatal day (PND) 8 offspring were tested on the ultrasonic vocalization task to assess communicative behaviour. Following behaviour, mice were perfused, and brains were prepared for *ex-vivo* MRI.

5.4.2 Magnetic resonance image acquisition and processing

All samples were shipped to the Mouse Imaging Centre (Toronto, ON) for scanning. A multi-channel 7.0-T MRI scanner with a 40 cm diameter bore (Varian Inc., Palo Alto, CA) was used to acquire anatomical images of the entire embryo (whole body), or of the neonate brains within skulls. A custom-built 16-coil solenoid array was used to acquire 40 μm^3 resolution images from 16 samples concurrently (Dazai et al., 2011; Lerch et al., 2011) (see **Supplement 5.8.2** for details).

Preprocessed embryo brain images (https://github.com/CoBrALab/documentation/wiki/Embryo-scan-preprocessing) of all subjects in the study were aligned by unbiased deformation based morphometry using the antsMultivariateTemplateConstruction2.sh tool (https://github.com/CoBrALab/twolevel_ants_dbm) (Avants et al. 2011). The output of this iterative group-wise registration procedure is a study average against which groups can be compared, as well as deformation fields that map each individual subject to the average at the voxel level. Relative Jacobian determinants (Chung et al. 2001), which explicitly model only the non-linear deformations and remove global linear transformation (attributable to differences in total brain size) were blurred at 80 µm full-width-at-half-maximum to better conform to Gaussian assumptions for downstream statistical testing. The same procedure was applied to the neonate brains (see **Supplement 5.8.3** for details).

Table 5.1. Final sample size for embryo and neonate MRI acquisition and neonate USV data following quality control.

	Embryos			**Neonates**				
Group	**Males MRI (Batch 2)**	**Females MRI (Batch 2)**	**Litters (Batch 2)**	**Males (MRI)**	**Females (MRI)**	**Males (USV)**	**Females (USV)**	**Litters**
SAL E	14 (1)	15 (2)	6 (1)	7	9	13	10	5
SAL L	12 (2)	12 (6)	4 (2)	8	11	14	16	4
POL E	11 (5)	17 (8)	7 (3)	13	13	23	17	6
POL L	14 (3)	17 (2)	7 (1)	16	13	25	14	6

5.4.3 Behavioural testing: ultrasonic vocalization task

Isolation-induced ultrasonic vocalizations of neonate offspring were assessed with standard procedures (Cossío et al. 2017; Baharnoori, Bhardwaj, and Srivastava 2010). Testing was performed on PND 8, as the rate of calling peaks around this time in mouse pups (Scattoni et al., 2008) using the Noldus UltraVox™ system (Noldus Information Technology, Leesburg, VA). Duration of each individual call per session per animal were recorded (raw data), as were the following summary measures per animal: total number of calls, maximum and minimum duration of calls, maximum and minimum call interval. See **Table 5.1** for sample size, and **Supplement 5.8.4** for details.

5.4.4 Electron microscopy

After MRI scanning, embryo brains (n=4males/4females per sex/group) were extracted from the fixed samples and further post-fixed with 3.5% acrolein in phosphate buffer [100mM] (pH 7.4) overnight at 4°C. Post-fixed brains were sectioned to 50 µm sagittal slices using a VT1200S vibratome (Leica Biosystem), and stored in cryoprotectant at -20 °C (30% glycerol, 30% ethylene glycol in PBS [50mM] (pH 7.4)). Three brain sections in which the dorsal hippocampus was present (Coronal section 12-15 (Schambra 2008) roughly equivalent to lateral 2.0-2.8 mm (Franklin and Paxinos 2008)) were processed for electron microscopy (n=3-4 animals/sex/group) using OTO post-fixation (Ellisman et al. 2011) (see **Supplement 5.8.5**). Samples were sectioned into ~70-75 nm ultrathin sections using a Leica Ultracut UC7 ultramicrotome (Leica Biosystems). Three levels of section-rubans were collected at an interval of 10 µm, glued on a specimen mount and imaged by array tomography at 25 nm resolution with an acceleration voltage of 1.4kV and current of 1.2nA using a Zeiss Crossbeam 540 Gemini scanning EM (Zeiss) (~3-7 images per embryo).

Images from the POL L and SAL L embryos were analyzed blind to the experimental conditions using QuPath (v0.2.0-m3) software (Bankhead et al. 2017). For each picture, region areas were traced and measured to calculate cell and blood vessel density. Total cell numbers and dark cells (neuronal and glial cells) were then counted within the dorsal hippocampus (CA1, CA3, and dentate gyrus). The percentage of dark cell population was calculated as a ratio over the total cell population (details in **Supplement 5.8.6**).

5.4.5 Statistical analyses

5.4.5.1 Neuroimaging data analysis

Statistical analyses were performed using the R software package (R version 3.5.1, RMINC version 1.5.2.2 www.r-project.org). Once we confirmed there were no statistically significant differences between our two control groups, they were combined, leaving us with three treatment groups: saline (SAL), GD 9-poly I:C (POL E), and GD 17-poly I:C (POL L). To assess the effects of poly I:C exposure at different gestational timepoints on embryo neuroanatomy we ran a whole-brain voxel-wise linear mixed-effects model (lme4_1.1-21 package; (Bates et al. 2015)) on the relative Jacobian determinant files using group and sex as fixed effects, and number of pups per litter, and collection batch as random intercepts. For neonates, the same procedure was followed, with group and sex as random effects, and number of pups per litter as a random intercept. SAL was set as the group reference, and males were set as the sex reference. The False Discovery Rate (FDR) correction was applied to correct for multiple testing (**Supplement 5.8.7** for details). Sex differences were explored as a follow up analysis for both embryos and neonates (**Supplement 5.8.7**). Putative differences in organs for the embryos were also investigated (**Supplement 5.8.4.1)**, however no differences were observed (**Supplement 5.9.2**).

5.4.5.2 Neonate USV data analysis

Since there were no differences in group means (see **Supplement 5.8.7.2** for analysis details), we used a shift function in order to maximize the data collected for each mouse (Rousselet, Pernet, and Wilcox 2017). This allows us to quantify how two distributions differ based on deciles of the distributions, i.e. it describes how one distribution should be rearranged to match the other and estimates how and by how much one distribution must be shifted. Three pairwise comparisons were made (SAL - POL E, SAL - POL L, POL L - POL E) on the distributions for the duration for each call for each animal in the 5 minute recording period, thresholded between 5 ms and 300 ms (a range previously used to filter out noise (Scattoni, Crawley, and Ricceri 2009)). A percentile bootstrap technique was used to derive confidence intervals based on differences in distribution at each decile of the distribution. This was then repeated to assess sex differences, so the same comparisons were made in only males, and only females, followed by the same percentile

bootstrap procedure. Sex differences were also investigated in the same way with the same pairwise comparisons as above for males and females separately.

5.4.5.3. Partial least squares analysis

A partial least squares (PLS) analysis was used to investigate putative brain-behaviour relationships between neonate neuroanatomy and USV behaviour. This is a multivariate technique for relating two sets of variables to each other by finding the optimal weighted linear combinations of variables that maximally covary with each other (Zeighami et al. 2019; McIntosh and Mišić 2013; McIntosh and Lobaugh 2004). The two variables used in this study were voxel-wise brain volumes (brain matrix) and USV call frequency binned by decile of distribution, as well as sex, and number of pups per litter (behaviour matrix). The behaviour matrix was z-scored and correlated to the brain matrix to create a brain-behaviour covariance matrix. A singular value decomposition was applied to generate a set of orthogonal latent variables (LVs), which describe linked patterns of covariation between the input brain and behaviour matrices. Permutation testing and bootstrap resampling were applied to assess LV significance and reliability (further details in **Supplement 5.8.7.3**).

5.4.5.4. Electron microscopy

A generalized linear mixed-effects model for Poisson distribution, robust to non-Gaussian distributions (Li et al. 2020), was applied to assess for differences in the density of dark glial and neuronal cells. To be consistent with our neuroimaging analyses, we first tested for group differences for density measures from each slide per mouse (~3-7), included to maximize variance, covarying for sex, with mouse as a random intercept to account for repeated measures (from each slide). Next, we tested for a group-by-sex interaction for density measures, again, with mouse as a random intercept. Given that the distribution of cell density counts was quite variable between groups, we also applied the shift function to assess for differences in distribution for POL L and SAL L offspring in each sex.

5.5 Results

5.5.1 Embryo brain results

We observed a significant effect of GD 9 MIA-exposure on the GD 18 POL E fetal brain volume (t=4.242, <1%FDR), wherein POL E offspring had smaller volumes than SAL in the globus pallidus, the hippocampus, including the dentate gyrus as well as more posterior regions, the fornix, centromedian thalamic nucleus, and cerebellum. Larger volume was observed in the caudate-putamen, the sexually dimorphic nucleus, the basolateral amygdala, and the CA1 region of the hippocampus in the POL E group relative to SAL (**Figure 5.2**).

GD 17 MIA-exposure induced very striking volumetric alterations, particularly volumetric increases in the brain of POL L offspring at GD18 (t=3.234, <1%FDR). Regions of volume increase relative to SAL offspring include the ventral pallidum, septal plate and lateral septal nucleus, the medial and lateral preoptic nuclei, the caudate putamen, the globus pallidus, hippocampus, both dentate gyrus and CA1 regions, the cingulum, anterior commissure, corpus callosum, external capsule, centromedian thalamus, and cerebellum. Decreases in volume were observed in the ventral hippocampus, more anterior regions of the cortical plate, the bilateral amygdala, fornix, and ventromedial thalamus (**Figure 5.3**).

Interestingly, GD 9- and GD 17-MIA exposure were observed to have opposite effects on brain volume in some regions implicated in neurodevelopmental disorders and in pervious MIA studies (Guma et al. 2021; Crum et al. 2017), such as the dorsal hippocampus, wherein GD 9-exposure decreased in volume, and GD 17-exposure increased volume. Similar observations were made for the centromedian thalamic nucleus. The septal nucleus and caudate-putamen were increased in both MIA-exposed groups. Post-hoc investigation of sex differences revealed no significant sex-by-group interactions.

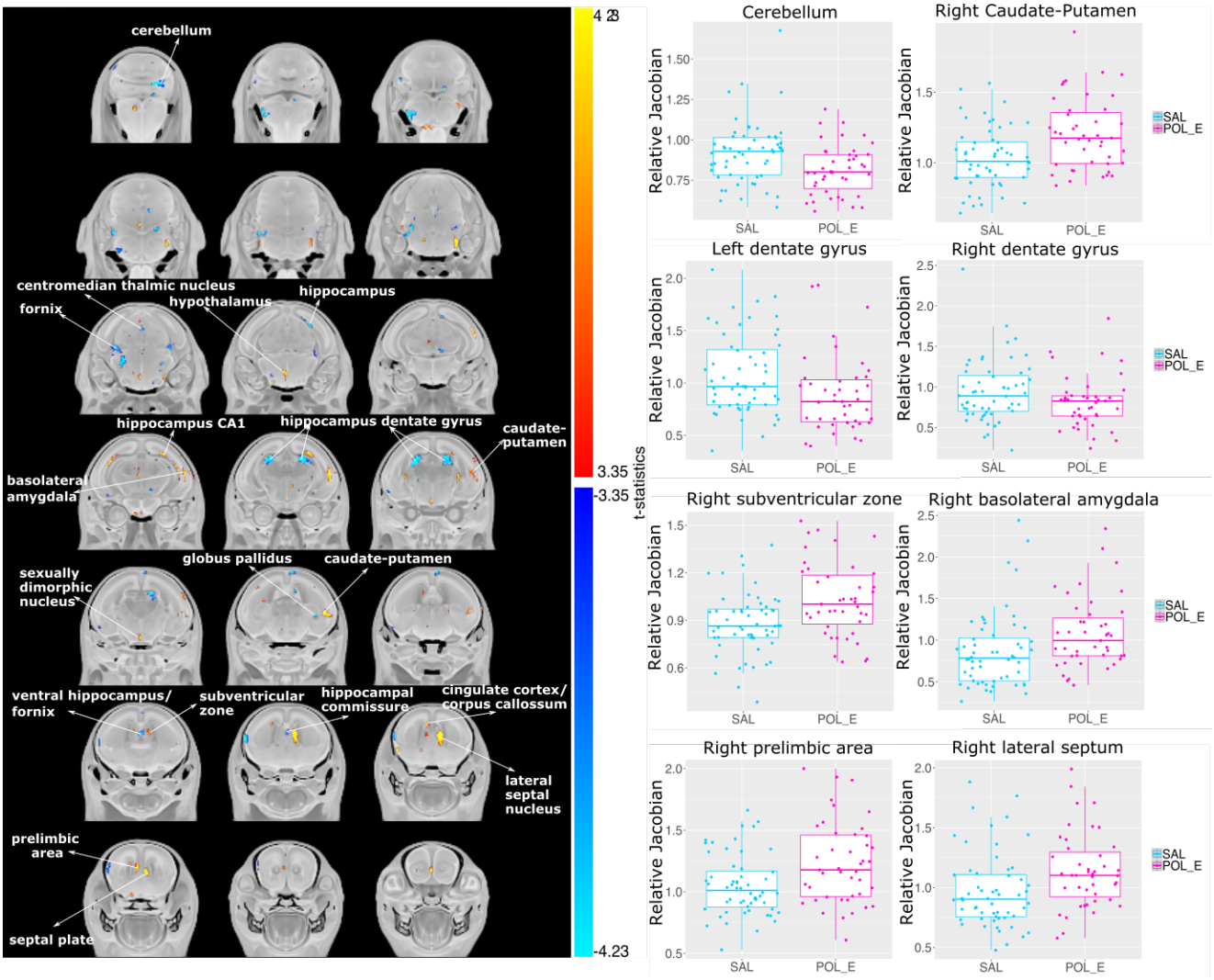


Figure 5.2. Neuroanatomical changes in the GD 18 embryo brain following GD 9-MIA exposure. **A.** t-statistic map of group (POL E vs SAL) thresholded at 5% (bottom, t=3.35) and 1% FDR (top, t=4.23) overlaid on the study average. **B.** Boxplot of peak voxels (voxels within a region of volume change showing largest effect) selected from regions of interest highlighted in white text in **A**. For all boxplots, the relative Jacobian determinants are plotted on the y-axis. Here a value of 1 means the voxel is no different than the average, anything above 1 is relatively larger, and below 1 is relatively smaller. For all boxplots, the midline represents the median of the data, the box represents the first and third quartiles, and the vertical line and dots represent the end range of the data.

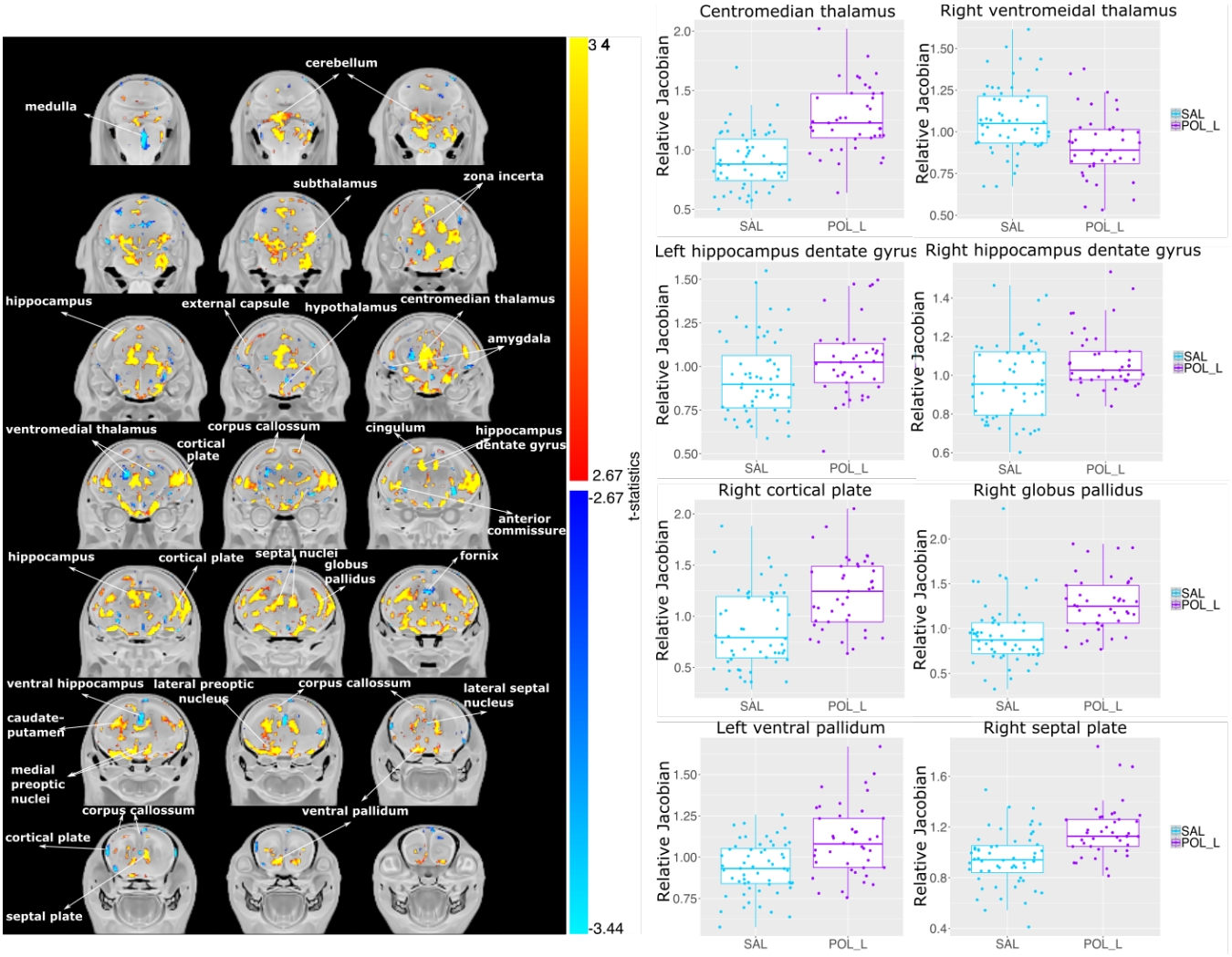


Figure 5.3. Neuroanatomical changes in the GD18 embryo brain following GD17-MIA exposure. **A.** t-statistic map of group (POL L vs SAL) thresholded at 5% (bottom, t=2.67) and 1% FDR (top, t=3.44) overlaid on the study average. **B.** Boxplot of peak voxels (voxels within a region of volume change showing largest effect) selected from regions of interest highlighted in white text in **A**. For all boxplots, the relative Jacobian determinants are plotted on the y-axis as in Figure 5.2.

5.5.3 Neonate brain results

Linear mixed-effects analysis of voxel wise volume difference revealed extremely subtle differences between SAL and POL E offspring (t=4.47, <20%FDR) wherein POL E offspring had a larger volume of a subregion of the right lateral amygdalar nucleus, a larger cluster of voxels in the right ventral hippocampus, and a smaller cluster of voxels in the right entorhinal cortex (**Figure 5.4**). POL L offspring had more significant, yet focal changes relative to the SAL offspring (t=5.47, <1%FDR). Larger volume in a cluster within the right prelimbic area, left amygdala, and right Crus I of the cerebellum were observed. Conversely, volume decreases were observed within a subregion of the left corpus callosum, and right fimbria, two white matter regions, as well as the right ventral subiculum. Post-hoc investigation of sex differences revealed subtle effects in the POL L group relative to SAL (t=4.23, <20%FDR); in the medial septum, pontine reticular nucleus, and cerebellum, volume for male POL L offspring was smaller than SAL, whereas the opposite was true for females, with larger volume for POL L than SAL offspring (**Supplement 5.9.2 and figure 5.S1)**.

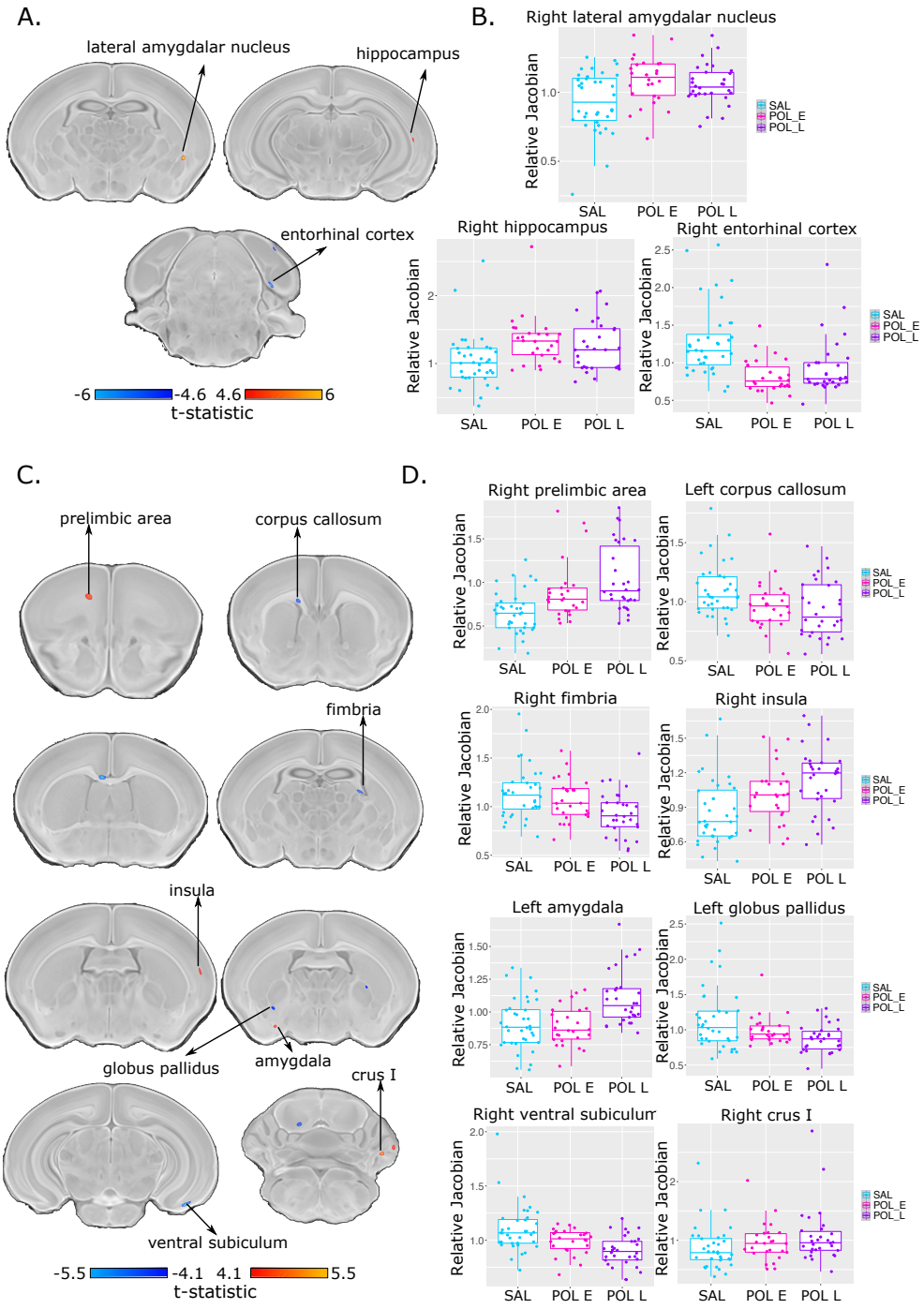


Figure 5.4. Neuroanatomical changes in the PND 8 neonate brain following GD 9 (**AB**) or GD 17 (**CD**) exposure. **A.** t-statistic map of group (POL E vs SAL) thresholded at 20% (bottom, t=4.60 to max, top, t=6.00) overlaid on the study average. **B.** Boxplot of peak voxels (voxels within a region of volume change showing largest effect) selected from regions of interest highlighted in **A**. **C.** t-statistic map of group (POL L vs SAL) thresholded at 5% (bottom, t=4.10) and 1% FDR (top, t=5.50) overlaid on the study average with peak voxels plotted in **D**.

5.5.4. Neonate ultrasonic vocalization behaviour results

Since there were no overall differences in means (see **Supplement 5.9.3**) but the data distribution and variance appeared different, we used an alternative, and more sensitive approach. The shift function revealed significant differences in distribution between the SAL and POL E groups. POL E offspring made significantly fewer calls, with more subtle differences for shorter calls (decile 1, $p=0.035$, decile 2, $p=0.003$), and with greater differences for longer duration calls ($p<0.00001$). The difference per decile is outlined in **Supplementary table 5.S2**. Similar differences were observed between POL L and POL E offspring, again with POL E making significantly fewer calls, particularly longer calls (**Supplementary table 5.S3**). Finally, there was no significant difference in distribution between POL L and SAL offspring at any of the deciles (**Supplementary table 5.S4**). Investigation of possible sex differences revealed that POL E females made significantly fewer call across all deciles, while POL L females made significantly more calls than SAL females across a few deciles of distribution; subtle differences were observed for POL E and POL L male offspring relative to SAL (**Supplement 5.9.4 and figure 5.2).**

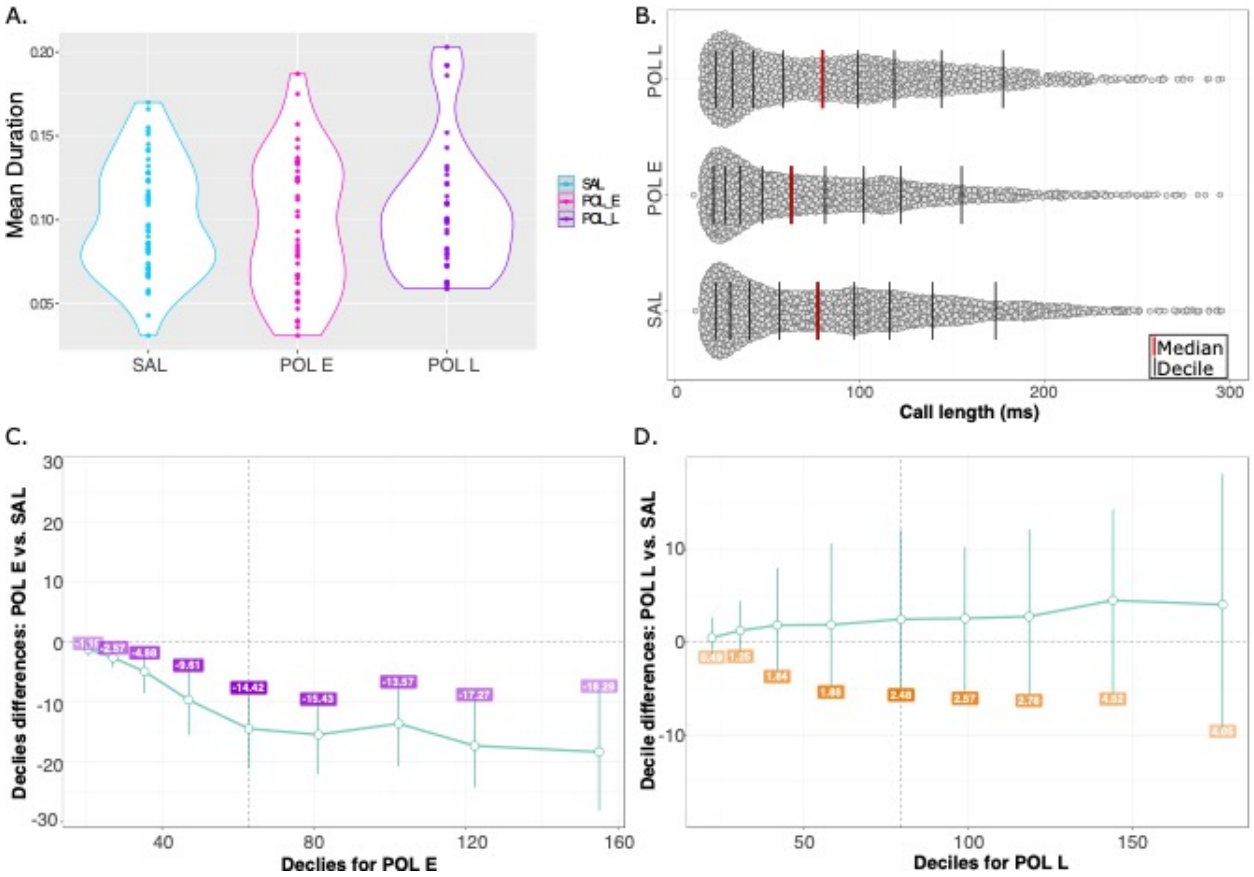


Figure 5.5. Results for ultrasonic vocalizations. **A.** Violin plot for mean call duration for each group (SAL, POL E, POL L) showing no overall differences. **B.** Distribution of call length (ms) for all calls made by all mice per group in the 5-minute recording period. The red line identifies the median of the data, while each black bar denotes a decile of distribution. **C.** Percentile bootstrapping technique applied to identify the difference in decile between the POL E group and SAL, showing significantly fewer calls made by the POL E group across the range of distributions. **D.** Percentile bootstrapping analysis reveals no significant difference between distributions for POL L relative to SAL as error bars cross the zero line.

5.5.5 Neonate USV-brain PLS

PLS analysis of voxel-level volume changes and USV call frequency binned into deciles (as with the distribution analysis) yielded three significant latent variables (LV)s. The first accounted for 46% covariance between matrices (p=0.003). This showed a pattern of increased call frequency associated with larger volume in the left thalamus, left lateral septum, left auditory cortex, left ventral hippocampus, and fourth ventricle, and smaller volume in the right primary motor and somatosensory cortex, right amygdala, right and left corpus callosum, right and left thalamus. The POL E and POL L groups appear to load onto the latent variable slightly more than the SAL group (although this relationship is reasonably subtle) (**Figure 5.6D**). The second LV accounted for 23% of the covariance (p=0.04) describing a pattern of increased corpus callosum, cingulate cortex, and subiculum volume, decreased thalamic and cerebellar volume associated with a greater number of shorter calls, and female sex. Again, the POL E and POL L group load onto the latent variable slightly more than the SAL group (**Figure 5.6H**). Finally, the third LV accounted for 10% of the covariance (p=0.02) and is further explained in **Supplementary materials 5.9.5** and **Supplementary figure 5.S3**.

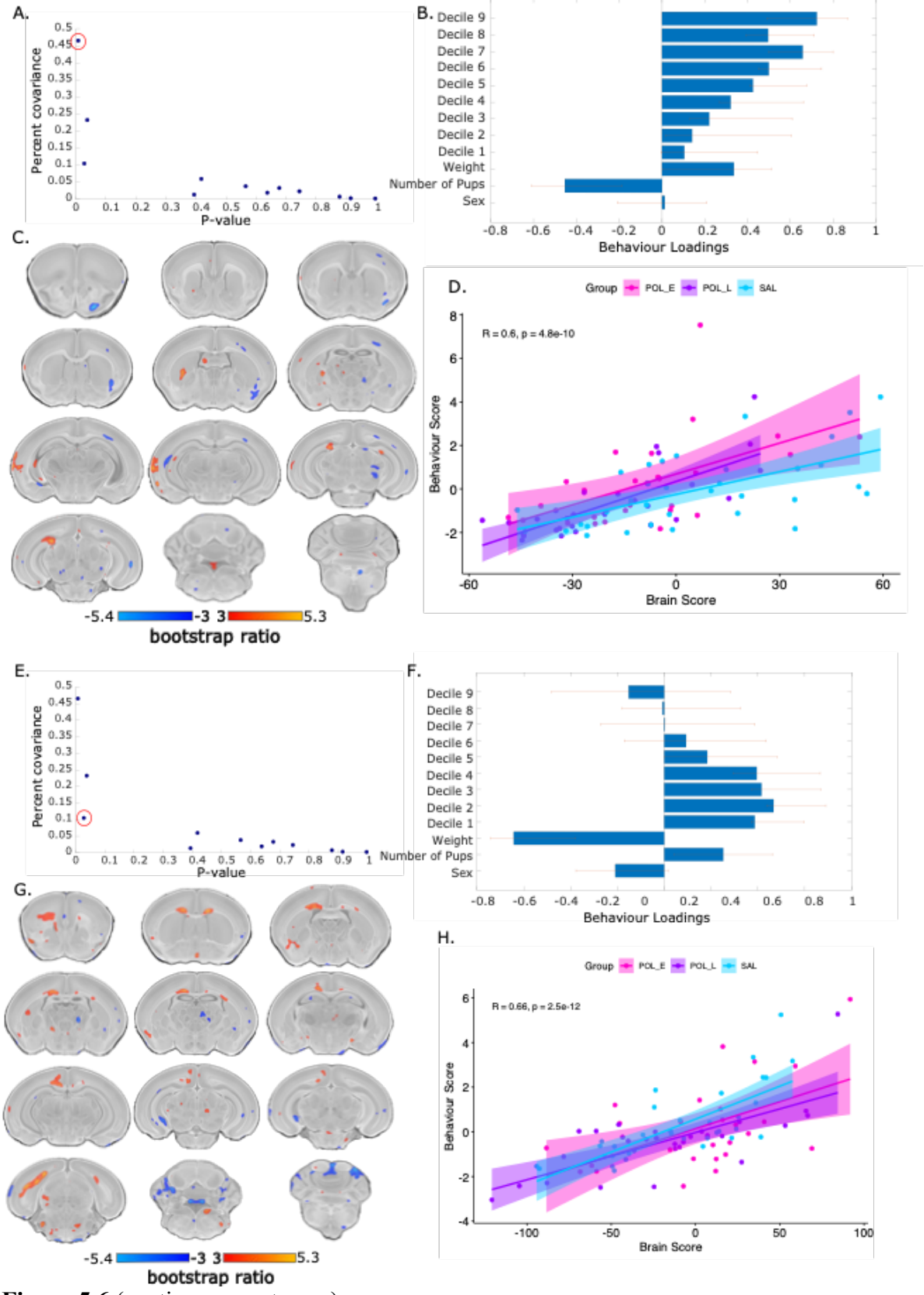


Figure 5.6 (caption on next page).

Figure 5.6 (continued). Partial least squares (PLS) analysis results for first and second significant latent variables (LV). **A**. Covariance explained (y-axis) and permutation p-values (x-axis) for all 12 LVs in the PLS analysis. LV1 is circled in red (p=0.003, %covariance=46%). **B.** Behaviour weight for each decile of distribution for the USC calls included in the analysis showing how much they contribute to the pattern of LV1. Singular value decomposition estimates the size of the bars whereas confidence intervals are estimated by bootstrapping. Bars with error bars that cross the 0 line should not be considered. **C**. Brain loading bootstrap ratios for the LV1 deformation pattern overlaid on the population average, with positive bootstrap ratios in orange yellow (indicative or larger volume), and negative in blue (indicative of smaller volume). Colored voxels make significant contributions to LV1. **D**. Correlation of individual mouse brain and behaviour score, color coded by treatment group with a trend line per group. Early poly I:C (POL-E) offspring (magenta) express this pattern more strongly than the saline controls (SAL) and late poly I:C (POL-L) groups. **E.** LV2 is circled in red on the same plots as in **A** (p=0.04, %covariance=23%). Behaviour weights (**F**), brain weights (**G**), and brain-behaviour correlations (**H)** represented as for LV1.

5.5.6 Electron microscopy of embryo dorsal hippocampus of POL L offspring

The post-mortem work was first carried out in the POL L and SAL L groups as this is where we observed the most striking volumetric changes. There were no significant overall group differences in either glial or neuronal cell density (glia: t=-0.484, p=0.628; neurons: t=-0.184, p=0.854). Interestingly, we observed a significant group-by-sex interaction for dark glial cell density (t=2.85, p=0.0044), and a main effect of group (t=-2.417, p=0.0157), but observed no significant interaction for the dark neurons (t=1.595, p=0.111). Comparison of glial and neuronal dark cell density distributions revealed significant differences between POL L and SAL L offspring within each sex, wherein, for both neurons and glia, male POL L offspring had significantly fewer dark cells than SAL offspring (glia: deciles 1-7, $p<0.05$, neurons: deciles 1-8, $p<0.05$), whereas POL L females had significantly more dark cells (all deciles for both neurons and glia $p>0.05$) (**Figures 5.7 & 5.8**, **Supplementary tables 5.S11-S14** for statistics at each decile).

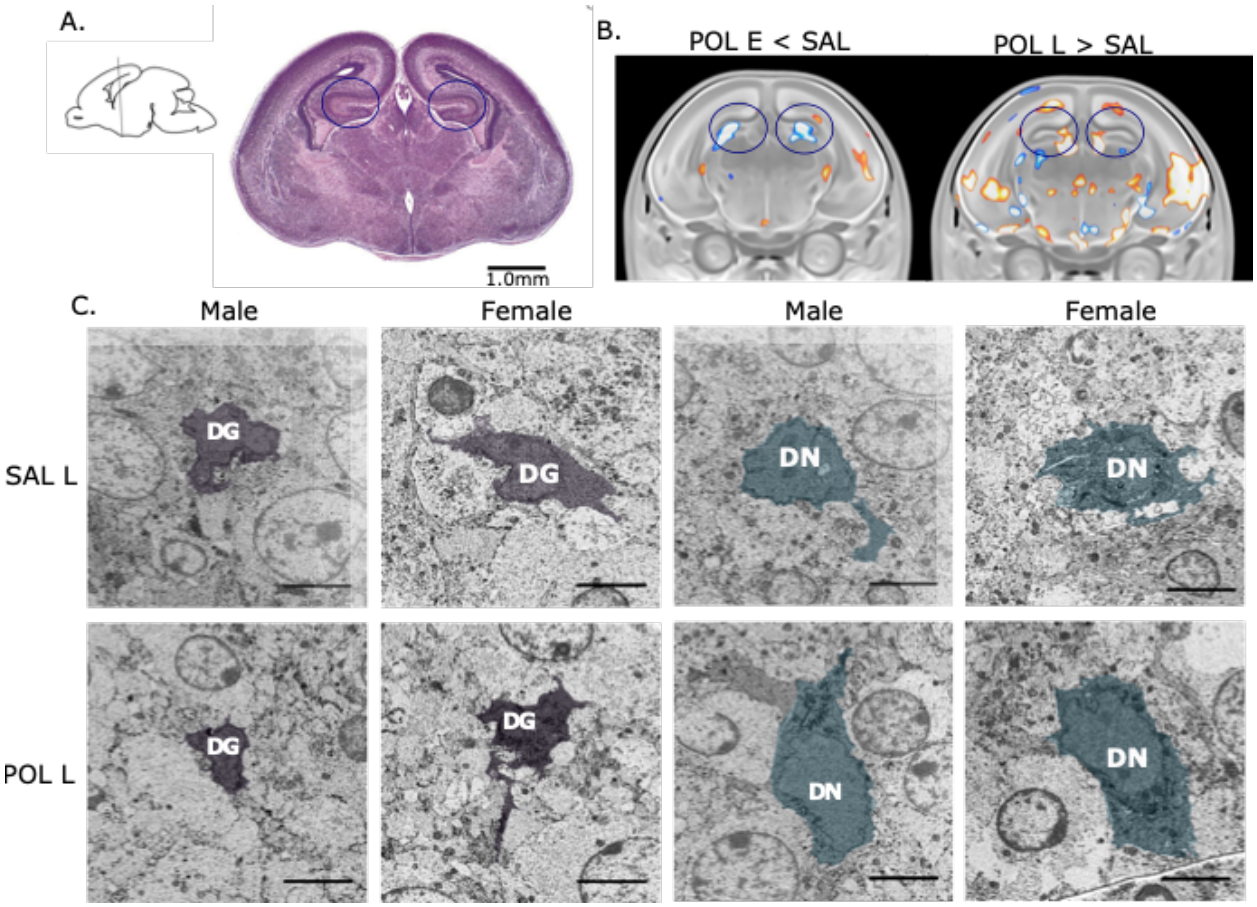


Figure 5.7. Representative image electron microscopy region of interest and of dark neuronal and glial cells. **A.** Sagittal slice orienting the region of the hippocampus selected, with the corresponding brain slice stained with Cresyl Violet from the GD18 mouse brain atlas (1mm scale bar), coronal slice 14 (Schambra 2008). The region of interest is highlighted in the circles. **B.** Representative slices of the hippocampus from the MRI results for POLE relative to SAL and POL L relative to SAL. The region of interest is highlighted in the circles. **C**. Images obtained with electron microscopy (25nm resolution) in the dorsal hippocampus from representative SAL L and POL L female and offspring (equivalent to coronal slice 14 from **A**) highlighting dark glial cells in the right 4 panels (dark gray cells, annotated with DG), and dark neurons (dark green cells, annotated with DN). Scale bar equivalent to 5 µm.

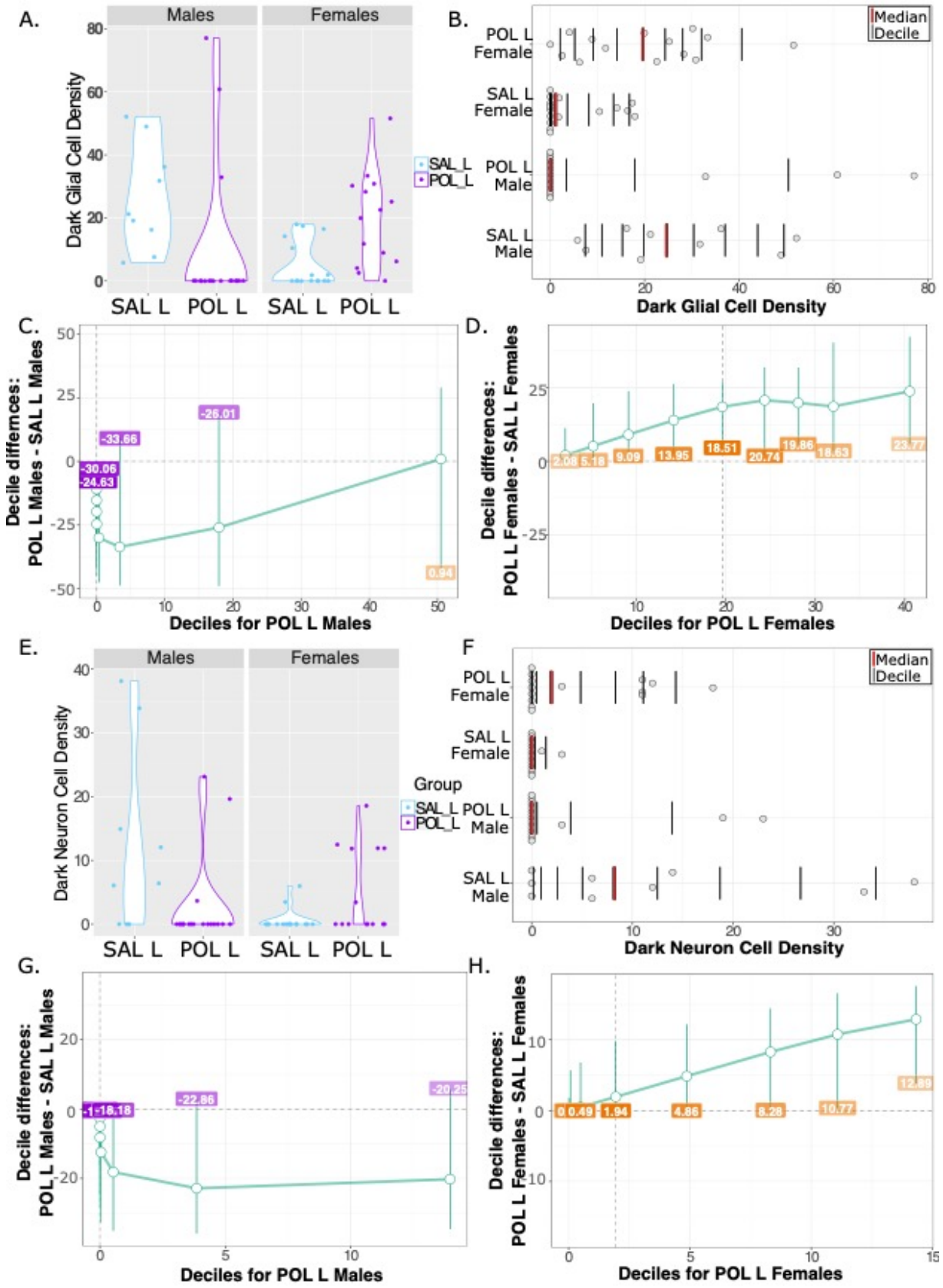


Figure 5.8 (caption on next page).

Figure 5.8 (continued). Sex differences in dark cell density in the embryo (GD18) hippocampus. **A.** Violin plot for dark glial cell density for each group (SAL, POL E, POL L) split by sex. **B.** Distribution of dark glial cell density for all hippocampal slices per animal. The red line identifies the median of the data, while each black bar denotes a decile of distribution. **C.** Percentile bootstrapping technique applied to identify the difference in decile between the POL L and SAL L, showing significantly less glial cell density in POL L males. **D.** Percentile bootstrapping analysis shows significantly more dark glial cells for females. **E**. Violin plot for dark neuron cell density per sex per group. **F.** Distribution of dark neuron cell density for all hippocampal slices per animal (same as in B). **G.** Decreased dark neuron cell density in POL L males across lower deciles of distribution. **H**. Increased dark neuron cell density in POL L females across all deciles of distribution.

5.6 Discussion

There is a well-established link between MIA-exposure *in utero* and latent neuroanatomical and behavioural abnormalities that emerge in adolescence or adulthood, with relevance to schizophrenia and ASD pathology (Knuesel et al. 2014; Estes and Kimberley McAllister 2016; Brown and Meyer 2018). However, limited work has been conducted in the early neurodevelopmental period (Guma, Plitman, and Chakravarty 2019). We leveraged high-resolution *ex vivo* MRI and electron microscopy to characterize the effects of MIA-exposure at two gestational timepoints on the embryo brain at GD 18. To determine whether the alterations observed *in utero* are sustained postnatally, we also assayed communicative behaviours and performed high-resolution *ex vivo* MRI in neonates at PND 8. Our results suggest that the embryo brain undergoes significant remodeling in response to MIA, particularly due to late gestational exposure, and coupled with sex-dependent changes in the presence of dark glia and neurons in the hippocampus. Conversely, the neonate brain remains largely unchanged, while subtle communication deficits emerge due to early gestational MIA-exposure. Elucidating the neurodevelopmental changes across embryonic and neonatal periods following MIA-exposure is an important step towards our understanding of MIA-exposure as a primer for downstream for late psychopathology and as a risk factor for an array of neuropsychiatric disorders.

Our results show that the embryo brain undergoes substantial volume changes following MIA. Broadly, we see volume reductions in the early exposed group, and striking volume expansions in the late exposed group, in many of the regions showing reductions in the early

exposed group. Since the late exposed embryo brains were harvested 24 hours following immune exposure, we may be capturing an acute neuroinflammatory or stress response, or an acceleration in brain development in response to the immune stimulus. However, given that there was no difference based on SAL timing, it is likely that any acute effects are attributable to the MIA itself. Interestingly, there is homology between regions affected in the embryo brains, and those in which we observed altered neurodevelopmental trajectories from childhood to adulthood in our previously published work (Guma et al. 2021). Some of these regions include the striatum/caudate-putamen, hippocampus, lateral septum, cingulate cortex, and cerebellum. Previous animal studies also report that MIA in late gestation increases neuroinflammation in the embryo rat brain and decreases placental function as measured by T2-signal intensity (Girard et al. 2010). These findings suggest that increased neuroinflammation and decreased placental function could, in part, be driving some of the volumetric increases in the late exposed embryo brain, which may provide some mechanisms underlying disease pathology.

In contrast, in the neonatal period we uncover very subtle effects due to MIA-exposure, which suggests that the neuroanatomical remodeling observed in the embryo may be transient, and may only emerge again later in adolescence, as identified by our previous work (Guma et al. 2021) and work of others (Crum et al. 2017; Piontkewitz, Arad, and Weiner 2011). If the neuroanatomical changes observed in the embryo do indeed capture an acute response to inflammation, it is possible that by PND 8 the neuroinflammation may have resolved, allowing the brain time to recover and normalize. In contrast with our findings, brain imaging studies report neuroanatomical and functional changes associated with chronic exposure to inflammation *in utero* in human neonates (Rudolph et al. 2018; Graham et al. 2018; Spann et al. 2018); additionally, some studies report neuroanatomical differences in individuals with ASD as early as 6 months of age (Shen and Piven 2017). Furthermore, neonatal rhesus monkeys whose mothers were exposed to maternal immunoglobulin G (isolated from mothers whose offspring had been diagnosed with ASD) in the first two trimesters displayed acceleration in brain growth, driven by white matter expansion particularly in the frontal and occipital lobes (Bauman et al. 2013). Further, MIA-exposure in late gestation in the rabbit has also been shown to increase neuroinflammation in the first two postnatal weeks of life (measured by TSPO PET imaging) (Zhang et al. 2018; Kannan, Saadani-Makki, Balakrishnan, Chakraborty, et al. 2011), and to decrease cortical serotonin binding (Kannan, Saadani-Makki, Balakrishnan, Dai, et al. 2011). Differences in the nature of MIA-exposure may

explain some of these differences, as both the human and rhesus monkey studies identified differences following more chronic inflammation. Differences in neurodevelopment between species could explain some of these discrepancies in findings (Kentner et al. 2019). Naturally the relatively minor alterations here require further investigation at both the structural and functional levels.

To gain more insight into the putative cellular underpinnings of the volumetric changes, we performed electron microscopy experiments in the dorsal hippocampus, a region highly implicated in neurodevelopmental pathology (Lieberman et al. 2018). In addition to these findings, we previously observed interesting MIA-related changes in our previous work in adolescence in a similar region - although following a different gestational exposure (Guma et al. 2021). Identifying the cellular processes triggered by MIA-exposure is critical to our understanding of how this risk factor may alter offspring neurodevelopmental trajectories. By leveraging high-resolution electron microscopic techniques, we have an unprecedented opportunity to investigate the brain parenchyma at nanoscale resolution (Nahirney and Tremblay 2021). This allows for identification of different cell types and their unique features, otherwise harder to detect and to connect to the later stages of the embryo morphogenesis. We focused our analyses or dark cells, in both neurons and glia, as these have been frequently observed in the brain parenchyma in response to stress (Henry et al. 2018), aging (Tremblay et al. 2012), and neurodegeneration (Bisht, Sharma, Lacoste, et al. 2016). They are thought to play a role in pathological circuit remodeling associated with disease (Bisht, Sharma, Lacoste, et al. 2016)**.** Dark neurons are typically defined by a darker appearance, with an accumulation of mitochondria and nuclear indentations, associated with structural plasticity (Versaevel et al. 2014), as well as markers of cellular stress (dilated endoplasmic reticulum and Golgi apparatus) (Nahirney and Tremblay 2021). Dark glial cells, particularly microglia, also display cellular markers of stress and have been shown to have hyper-ramified processes, to interact more frequently with synapses, and to have greater phagocytic capacity (Bisht et al. 2016; St-Pierre et al. 2020). Interestingly, sex-dependent effects on dark cell density, specifically dark microglia, have been reported in the brains of adult offspring exposed to poly I:C at GD9, with greater effects in male mice (Hui et al. 2018). We also observed sex-dependent effects, with increases in female embryos associated with GD17 exposure. This suggests that the directionality of the sex-dependent changes may be differentially affected by MIA timing and age of investigation in offspring (Ozaki et al. 2020). Further, reports of microglial

activation and density in the brains of prenatally immune-challenged animals early in life are mixed, with observations of increased density and motility, as well as no differences (Smolders et al. 2018). By focusing specifically on dark cells, we may gain better insight into the phenotypic variability of these reactive cells, and parse some of the heterogeneity in the literature.

In an attempt to better understand how the timing of MIA-exposure may differentially impact neurodevelopment, it is important to consider the possible processes it may be interfering with. In humans, the first trimester is a critical period for cell proliferation and neurogenesis; corticogenesis begins at ~4.5 weeks of gestation, followed by emergence of neuronal precursors shortly thereafter. At ~7 weeks of gestation, thalamo-cortical and dopaminergic fibers begin to develop, followed by the formation of the cortical plate at ~8 weeks of gestation (Selemon and Zecevic 2015). In the mouse, the neural tube forms, and neurogenesis begins at GD 9 and ends at GD 15. Microglia also begin to colonize the brain at GD 9, making this developmental period sensitive to potential inflammatory insults (Thion, Ginhoux, and Garel 2018; Manitz et al. 2013; Ginsberg et al. 2017). By GD 17, many neurodevelopmental processes are complete, however cell migration to form cortical layers, and myelination are ongoing (Selemon and Zecevic 2015).

Understanding the relationship between neuroanatomical abnormality and behaviour is critical. Social and communicative deficits are central to the pathology of many neurodevelopmental disorders, such as autism spectrum disorder. We experimentally modeled this by measuring ultrasonic vocalizations in our MIA-exposed neonates, and found abnormalities in our early exposed group, particularly in female offspring. Previous groups have also observed decreases in neonatal vocalization in both MIA-models (Baharnoori, Bhardwaj, and Srivastava 2010), and other animal models for neurodevelopmental disorders (Melancia et al. 2018; Malkova et al. 2012). Some studies also report sex-dependent differences, but with greater alterations in male exposed offspring (Gzielo et al. 2021; Cossío et al. 2017).

The results presented in this manuscript should be considered in light of their limitations. The design of our embryo study would be more complete with an assessment of neuroanatomy acutely following the GD 9-exposure at GD 10; this would allow us to detect whether volume increases, as those detected in the GD 17 -exposed offspring, are a response to acute inflammation, or specific to that gestational timepoint. Unfortunately, the embryo is too small for MRI acquisition at this gestational stage. Further, we observed sex-dependent effects on the density of dark neuronal and glial cells in the embryo brain, which were not recapitulated by volumetric changes.

However, it is possible that these cellular differences may manifest in sex-dependent changes in neuroanatomy as offspring develop. Employing longitudinal imaging on larger groups of offspring throughout the neonatal period may provide more sensitivity in detecting developmental changes; this approach has been successful at uncovering sex differences throughout mouse brain development (Qiu et al. 2018), and in previous work by our group examining offspring development from weaning to adulthood (Guma et al. 2021).

We comprehensively examined the effects of prenatal MIA-exposure, a known risk factor for neuropsychiatric disorders, at two gestational timepoints on embryo and neonate brain anatomy and neonate behaviour. We identified striking neuroanatomical remodeling in the embryo brain, particularly following exposure in late gestation; here we also observed sex-dependent alterations in the density of dark neuronal and glial cells in the dorsal hippocampus, with greater cell density in female offspring. This may reflect the initiation of pathological circuit remodeling. Neuroanatomical differences were mostly resolved by the neonatal period, where we observed deficits in communication in the early exposed offspring, again, with greater effects in female offspring. These findings show that MIA-exposure induces striking neurodevelopmental changes in embryonic and neonatal development, which may further our understanding of how this risk factor increases the likelihood of developing neuropsychiatric illnesses later in life.

5.7.1 Acknowledgements

The authors are grateful to the laboratory of Dr. Lalit Srivastava for lending us the Ultrasonic Vocalizations equipment, particularly to Teresa Joseph. Additionally, we would like to thank Lourdes de Cossio Fernandez for sharing her protocol for performing the behavioural assays. We would like to thank Roulin Gao for providing training in how to harvest embryos. We would like to thank Dr's Bruno Giros and Salah El Mestikawy for lending us their centrifuge. Finally, the authors would like to acknowledge their funding bodies, including the Canadian Institute of Health Research and Healthy Brains for Healthy Lives for providing support for this research. Additionally, we would like to thank the Fonds de Recherche du Québec en Santé for providing salary support for EG and MMC, as well as the Kappa Kappa Gamma Foundation of Canada for supporting EG's salary.

5.7.2 Conflict of interest statement

The authors report no conflicts of interest.

5.8 Supplementary methods

5.8.1 Animals and maternal immune activation (MIA)

C57BL/6J mice were used throughout the study and were bred in our facility under a 12-hour light cycle (8am-8pm), with food and water access *ad libitum*. C57BL/6J female and male mice of breeding age (8-12 weeks old) were subject to timed mating procedures. One male and one female were placed in a new cage in the afternoon, checked for appearance of plug, weighed, and separated the following morning. When seminal plug was observed, this was considered gestational day (GD) 0 (mice were allowed only 1 night together to improve the accuracy of GD 0 detection).

For embryo sample collection, pregnant dams were randomly assigned to one of four treatment groups: (1) poly I:C (P1530-25MG polyinosinic:polycytidylic acid sodium salt TLR ligand tested; Sigma Aldrich) (5mg/kg, intraperitoneally) at gestational day (GD) 9 (POL E; n = 10), (2) 0.9% sterile NaCl solution at GD 9 (SAL E; n = 7), (3) poly I:C at GD17 (POL L; n = 6 batch 1, 2 batch 2), or (4) saline at GD17 (SAL L; n = 4 batch 1, 2 batch 2). Collection was performed in two rounds with two different batches of poly I:C (same product). **Table 5.1** outlines total sample size for embryo groups, with those from batch 2 in brackets.

This procedure was repeated for neonatal sample collection, with pregnant dams being randomly assigned to the same four treatment groups: (1) poly I:C GD9 (POL E; n=6), (2) saline at GD9 (SAL E; n=4), (3) poly I:C at GD17 (POL L; n=6), (4) saline at GD17 (SAL L; n=4). GD9 corresponds roughly to the end of the first trimester in human gestation and GD17 corresponds to the end of the second trimester (Semple et al. 2013; Clancy, Darlington, and Finlay 2001). All injections were formed at 12pm (+/-1hour). All procedures were approved by McGill University's Animal Care Committee under the guidelines of the Canadian Council on Animal Care.

In a separate group of dams, poly I:C or saline was injected as described above (GD 9-POL: n=3 batch 1, n=4 batch 2; GD 17-POL n=3 batch 1, n=4 batch 2; GD 9-SAL n=5, GD 17-SAL n=3). Three hours following injection, dams were sacrificed by decapitation without euthanasia, and trunk blood was collected in a 1.5 mL Eppendorf tube. The blood was allowed to coagulate at room temperature for 30 minutes, and then centrifuged for 10 minutes at 4 ℃, with 2000 revolutions per minute. Serum was collected and stored at -80 ℃ until ready for analysis. Serum samples were shipped to the University of Maryland Core Cytokine Facility

(http://www.cytokines.com/) for multiplex ELISA to measure levels of IL-6, TNF-α, IL-1β, IL-10 to the immunostimulatory potential of our poly I:C. We chose to use a separate group of dams to ensure we could collect enough blood for analysis, and so as not to introduce an additional stressful experience for the dam, thereby potentially confounding the neurodevelopmental trajectory of offspring. Detection ranges were as follows IL-6 (0.64-8000 pg/ml), TNF-α (0.64-3500 pg/ml), IL-1β (0.64-15000 pg/ml), IL-10 (0.64-20000 pg/ml).

5.8.2 Brain sample preparation

5.8.2.1 Embryos

Embryos were harvested at GD 18 as follows. Pregnant dams were euthanized by cervical dislocation without anesthesia, embryos were removed and placed in ice cold 1x phosphate buffered saline (PBS) solution. One at a time, embryos were moved into warm (37 °C) PBS, separated from the uterus and placenta, and allowed to bleed out under agitation. A piece of the yolk sac was collected for genotyping for each embryo in order to identify the sex of the mouse via presence of the SRY gene (performed by Transnetyx, Memphis, TN). Once blood was successfully drained, embryos were placed in 4% paraformaldehyde (PFA) with 2% Gadolinium (MRI contrast agent; Bracco Imaging S.p.A) in PBS, and post-fixed for 1 week. Finally, embryos were moved to a 2% Gadolinium, 0.02% Sodium Azide 1x PBS solution for long-term storage until scanning.

5.8.2.2 Neonates

On postnatal day (PND) 8, ~1 hour following behavioural testing neonates were given an i.p. injection of ketamine, xylazine, and acepromazine at a dose of 0.1 mL/100 g before being transcardially perfused. Perfusion was performed using 1x PBS solution with heparin, followed by 4% PFA with 2% Gadolinium in PBS. Skulls were extracted containing the brains of offspring, and post-fixed in the same PFA-gadolinium solution for 24 hours. After 24 hours, the brains were transferred from the PFA-PBS-gadolinium solution to a solution of 0.02% sodium azide, PBS, and 2% gadolinium.

4.8.3 Magnetic resonance image acquisition

Prior to imaging, the samples were removed from the contrast agent solution, blotted and placed in 13-mm diameter plastic tubes filled with a proton-free susceptibility-matching fluid (Fluorinert FC-77, 3M Corp. St. Paul, MN). An anatomical scan was performed using a T2-weighted, 3D fast spin echo sequence using a cylindrical k-space acquisition (Nieman, et al., 2005) with TR/TE=350/12 ms, echo train length=6, two averages, field-of-view 20 mm x 20 mm x 25 mm, matrix size=504 x 504 x 630 (Spencer Noakes, Henkelman, and Nieman 2017). Total imaging time was 14 hours producing images with 40 µm isotropic resolution. MR images of precision-machined phantoms were aligned towards a computed tomography (CT) scan of the same phantom to produce distortion correcting transformations to correct for geometric distortions in a coil-specific manner.

5.8.4 MRI processing

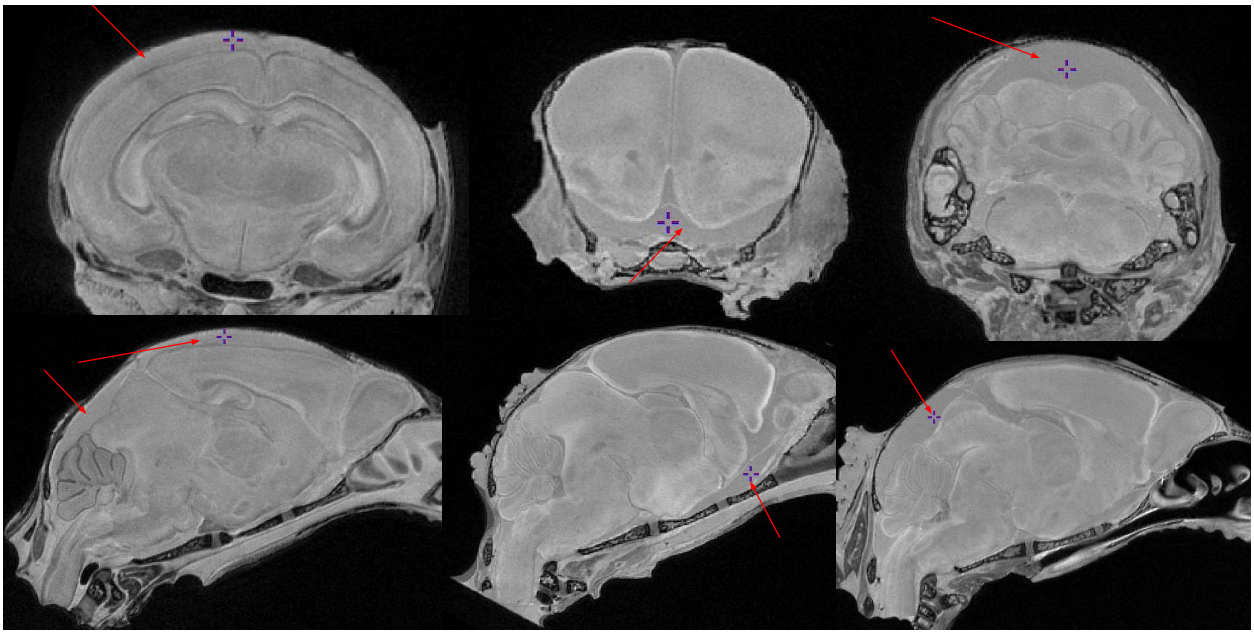
5.8.4.1 Embryos

Images were first cropped to center around the head of the embryos, an N4 correction for B1 bias field inhomogeneities (Tustison et al. 2010) and denoising using non-local means (minc_anlm) (Manjón et al. 2010) was applied, and the background was set to zero using minc tools. Next, brain images of all subjects in the study were aligned using the antsMultivariateTemplateConstruction2.sh tool (https://github.com/CoBrALab/twolevel_ants_dbm) (Avants et al. 2011). Briefly, images were aligned using rigid registration (translation and rotation), followed by affine (rigid, scaling, and shear), and finally, by nonlinear registration yielding a precise anatomical alignment in an automated, minimally biased fashion. The output of this iterative registration procedure is a study-specific average against which groups can be compared, as well as deformation fields that map each individual subject to the average at the voxel level. Further, the Jacobian determinants of each deformation field provide a measure of volume difference at each voxel in the image relative to the average. Relative Jacobian determinants (used for statistical analysis in this work) explicitly model only the non-linear part of the deformations and remove residual global linear transformations (attributable to differences in total brain size). Absolute Jacobians (without

removal of overall linear transformations) can be used to determine what localized changes in volume are attributable to global changes in volume. Prior to performing statistics, Jacobian determinants were blurred at 0.08 mm full-width-at-half-maximum to better conform to Gaussian assumptions for downstream statistical testing.

5.8.4.2 Neonates

The same automated image registration pipeline described above was used to process the neonate brain scans, with a few differences. Due to some perfusion artefacts, some brains presented with some extra matter that had similar intensity as the gray matter. To avoid misalignment during the registration, brain masks were manually segmented in order to target the automatic registrations to brain matter only (**Supplementary figure 5.S1)**.



Supplementary figure 5.S1. Examples of some of the perfusion artifacts observed in neonate scans. Whole-brain masks were manually segmented to ensure they did not bias registrations.

5.8.5 Behavioural testing: ultrasonic vocalization task

Isolation-induced ultrasonic vocalizations of GD 9- and 17- poly I:C and saline exposed offspring were assessed with standard procedures (Cossío et al. 2017; Baharnoori, Bhardwaj, and Srivastava 2010). Testing was performed on PND 8, as the rate of calling peaks around this time in mouse pups (Scattoni et al., 2008). Fifteen minutes after the dam was removed from the home

cage, each pup was individually transferred from the home cage to a plexiglass box (20.3 cm^3), where ultrasonic vocalizations were recorded continuously for 5 minutes using the Noldus UltraVox™ system (Noldus Information Technology, Leesburg, VA). An ultrasound detector (UltraSound Advice's Mini-3 bat detector) was tuned to detect incoming calls from 42 to 58 Hz. This was placed 15 cm above the bottom of the plexiglass box during each recording. A one-channel audio filter, which transmits a signal from the ultrasound detector to the computer if the signal is within the defined frequency amplitude range was set to detect calls if they lasted at least 5 ms and breaks in calls that lasted at least 1 ms. This allowed us to maximize sensitivity to sound increases but avoid background noise. After each completed acquisition, pups were removed from the acquisition chamber, weighed, and returned to their home cage with their littermates. The acquisition box was cleaned with 20% ethanol between each session. The duration of each individual call per session per animal was recorded (raw data), as were the following summary measures per animal: total number of calls, maximum and minimum duration of calls, maximum and minimum call interval. POL E = 23 males, 17 females, SAL E = 12 males, 10 females, POL L = 25 males, 14 females, SAL L = 14 males, 16 females.

5.8.6 Electron microscopy

After post-fixation (described in the main text, **5.8.6**), tissues were incubated in 3% ferrocyanide (BioShop, cat# PFC232.250) combined with 4% osmium tetroxide (Electron Microscopy Sciences, cat#19170) (1:1) for 1 hour, 1% thiocarbohydrazide (in PBS; Electron Microscopy Sciences, cat# 2231-57-4) for 20 minutes, and in 2% osmium tetroxide for 30 minutes. OTO post-fixed sections were then dehydrated (ethanol (2× in 35%, 1× in 50%, 1× in 70%, 1× in 80%, 1× in 90%, 2× in 100%) followed by 3× in propylene oxide, for 5 minutes each), embedded in Durcupan ACM resin (MilliporeSigma, cat# 44611-44614) for 24 hours, and transferred between two fluoropolymer ACLAR® sheets (Electron Microscopy Sciences, cat# 50425-25). ACLAR sheets containing tissues were let polymerized at 55-60 °C for 5 days. Using a binocular microscope, the dorsal hippocampus was excised from tissue sections using a razor blade, superglued onto a resin block, and left to incubate overnight at 55-60 °C. Next, the resin block was trimmed with a razor blade into a pyramidal shape to allow for more stable and precise sectioning. Following glue removal tissue was sectioned into ~70-75 nm ultrathin sections using a Leica

Ultracut UC7 ultramicrotome (Leica Biosystems). Three levels of section-rubans were collected at an interval of 10 µm, which represented different levels of the dorsal hippocampus. Section-rubans were collected on a silicon nitride chip and glued on a specimen mounts and imaged by array tomography at 25 nm resolution with an acceleration voltage of 1.4kV and current of 1.2nA using a Zeiss Crossbeam 540 Gemini scanning EM (Zeiss).

5.8.7 Statistical analyses

5.8.7.1 Neuroimaging analysis

Our first goal was to determine whether there were neuroanatomical differences in our two control groups, SAL E (GD9) and SAL L (GD17). We ran a whole-brain (delimited by a brain mask) voxel-wise linear mixed effects model on the relative Jacobian determinant files of each subject with injection timing and sex as fixed effects, and litter and number of pups per litter as random intercept to account for litter-specific variation. A False Discovery Rate (FDR) correction was applied. The same procedure was performed to assess whether there were any differences between the control neonate offspring (batch was not a factor for this analysis). In both cases, there were no differences, and SAL E and SAL L offspring were combined and set as the reference group. Finally, sex differences were explored, investigating the interaction between group and sex, with the same covariates as above. The statistical models applied to the relative Jacobian determinants to assess for group differences, described in **5.8.7.1** are detailed below:

Embryo model: $Y_{subject,j} = \beta_0 + \beta_1 sexF_{subject,j} + \beta_2 groupPOL_E_{subject,j} + \beta_3 groupPOL_L_{subject,j} + \mathbf{b_1}$collection size + $\mathbf{b_2}$litter size + $\epsilon_{subject,j}$

Neonate model: $Y_{subject,j} = \beta_0 + \beta_1 sexF_{subject,j} + \beta_2 groupPOL_E_{subject,j} + \beta_3 groupPOL_L_{subject,j} + \mathbf{b_1}$litter size + $\epsilon_{subject,j}$

Embryo sex interaction model: $Y_{subject,j} = \beta_0 + \beta_1 sexF_{subject,j} + \beta_2 groupPOL_E_{subject,j} + \beta_3 groupPOL_L_{subject,j} + \beta_4 sexF{:}groupPOL_E_{subject,j} + \beta_5 sexF{:}groupPOL_L_{subject,j} + \mathbf{b_1}$collection size + $\mathbf{b_2}$litter size + $\epsilon_{subject,j}$

Neonate sex interaction model: model: $Y_{subject,j} = \beta_0 + \beta_1 sexF_{subject,j} + \beta_2 groupPOL\_E_{subject,j} + \beta_3 groupPOL\_L_{subject,j} + \beta_4 sexF{:}groupPOL\_E_{subject,j} + \beta_5 sexF{:}groupPOL\_L_{subject,j} + + \mathbf{b_1} litter\ size + \epsilon_{subject,j}$

Y= outcome measures (i.e. blurred absolute Jacobian determinants); β_i= fixed effect coefficient; β_0 = equation intercept; **b** = random predictor; **ϵ** = random error; **j** = repeated measure per subject; **:** = interaction; POL_E = early poly I:C group relative to SAL as reference; POL_L = late poly I:C group relative to SAL as reference; SexF = female sex relative to male as reference

As an exploratory analysis, we investigated whether there were any volume differences in the organs due to GD9 or 17 MIA exposure. We ran the same model as above on the voxels within the organ cavity defined manually with the same models described above.

5.8.7.2. Neonate USV data analysis

For the USV data, summary measures were first tested for normality using a Shapiro-Wilks test. Since the data were not normally distributed, means were first compared using the Kruskal Wallis test. We determined that this was not the most accurate way to represent our data. Therefore, instead of using the summary measures provided by Ultravox, we examined the duration for each individual call made by each animal in the 5-minute recording period.

5.8.7.3 Partial least squares analysis

Input imaging and behavioural data were organized into two matrices X (imaging), and Y (behaviour), with subjects as rows and variables in columns. The behavioural matrix was first z-scored (mean subtracted from each column and divided by standard deviation). A covariance matrix was then computed from the brain and z-scored behavioural matrices to represent all voxel deformation values and behavioural measures per subject. Singular value decomposition (SVD) was then applied to the covariance matrix (Eckart and Young 1936). This yields a set of orthogonal latent variables (LVs), which are patterns that describe the relationship between the brain and behaviour data. From these you get a set of 'brain scores' describing how each brain variable weights into a given LV, a set of 'behaviour scores' describing how each behaviour variable weights into a given LV, and a singular value, which describes the proportion of variance explained by the LV.

Permutation testing was applied to assess the statistical significance of each LV wherein the rows (subjects) of the brain data matrix were randomly shuffled (n=1000 repetitions) to

generate a null distribution of possible brain-behaviour correlations. The random shuffling allows for dependencies between brain and behaviour to be nullified. SVD was then applied to these "null" correlations, generating a distribution of singular values under the null hypothesis. A p-value can then be generated by looking at the probability that a permuted singular value exceeds the original, non-permuted singular value (Zeighami et al. 2019; Patel et al. 2020).

Next, ***bootstrap resampling*** was applied to assess the contribution of individual variables (both voxel and behavioural metrics) to each LV. Subjects (rows for both brain and behaviour matrices) were randomly sampled and replaced (n=1000) to generate a set of resampled correlation matrices to which SVD was applied to generate a sampling distribution for each weight of the singular vectors. The ratio of each singular vector weight and its bootstrap-estimated standard error were used to calculate a "bootstrap ratio" for each voxel. Voxels that make large contributions to certain patterns can therefore be identified by large bootstrap ratios. For this analysis, bootstrap ratios were thresholded at values corresponding to 95% confidence interval, as previously done (Zeighami et al. 2019). Next, weighted 'brain' and 'behaviour scores' were projected onto individual patient data to generate patient-specific scores.

5.9 Supplementary results

5.9.1 Poly I:C injection does increase pro-inflammatory cytokines

We observed an increase in levels of pro-inflammatory cytokines IL-6, IL-1β**,** and IL-10, but not TNF-α, in a separate cohort of pregnant dams 3-hours post poly I:C injection with our first batch on GD 9 relative to saline control on GD 9. For the second batch of poly I:C injected on GD 9, all 4 pro-inflammatory cytokines were increased (IL-6, IL-1β, TNF-α) 3 hours post-injection, but the anti-inflammatory cytokine IL-10 was not.

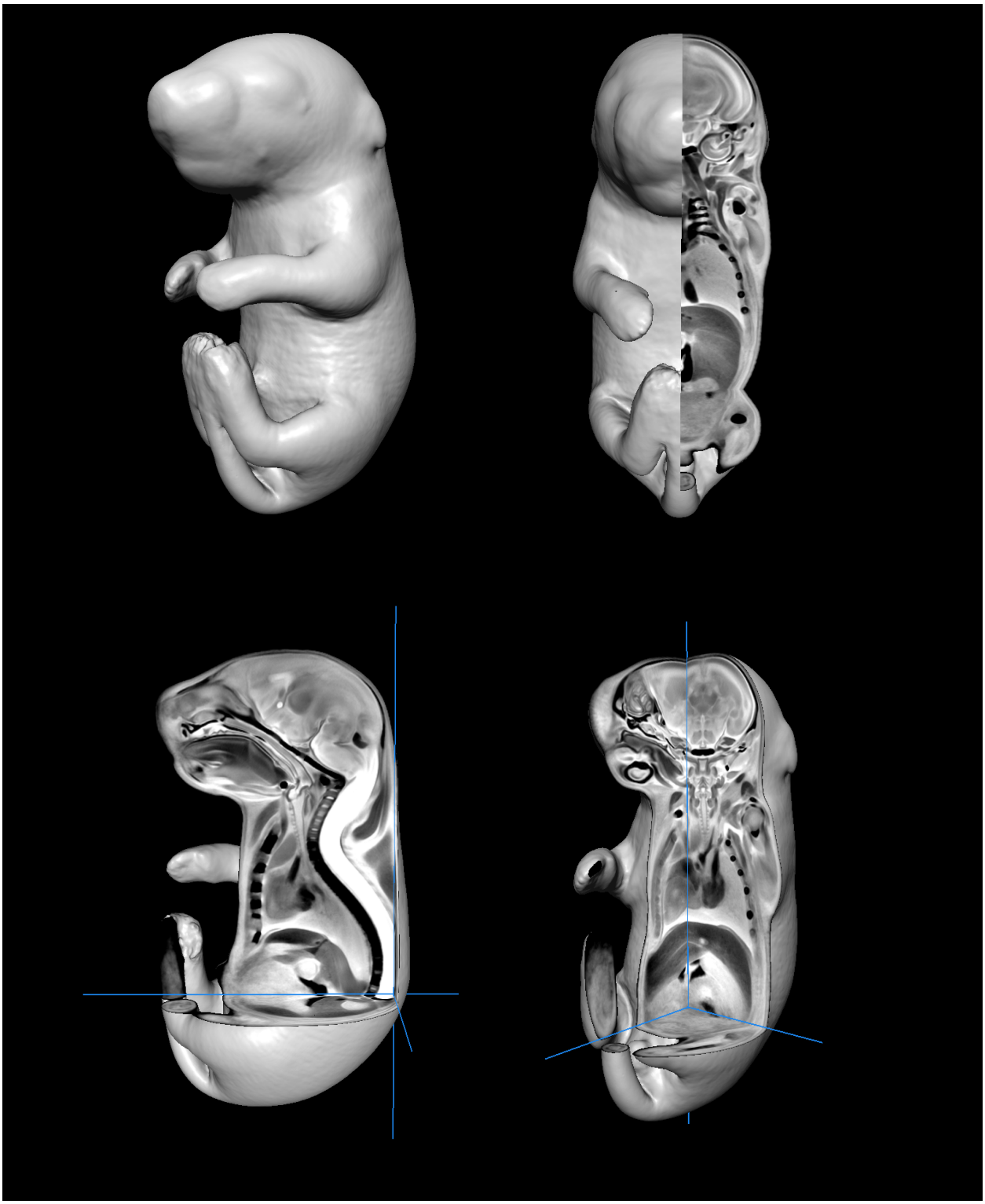
Exposure to our first batch of poly I:C on GD 17 increased levels of pro-inflammatory cytokines IL-6 relative to all but one SAL L dam who had exceptionally high IL-6 values. No differences were observed for TNF-α, IL-1β, or IL-10 relative to GD 17 saline controls. Our second batch of poly I:C similarly only increased IL-6, and had no effect on the other cytokine levels, however, for IL-1β and IL-10 values were below the detection threshold (<0.64) (**Supplementary Table 5.S1)**.

Supplementary Table 5.S1. Maternal serum cytokine levels for our 4 treatment groups, mean [range]; * below detection range

	IL-1β (pg/ml)	**IL-6 (pg/ml)**	**IL-10 (pg/ml)**	**TNF-α (pg/ml)**
SAL E (n=5)	21.58 [0.64-96.62]	45.54 [30.58-56.59]	21.86 [0.64-56.22]	14.30 [0.64-26.05]
SAL L (n=3)	4.62 [0.64-6.88]	746.33 [22.2-2192.47]	42.13 [8.30-83.37]	17.76 [5.99-39.59]
POL E batch 1 (n=3)	11.56 [9.80-12.95]	3578.84 [3624.87-3740.81]	44.05 [36.93-49.70]	8.20 [7.91-8.35]
POL L batch 1 (n=3)	5.45 [5.00-6.34]	1298.69 [54.36-3173.25]	16.73 [13.67-19.58]	20.35 [14.21-31.18]
POL E batch 2 (n=4)	16.26 [0.64-25.4]	14086.22 [1000.00- 18033.31]	76.79 [35.18-174.44]	69.05 [7.02-131.31]
POL L batch 2 (n=4)	0.64* [0.64-0.64]	1503.49 [0.64-1000]	0.64 [0.64-0.64]	4.95 [0.64-14.98]

5.9.2 No volumetric differences in the embryo organs

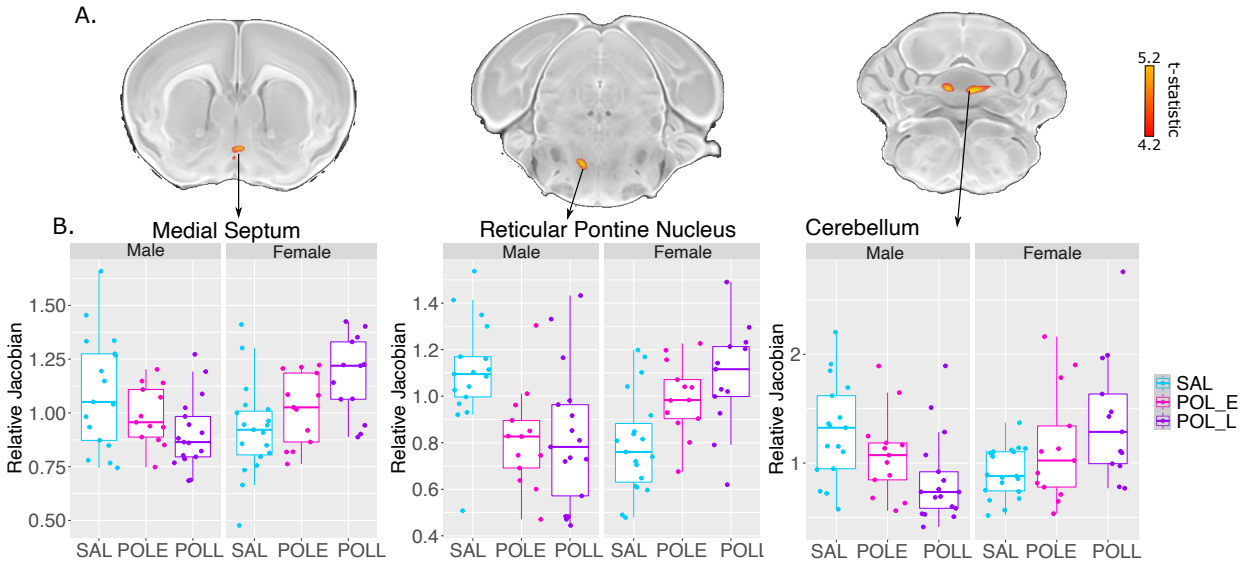
No significant differences were observed in the volume of the organ cavity for POL E or POL L exposed embryos at GD 18 (data not shown). The average for the whole-body registration is presented below in **Supplementary figure 5.S2.**



Supplementary figure 5.S2. Representative 3D view of whole-body nonlinear average for the GD 18 embryo (40 µm^3 resolution).

5.9.3 Sex differences neonate neuroanatomy

As described in the main text, post-hoc investigation of sex differences revealed subtle effects in the POL L group relative to SAL (t=4.23, q=0.20) wherein POL L males and smaller volume in the medial septum, reticular pontine nucleus, and cerebellum relative to SAL, with the opposite patter in females (**Supplementary figure 5.S3)**.



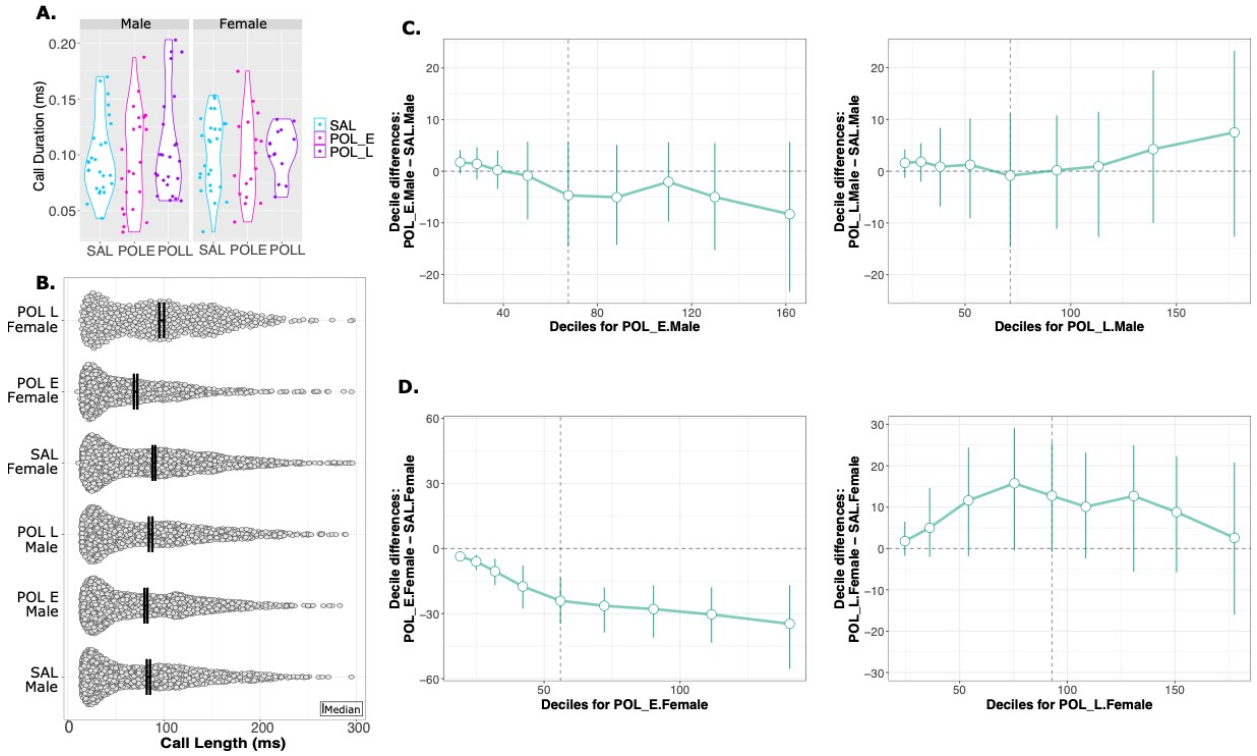
Supplementary figure 5.S3. Sex differences in neuroanatomy in the PND8 neonate brain following GD17 exposure. **A.** t-statistic map of group (POL E vs SAL) thresholded at 20% (bottom, t=4.20) overlaid on the study average. **B.** Boxplot of peak voxels (voxels within a region of volume change showing largest effect) selected from regions of interest.

5.9.4 Analysis of summary USV data

Summary measures for USV data was not normally distributed based on the Shapiro-Wilks test for the following measures of interest: number of calls (W=0.821, p=2.43e-11), mean call duration (W=0.975, p=0.0155), and maximum call duration (W=0.849, p=3.07e-10). The Kruskall-Wallis rank sum test to compare group differences revealed no significant differences between the SAL, POL E, and POL L groups for number of calls (KW chi-squared=0.600, df=2, p=0.896), mean call duration (KW chi-squared=0.443, df=2, p=0.801), and maximum call duration (KW chi-squared=0.067, df=2, p=0.967).

5.9.5 Sex differences in USVs

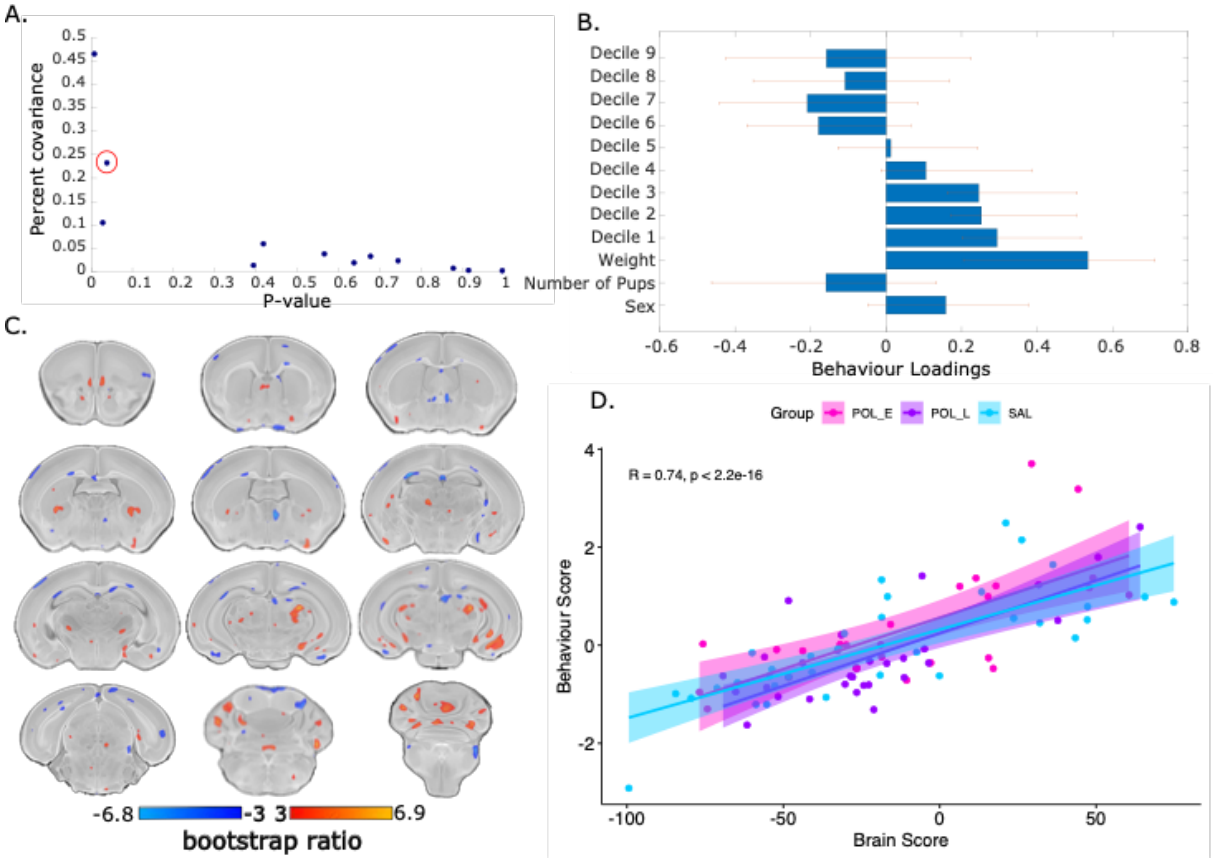
Investigation of possible sex differences revealed that POL E females made significantly fewer call across all deciles (all deciles, p<0.00001). POL E males made significantly more calls than SAL offspring at the first decile of distribution (p=0.025), but no significant differences were observed otherwise. POL L females made significantly more calls than SAL females from the second to eighth deciles (p>0.02), whereas POL L males, similar to the POL E males, made significantly more calls the SAL males only at the first decile of distribution (p=0.043) (**Supplementary figure 5.S4; Supplementary tables 5.S5-5.S10**).



Supplementary figure 5.S4. Sex differences in ultrasonic vocalizations. **A.** Violin plot for mean call duration for each group (SAL, POL E, POL L) split by males and females showing no significant differences. **B.** Distribution of call length (ms) for all calls made by all mice per group and per sex in the 5-minute recording period. The dark line identifies the median of the data, wherein the POL E females have a lower median than the other groups. **C.** Percentile bootstrapping technique applied to identify the difference in decile between the POL E and SAL males (right), and POL L and SAL males (right) showing no significant differences **D.** Percentile bootstrapping technique applied to identify the difference in decile between the POL E and SAL females (right), and POL L and SAL females (right) showing POL E females make significantly fewer calls than SAL females, with no differences in the POL L group.

5.9.6 Partial least squares results

The third significant latent variable described a pattern of increased striatal, thalamic, ventral hippocampal, septal, and amygdala volume and decreased dorsal hippocampus and corpus callosum volume with a greater number of short calls, and heavier body weight (**Supplementary figure 5.S5)**.



Supplementary figure 5.S5. Partial least squares (PLS) analysis results for third significant latent variable (LV3). **A**. Covariance explained (y-axis) and permutation p-values (x-axis) for all 12 LVs in the PLS analysis. LV3 is circled in red (p=0.02, %covariance=10%). **B.** Behaviour weight for each behavioural measure included in the analysis showing how much they contribute to the pattern of LV3. Singular value decomposition estimates the size of the bars whereas confidence intervals are estimated by bootstrapping. Bars with error bars that cross the 0 line should not be considered. **C**. Brain loading bootstrap ratios for the LV3 deformation pattern overlaid on the population average, with positive bootstrap ratios in orange-yellow (indicative or larger volume), and negative in blue (indicative of smaller volume). Colored voxels make significant contributions to LV3. **D**. Correlation of individual mouse brain and behaviour score, color coded by treatment group with a trend line per group. Early poly I:C (POL-E) offspring (magenta) express this pattern more strongly than the saline controls (SAL) and late poly I:C (POL-L) groups.

5.9.7 Supplementary tables

For supplementary tables **5.S2-5.S14**, CI (confidence interval), p crit (uncorrected p-value), p-value (bootstrap corrected p-value); SAL (saline); POL E (early poly I:C group); POL L (late poly I:C group).

Supplementary Table 5.S2. USV decile differences between SAL vs POL E

Decile	SAL	POL E	difference	CI lower	CI upper	p crit	p value
1	21.714	20.525	1.1895	0.092	2.329	0.05	0.035
2	29.633	27.064	2.568	0.693	4.203	0.025	0.003
3	40.247	35.368	4.879	1.728	8.188	0.017	0.000
4	56.539	46.926	9.613	3.755	15.523	0.013	0.000
5	77.229	62.806	14.423	8.001	21.050	0.010	0.000
6	96.559	81.133	15.426	9.131	21.920	0.0083	0.000
7	115.807	102.242	13.565	6.503	20.644	0.0071	0.000
8	139.515	122.2434	17.271	9.606	24.053	0.0063	0.000
9	173.329	155.035	18.295	6.887	27.941	0.0056	0.000

Supplementary Table 5.S3. USV decile differences between POL L vs POL

Decile	POL L	POL E	Difference	CI lower	CI upper	p crit	p value
1	22.208	20.525	1.684	-0.241	4.049	0.0011	0.005
2	30.885	27.064	3.821	0.552	7.152	0.00056	0.000
3	42.085	35.368	6.717	1.101	13.187	0.00037	0.000
4	58.419	46.926	11.492	3.300	19.717	0.00028	0.000
5	79.713	62.806	16.907	7.340	27.907	0.0002	0.000
6	99.130	81.133	17.997	9.423	28.860	0.00019	0.000
7	118.582	102.242	16.341	4.725	25.848	0.00016	0.000
8	144.034	122.244	21.791	11.372	32.380	0.000134	0.000
9	177.383	155.035	22.349	6.880	37.106	0.00012	0.000

Supplementary Table 5.S4. USV decile differences between SAL vs POL L

Decile	SAL	POL L	Difference	CI lower	CI upper	p crit	p value
1	21.714	22.208	-0.494	-2.556	1.450	0.0019	0.383
2	29.633	30.885	-1.252	-4.541	1.429	0.00069	0.187
3	40.247	42.085	-1.838	-8.138	3.594	0.0014	0.360
4	56.539	58.419	-1.880	-10.332	5.278	0.0056	0.478
5	77.229	79.713	-2.484	-11.559	6.460	0.0028	0.384
6	96.559	99.130	-2.570	-11.180	5.096	0.0008	0.327
7	115.807	118.582	-2.776	-12.281	5.927	0.0009	0.380
8	139.515	144.034	-4.519	-17.299	5.480	0.00062	0.142
9	173.329	177.383	-4.054	-18.817	7.7823	0.0011	0.333

Supplementary Table 5.S5. USV decile differences between SAL males vs POL E males

Decile	SAL Male	POL E Male	Difference	CI lower	CI upper	p crit	p value
1	19.933	21.650	-1.717	-4.017876	8	0.0056	0.025
2	27.340	28.767	-1.427	-4.615	1.663	0.008	0.219
3	37.337	37.550	-0.212	-3.886	3.472	0.05	0.883
4	51.184	50.306	0.878	-5.670	9.181	0.025	0.777
5	72.241	67.573	4.6691	-5.560	13.618	0.01	0.234
6	93.214	88.170	5.045	-5.152	14.264	0.007	0.176
7	112.213	110.150	2.063	-5.824	9.6212	0.017	0.552
8	134.826	129.820	5.005	-5.852	15.361	0.01	0.215
9	169.970	161.640	8.329	-6.038	24.231	0.006	0.115

Supplementary Table 5.S6. USV decile differences between SAL males vs POL L males

Decile	SAL Male	POL_L Male	Difference	CI lower	CI upper	p crit	p value
1	19.933	21.535	-1.602	-3.652	0.778	0.0007	0.043
2	27.340	29.170	-1.830	-5.608	1.975	0.0008	0.165
3	37.337	38.211	-0.873	-8.430	6.296	0.001	0.703
4	51.184	52.423	-1.238	-10.188	9.537	0.0012	0.756
5	72.241	71.3778	0.863	-11.778	13.412	0.003	0.893
6	93.214	93.391	-0.177	-11.402	9.847	0.006	0.961
7	112.213	113.1518	-0.939	-12.256	9.914	0.002	0.809
8	134.826	139.070	-4.244	-19.628	9.285	0.001	0.362
9	169.969	177.468	-7.499	-22.819	12.207	0.001	0.259

Supplementary Table 5.S7. USV decile differences between SAL females vs POL E females

Decile	SAL Female	POL E Female	Difference	CI lower	CI upper	p crit	p value
1	22.761	24.552	-1.791	-6.782	1.777	5.51E-06	0.185
2	31.065	36.079	-5.0147	-15.719	1.006	2.20E-06	0.013
3	42.454	54.088	-11.633	-27.29872	0.829	1.57E-06	0.005
4	59.665	75.432	-15.767	-29.412113	2.100	1.22E-06	0.002
5	80.100	92.849	-12.749	-28.968745	-1.298	1.38E-06	0
6	98.530	108.6201	-10.090	-24.424757	1.361	1.84E-06	0.011
7	118.166	130.870	-12.704	-27.804626	3.778	2.76E-06	0.013
8	141.988	150.794	-8.806	-23.537783	3.433	3.67E-06	0.029
9	175.074	177.658	-2.584	-24.779848	15.394	1.10E-05	0.614

Supplementary Table 4.S8. USV decile differences between SAL females vs POL L females

Decile	SAL Female	POL L Female	Difference	CI lower	CI upper	p crit	p value
1	22.761	24.552	-1.791	-6.782	1.777	5.51E-06	0.185
2	31.065	36.080	-5.0147	-15.719	1.006	2.20E-06	0.013
3	42.454	54.088	-11.633	-27.299	0.829	1.57E-06	0.005
4	59.665	75.432	-15.767	-29.412	2.100	1.22E-06	0.002
5	80.100	92.849	-12.7492	-28.968	-1.298	1.38E-06	0
6	98.530	108.621	-10.090	-24.425	1.36145	1.84E-06	0.011
7	118.166	130.870	-12.704	-27.805	3.778	2.76E-06	0.013
8	141.988	150.794	-8.806	-23.538	3.433	3.67E-06	0.029
9	175.074	177.658	-2.584	-24.780	15.394	1.10E-05	0.614

Supplementary Table 4.S9. USV decile differences between POL L males vs POLE males

Decile	POL L Female	POL_E Female	Difference	CI lower	CI upper	p crit	p value
1	21.535	21.650	-0.115	-2.942	2.264	8.93E-04	0.858
2	29.170	28.767	0.403	-5.483	4.725	4.46E-04	0.742
3	38.211	37.550	0.662	-5.016	7.429	2.98E-04	0.725
4	52.423	50.306	2.117	-7.071	11.555	2.23E-04	0.474
5	71.378	67.573	3.805	-12.423	15.268	1.49E-04	0.373
6	93.391	88.170	5.221	-6.892	17.637	1.28E-04	0.192
7	113.152	110.150	3.002	-9.600	15.952	1.79E-04	0.400
8	139.069	129.820	9.249	-5.530	23.945	1.12E-04	0.041
9	177.468	161.640	15.828	-1.051	30.105	9.92E-05	0.001

Supplementary Table 5.S10. USV decile differences between POL L females vs POL E females

Decile	POL L Female	POL E Female	Difference	CI lower	CI upper	p crit	p value
1	24.552	19.115	5.438	1.371	10.853	1.10E-05	0
2	36.079	25.013	11.067	4.826	18.369	5.51E-06	0
3	54.088	31.940	22.149	6.850	37.049	3.67E-06	0
4	75.432	42.167	33.265	16.423	45.459	2.76E-06	0
5	92.849	56.006	36.844	22.777	49.530	2.20E-06	0
6	108.621	72.123	36.498	24.534	51.766	1.84E-06	0
7	130.870	90.240	40.631	20.594	54.509	1.57E-06	0
8	150.794	111.587	39.207	24.807	53.958	1.38E-06	0
9	177.658	140.331	37.327	15.605	60.514	1.22E-06	0

Supplementary Table 5.S11. Dark glial cell density decile differences between POL L males vs SAL L males

Decile	POL L Male	SAL L Males	Difference	CI lower	CI upper	p crit	p value
1	1.96E-11	7.399	-7.399	-21.499	-5.829	0.0100	0
2	1.83E-08	10.916	-10.916	-28.836	-5.970	0.008	0
3	4.34E-06	15.277	-15.277	-33.177	-6.743	0.007	0
4	3.96E-04	19.771	-19.77	-41.465	-7.540	0.006	0
5	1.64E-02	24.650	-24.634	-45.200	-7.857	0.006	0
6	3.30E-01	30.394	-30.064	-47.616	-2.849	0.0125	0.01
7	3.39E+00	37.046	-33.660	-48.822	9.0878	0.017	0.049
8	1.79E+01	43.950	-26.008	-49.016	19.312	0.025	0.253
9	5.05E+01	49.551	0.9401	-43.765	29.042	0.050	0.965

Supplementary Table 5.S12. Dark glial cell density decile differences between POL L Females vs SAL L Females

Decile	POL L Female	SAL L Female	Difference	CI lower	CI upper	p crit	p value
1	2.075	3.15E-05	2.075	0.0482	11.342	0.008	0
2	5.178	1.68E-03	5.177	0.424	19.715	0.007	0.001
3	9.115	2.80E-02	9.087	1.867	23.817	0.01	0.002
4	14.177	2.27E-01	13.950	2.471	26.214	0.017	0.004
5	19.612	1.10E+00	18.508	4.591	26.911	0.05	0.006
6	24.353	3.61E+00	20.742	1.912	31.900	0.013	0.004
7	28.087	8.22E+00	19.864	5.829	31.837	0.025	0.005
8	32.0278	1.34E+01	18.633	3.559	40.321	0.006	0
9	40.542	1.68E+01	23.766	5.927	42.268	0.00555556	0

Supplementary Table 5.S13. Dark neuronal cell density decile differences between POL L males vs SAL L males

Decile	POL L Male	SAL L Male	Difference	CI lower	CI upper	p crit	p value
1	1.80E-12	0.1602603	-0.1602603	-6.639168	-1.59E-05	0.008	0
2	1.69E-09	0.9108863	-0.9108863	-10.518336	-5.19E-04	0.010	0
3	4.07E-07	2.5696511	-2.5696507	-16.722343	-3.94E-03	0.006	0
4	3.84E-05	5.0337625	-5.0337241	-24.733094	-7.76E-02	0.006	0.001
5	1.71E-03	8.197997	-8.1962825	-28.371954	-2.89E-01	0.007	0.001
6	4.04E-02	12.4983076	-12.457925	-32.705342	-5.71E-01	0.013	0.008
7	5.32E-01	18.7118055	-18.17973	-35.165421	-3.03E-01	0.017	0.016
8	3.85E+00	26.7111389	-22.856876	-35.916205	2.95E+00	0.025	0.054
9	1.40E+01	34.2061908	-20.247068	-34.60401	5.95E+00	0.050	0.127

Supplementary Table 5.S14. Dark neuronal cell density decile differences between POL L females vs. SAL L females

Decile	POL L Female	SAL L Female	Difference	CI lower	CI upper	p crit	p value
1	1.32E-04	1.08E-13	1.32E-04	-5.10E-11	0.288	0.017	0.027
2	5.25E-03	9.06E-11	5.25E-03	-4.40E-09	1.795	0.01	0.019
3	7.19E-02	2.18E-08	7.19E-02	-4.72E-05	5.692	0.0125	0.03
4	4.91E-01	2.22E-06	4.91E-01	4.04E-04	6.807	0.05	0.0225
5	1.94E+00	1.10E-04	1.94E+00	7.27E-04	9.838	0.025	0.021
6	4.86E+00	2.83E-03	4.86E+00	-1.52E-03	12.180	0.008	0.01
7	8.32E+00	3.95E-02	8.28E+00	5.81E-02	14.439	0.007	0.004
8	1.11E+01	3.13E-01	1.08E+01	3.65E-01	16.574	0.006	0.003
9	1.43E+01	1.42E+00	1.29E+01	3.86E+00	17.596	0.006	0

References

Avants, Brian B., Nicholas J. Tustison, Gang Song, Philip A. Cook, Arno Klein, and James C. Gee. 2011. "A Reproducible Evaluation of ANTs Similarity Metric Performance in Brain Image Registration." *NeuroImage* 54 (3): 2033–44.

Baharnoori, Moogeh, Sanjeev K. Bhardwaj, and Lalit K. Srivastava. 2010. "Neonatal Behavioral Changes in Rats with Gestational Exposure to Lipopolysaccharide: A Prenatal Infection Model for Developmental Neuropsychiatric Disorders." *Schizophrenia Bulletin* 38 (3): 444–56.

Bankhead, Peter, Maurice B. Loughrey, José A. Fernández, Yvonne Dombrowski, Darragh G. McArt, Philip D. Dunne, Stephen McQuaid, et al. 2017. "QuPath: Open Source Software for Digital Pathology Image Analysis." *Scientific Reports* 7 (1): 16878.

Barron, Helen C., Rogier B. Mars, David Dupret, Jason P. Lerch, and Cassandra Sampaio-Baptista. 2021. "Cross-Species Neuroscience: Closing the Explanatory Gap." *Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences* 376 (1815): 20190633.

Bates, Douglas, Martin Mächler, Ben Bolker, and Steve Walker. 2015. "Fitting Linear Mixed-Effects Models Usinglme4." *Journal of Statistical Software*. https://doi.org/10.18637/jss.v067.i01.

Bauman, M. D., A-M Iosif, P. Ashwood, D. Braunschweig, A. Lee, C. M. Schumann, J. Van de Water, and D. G. Amaral. 2013. "Maternal Antibodies from Mothers of Children with Autism Alter Brain Growth and Social Behavior Development in the Rhesus Monkey." *Translational Psychiatry* 3 (July): e278.

Bisht, Kanchan, Kaushik Sharma, Baptiste Lacoste, and Marie-Ève Tremblay. 2016. "Dark Microglia: Why Are They Dark?" *Communicative & Integrative Biology* 9 (6): e1230575.

Bisht, Kanchan, Kaushik P. Sharma, Cynthia Lecours, Maria Gabriela Sánchez, Hassan El Hajj, Giampaolo Milior, Adrián Olmos-Alonso, et al. 2016. "Dark Microglia: A New Phenotype Predominantly Associated with Pathological States." *Glia* 64 (5): 826–39.

Boksa, Patricia. 2010. "Effects of Prenatal Infection on Brain Development and Behavior: A Review of Findings from Animal Models." *Brain, Behavior, and Immunity* 24 (6): 881–97.

Brown, Alan S., Melissa D. Begg, Stefan Gravenstein, Catherine A. Schaefer, Richard J. Wyatt, Michaeline Bresnahan, Vicki P. Babulas, and Ezra S. Susser. 2004. "Serologic Evidence of Prenatal Influenza in the Etiology of Schizophrenia." *Archives of General Psychiatry* 61 (8): 774–80.

Brown, Alan S., Patricia Cohen, Jill Harkavy-Friedman, Vicki Babulas, Dolores Malaspina, Jack M. Gorman, and Ezra S. Susser. 2001. "Prenatal Rubella, Premorbid Abnormalities, and Adult Schizophrenia." *Biological Psychiatry* 49 (6): 473–86.

Brown, Alan S., and Urs Meyer. 2018. "Maternal Immune Activation and Neuropsychiatric Illness: A Translational Research Perspective." *The American Journal of Psychiatry* 175 (11): 1073–83.

Canales, Cesar P., Myka L. Estes, Karol Cichewicz, Kartik Angara, John Paul Aboubechara, Scott Cameron, Kathryn Prendergast, et al. 2021. "Sequential Perturbations to Mouse Corticogenesis Following in Utero Maternal Immune Activation." *eLife* 10 (March). https://doi.org/10.7554/eLife.60100.

Canetta, Sarah, and Alan Brown. 2012. "Prenatal Infection, Maternal Immune Activation, and

Risk for Schizophrenia." *Translational Neuroscience*. https://doi.org/10.2478/s13380-012-0045-6.

Choi, G. B., Y. S. Yim, H. Wong, S. Kim, H. Kim, S. V. Kim, C. A. Hoeffer, D. R. Littman, and J. R. Huh. 2016. "The Maternal Interleukin-17a Pathway in Mice Promotes Autism-like Phenotypes in Offspring." *Science*. https://doi.org/10.1126/science.aad0314.

Chung, M. K., K. J. Worsley, T. Paus, C. Cherif, D. L. Collins, J. N. Giedd, J. L. Rapoport, and A. C. Evans. 2001. "A Unified Statistical Approach to Deformation-Based Morphometry." *NeuroImage* 14 (3): 595–606.

Clancy, B., R. B. Darlington, and B. L. Finlay. 2001. "Translating Developmental Time across Mammalian Species." *Neuroscience* 105 (1): 7–17.

Cossío, Lourdes Fernández de, Lourdes Fernández de Cossío, Andrea Guzmán, Suzanne van der Veldt, and Giamal N. Luheshi. 2017. "Prenatal Infection Leads to ASD-like Behavior and Altered Synaptic Pruning in the Mouse Offspring." *Brain, Behavior, and Immunity*. https://doi.org/10.1016/j.bbi.2016.09.028.

Crum, William R., Stephen J. Sawiak, Winfred Chege, Jonathan D. Cooper, Steven C. R. Williams, and Anthony C. Vernon. 2017. "Evolution of Structural Abnormalities in the Rat Brain Following in Utero Exposure to Maternal Immune Activation: A Longitudinal in Vivo MRI Study." *Brain, Behavior, and Immunity* 63 (July): 50–59.

Eckart, Carl, and Gale Young. 1936. "The Approximation of One Matrix by Another of Lower Rank." *Psychometrika* 1 (3): 211–18.

Ellisman, M., X. Shu, V. Lev-Ram, T. Deerinck, R. Tsien, S. Lamont, J. Martinez, et al. 2011. "Bridging Gaps in Imaging by Applying EM Tomography and Serial Block Face SEM, Including a New Genetically Encoded Tag for Correlated Light and 3D Electron Microscopy of Intact Cells, Tissues and Organisms: Integrating the Resulting Correlated Image Data Using the Whole Brain Catalog." *Microscopy and Microanalysis*. https://doi.org/10.1017/s1431927611000882.

Ellman, Lauren M., Raymond F. Deicken, Sophia Vinogradov, William S. Kremen, John H. Poole, David M. Kern, Wei Yann Tsai, Catherine A. Schaefer, and Alan S. Brown. 2010. "Structural Brain Alterations in Schizophrenia Following Fetal Exposure to the Inflammatory Cytokine Interleukin-8." *Schizophrenia Research* 121 (1-3): 46–54.

Estes, Myka L., and A. Kimberley McAllister. 2016. "Maternal Immune Activation: Implications for Neuropsychiatric Disorders." *Science* 353 (6301): 772–77.

Franklin, K. B., and George Paxinos. 2008. *The Mouse Brain in Stereotaxic Coordinates, Compact. The Coronal Plates and Diagrams*. Amsterdam: Elsevier Academic Press.

Ginsberg, Yuval, Nizar Khatib, Zeev Weiner, and Ron Beloosesky. 2017. "Maternal Inflammation, Fetal Brain Implications and Suggested Neuroprotection: A Summary of 10 Years of Research in Animal Models." *Rambam Maimonides Medical Journal* 8 (2). https://doi.org/10.5041/RMMJ.10305.

Girard, Sylvie, Luc Tremblay, Martin Lepage, and Guillaume Sébire. 2010. "IL-1 Receptor Antagonist Protects against Placental and Neurodevelopmental Defects Induced by Maternal Inflammation." *Journal of Immunology* 184 (7): 3997–4005.

Graham, Alice M., Jerod M. Rasmussen, Marc D. Rudolph, Christine M. Heim, John H. Gilmore, Martin Styner, Steven G. Potkin, et al. 2018. "Maternal Systemic Interleukin-6 During Pregnancy Is Associated With Newborn Amygdala Phenotypes and Subsequent Behavior at 2 Years of Age." *Biological Psychiatry* 83 (2): 109–19.

Guma, Elisa, Pedro do Couto Bordignon, Gabriel A. Devenyi, Daniel Gallino, Chloe

Anastassiadis, Vedrana Cvetkovska, Amadou Barry, et al. 2021. "Early or Late Gestational Exposure to Maternal Immune Activation Alters Neurodevelopmental Trajectories in Mice: An Integrated Neuroimaging, Behavioural, and Transcriptional Study." *Biological Psychiatry*, March. https://doi.org/10.1016/j.biopsych.2021.03.017.

Guma, Elisa, Eric Plitman, and M. Mallar Chakravarty. 2019. "The Role of Maternal Immune Activation in Altering the Neurodevelopmental Trajectories of Offspring: A Translational Review of Neuroimaging Studies with Implications for Autism Spectrum Disorder and Schizophrenia." *Neuroscience and Biobehavioral Reviews*. https://www.sciencedirect.com/science/article/pii/S0149763419302088.

Gumusoglu, Serena B., and Hanna E. Stevens. 2019. "Maternal Inflammation and Neurodevelopmental Programming: A Review of Preclinical Outcomes and Implications for Translational Psychiatry." *Biological Psychiatry* 85 (2): 107–21.

Gzielo, Kinga, Agnieszka Potasiewicz, Ewa Litwa, Diana Piotrowska, Piotr Popik, and Agnieszka Nikiforuk. 2021. "The Effect of Maternal Immune Activation on Social Play-Induced Ultrasonic Vocalization in Rats." *Brain Sciences* 11 (3): 344.

Henry, Mathilde S., Kanchan Bisht, Nathalie Vernoux, Louis Gendron, Angélica Torres-Berrio, Guy Drolet, and Marie-Ève Tremblay. 2018. "Delta Opioid Receptor Signaling Promotes Resilience to Stress Under the Repeated Social Defeat Paradigm in Mice." *Frontiers in Molecular Neuroscience* 11 (April): 100.

Hui, Chin W., Abygaël St-Pierre, Hassan El Hajj, Yvan Remy, Sébastien S. Hébert, Giamal N. Luheshi, Lalit K. Srivastava, and Marie-Ève Tremblay. 2018. "Prenatal Immune Challenge in Mice Leads to Partly Sex-Dependent Behavioral, Microglial, and Molecular Abnormalities Associated with Schizophrenia." *Frontiers in Molecular Neuroscience* 11 (February): 13.

Kalish, Brian T., Eunha Kim, Benjamin Finander, Erin E. Duffy, Hyunju Kim, Casey K. Gilman, Yeong Shin Yim, et al. 2021. "Maternal Immune Activation in Mice Disrupts Proteostasis in the Fetal Brain." *Nature Neuroscience* 24 (2): 204–13.

Kannan, Sujatha, Fadoua Saadani-Makki, Bindu Balakrishnan, Pulak Chakraborty, James Janisse, Xin Lu, Otto Muzik, Roberto Romero, and Diane C. Chugani. 2011. "Magnitude of [(11)C]PK11195 Binding Is Related to Severity of Motor Deficits in a Rabbit Model of Cerebral Palsy Induced by Intrauterine Endotoxin Exposure." *Developmental Neuroscience* 33 (3-4): 231–40.

Kannan, Sujatha, Fadoua Saadani-Makki, Bindu Balakrishnan, Hui Dai, Pulak K. Chakraborty, James Janisse, Otto Muzik, Roberto Romero, and Diane C. Chugani. 2011. "Decreased Cortical Serotonin in Neonatal Rabbits Exposed to Endotoxin in Utero." *Journal of Cerebral Blood Flow and Metabolism: Official Journal of the International Society of Cerebral Blood Flow and Metabolism* 31 (2): 738–49.

Kentner, Amanda C., Staci D. Bilbo, Alan S. Brown, Elaine Y. Hsiao, A. Kimberley McAllister, Urs Meyer, Brad D. Pearce, Mikhail V. Pletnikov, Robert H. Yolken, and Melissa D. Bauman. 2019. "Maternal Immune Activation: Reporting Guidelines to Improve the Rigor, Reproducibility, and Transparency of the Model." *Neuropsychopharmacology: Official Publication of the American College of Neuropsychopharmacology* 44 (2): 245–58.

Knuesel, Irene, Laurie Chicha, Markus Britschgi, Scott A. Schobel, Michael Bodmer, Jessica A. Hellings, Stephen Toovey, and Eric P. Prinssen. 2014. "Maternal Immune Activation and Abnormal Brain Development across CNS Disorders." *Nature Reviews. Neurology* 10 (11): 643–60.

Li, Daijiang, Russell Dinnage, Lucas A. Nell, Matthew R. Helmus, and Anthony R. Ives. 2020. "Phyr: An R Package for Phylogenetic Species-distribution Modelling in Ecological Communities." *Methods in Ecology and Evolution / British Ecological Society* 11 (11): 1455–63.

Lieberman, J. A., R. R. Girgis, G. Brucato, H. Moore, F. Provenzano, L. Kegeles, D. Javitt, et al. 2018. "Hippocampal Dysfunction in the Pathophysiology of Schizophrenia: A Selective Review and Hypothesis for Early Detection and Intervention." *Molecular Psychiatry* 23 (8): 1764–72.

Lombardo, M. V., H. M. Moon, J. Su, T. D. Palmer, E. Courchesne, and T. Pramparo. 2018. "Maternal Immune Activation Dysregulation of the Fetal Brain Transcriptome and Relevance to the Pathophysiology of Autism Spectrum Disorder." *Molecular Psychiatry* 23 (4): 1001–13.

Malkova, Natalia V., Collin Z. Yu, Elaine Y. Hsiao, Marlyn J. Moore, and Paul H. Patterson. 2012. "Maternal Immune Activation Yields Offspring Displaying Mouse Versions of the Three Core Symptoms of Autism." *Brain, Behavior, and Immunity* 26 (4): 607–16.

Manitz, Marie Pierre, Manuela Esslinger, Simone Wachholz, Jennifer Plümper, Astrid Friebe, Georg Juckel, and Rainer Wolf. 2013. "The Role of Microglia during Life Span in Neuropsychiatric Disease — an Animal Study." *Schizophrenia Research*. https://doi.org/10.1016/j.schres.2012.10.028.

Manjón, José V., Pierrick Coupé, Luis Martí-Bonmatí, D. Louis Collins, and Montserrat Robles. 2010. "Adaptive Non-Local Means Denoising of MR Images with Spatially Varying Noise Levels." *Journal of Magnetic Resonance Imaging*. https://doi.org/10.1002/jmri.22003.

McIntosh, Anthony Randal, and Nancy J. Lobaugh. 2004. "Partial Least Squares Analysis of Neuroimaging Data: Applications and Advances." *NeuroImage* 23 Suppl 1: S250–63.

McIntosh, Anthony R., and Bratislav Mišić. 2013. "Multivariate Statistical Analyses for Neuroimaging Data." *Annual Review of Psychology* 64: 499–525.

Melancia, Francesca, Sara Schiavi, Michela Servadio, Veronica Cartocci, Patrizia Campolongo, Maura Palmery, Valentina Pallottini, and Viviana Trezza. 2018. "Sex-Specific Autistic Endophenotypes Induced by Prenatal Exposure to Valproic Acid Involve Anandamide Signalling." *British Journal of Pharmacology*. https://doi.org/10.1111/bph.14435.

Meyer, Urs, Myriel Nyffeler, Andrea Engler, Adrian Urwyler, Manfred Schedlowski, Irene Knuesel, Benjamin K. Yee, and Joram Feldon. 2006. "The Time of Prenatal Immune Challenge Determines the Specificity of Inflammation-Mediated Brain and Behavioral Pathology." *The Journal of Neuroscience: The Official Journal of the Society for Neuroscience* 26 (18): 4752–62.

Nahirney, Patrick C., and Marie-Eve Tremblay. 2021. "Brain Ultrastructure: Putting the Pieces Together." *Frontiers in Cell and Developmental Biology* 9 (February): 629503.

Ozaki, Kana, Daisuke Kato, Ako Ikegami, Akari Hashimoto, Shouta Sugio, Zhongtian Guo, Midori Shibushita, et al. 2020. "Maternal Immune Activation Induces Sustained Changes in Fetal Microglia Motility." *Scientific Reports* 10 (1): 21378.

Patel, Raihaan, Christopher J. Steele, Anthony G. X. Chen, Sejal Patel, Gabriel A. Devenyi, Jürgen Germann, Christine L. Tardif, and M. Mallar Chakravarty. 2020. "Investigating Microstructural Variation in the Human Hippocampus Using Non-Negative Matrix Factorization." *NeuroImage* 207 (February): 116348.

Piontkewitz, Yael, Michal Arad, and Ina Weiner. 2011. "Abnormal Trajectories of Neurodevelopment and Behavior Following in Utero Insult in the Rat." *Biological*

Psychiatry 70 (9): 842–51.
Qiu, Lily R., Darren J. Fernandes, Kamila U. Szulc-Lerch, Jun Dazai, Brian J. Nieman, Daniel H. Turnbull, Jane A. Foster, Mark R. Palmert, and Jason P. Lerch. 2018. "Mouse MRI Shows Brain Areas Relatively Larger in Males Emerge before Those Larger in Females." *Nature Communications* 9 (1): 2615.
Reisinger, Sonali, Deeba Khan, Eryan Kong, Angelika Berger, Arnold Pollak, and Daniela D. Pollak. 2015. "The Poly(I:C)-Induced Maternal Immune Activation Model in Preclinical Neuropsychiatric Drug Discovery." *Pharmacology & Therapeutics* 149 (May): 213–26.
Rousselet, Guillaume A., Cyril R. Pernet, and Rand R. Wilcox. 2017. "Beyond Differences in Means: Robust Graphical Methods to Compare Two Groups in Neuroscience." *The European Journal of Neuroscience* 46 (2): 1738–48.
Rudolph, Marc D., Alice M. Graham, Eric Feczko, Oscar Miranda-Dominguez, Jerod M. Rasmussen, Rahel Nardos, Sonja Entringer, Pathik D. Wadhwa, Claudia Buss, and Damien A. Fair. 2018. "Maternal IL-6 during Pregnancy Can Be Estimated from Newborn Brain Connectivity and Predicts Future Working Memory in Offspring." *Nature Neuroscience* 21 (5): 765–72.
Scattoni, Maria Luisa, Jacqueline Crawley, and Laura Ricceri. 2009. "Ultrasonic Vocalizations: A Tool for Behavioural Phenotyping of Mouse Models of Neurodevelopmental Disorders." *Neuroscience and Biobehavioral Reviews* 33 (4): 508–15.
Schambra, Uta. 2008. *Prenatal Mouse Brain Atlas: Color Images and Annotated Diagrams of: Gestational Days 12, 14, 16 and 18 Sagittal, Coronal and Horizontal Section*. Springer, Boston, MA.
Selemon, L. D., and N. Zecevic. 2015. "Schizophrenia: A Tale of Two Critical Periods for Prefrontal Cortical Development." *Translational Psychiatry* 5 (August): e623.
Selten, J. P., R. van Duursen, Y. van der Graaf, C. Gispen-de Wied, and R. S. Kahn. 1997. "736 - Second-Trimester Exposure to Maternal Stress Is a Possible Risk Factor for Psychotic Illness in the Child." *Schizophrenia Research* 24 (1): 258.
Semple, Bridgette D., Klas Blomgren, Kayleen Gimlin, Donna M. Ferriero, and Linda J. Noble-Haeusslein. 2013. "Brain Development in Rodents and Humans: Identifying Benchmarks of Maturation and Vulnerability to Injury across Species." *Progress in Neurobiology* 106-107 (July): 1–16.
Shen, Mark D., and Joseph Piven. 2017. "Brain and Behavior Development in Autism from Birth through Infancy." *Dialogues in Clinical Neuroscience* 19 (4): 325–33.
Smolders, Silke, Tina Notter, Sophie M. T. Smolders, Jean-Michel Rigo, and Bert Brône. 2018. "Controversies and Prospects about Microglia in Maternal Immune Activation Models for Neurodevelopmental Disorders." *Brain, Behavior, and Immunity* 73 (October): 51–65.
Solek, Cynthia M., Nasr Farooqi, Myriam Verly, Tony K. Lim, and Edward S. Ruthazer. 2018. "Maternal Immune Activation in Neurodevelopmental Disorders." *Developmental Dynamics*. https://doi.org/10.1002/dvdy.24612.
Spann, Marisa N., Catherine Monk, Dustin Scheinost, and Bradley S. Peterson. 2018. "Maternal Immune Activation During the Third Trimester Is Associated with Neonatal Functional Connectivity of the Salience Network and Fetal to Toddler Behavior." *The Journal of Neuroscience: The Official Journal of the Society for Neuroscience* 38 (11): 2877–86.
Spencer Noakes, T. Leigh, R. Mark Henkelman, and Brian J. Nieman. 2017. "Partitioning K-Space for Cylindrical Three-Dimensional Rapid Acquisition with Relaxation Enhancement Imaging in the Mouse Brain." *NMR in Biomedicine* 30 (11): e3802.

St-Pierre, Marie-Kim, Eva Šimončičová, Eszter Bögi, and Marie-Ève Tremblay. 2020. "Shedding Light on the Dark Side of the Microglia." *ASN Neuro* 12 (January): 1759091420925335.

Thion, Morgane S., Florent Ginhoux, and Sonia Garel. 2018. "Microglia and Early Brain Development: An Intimate Journey." *Science* 362 (6411): 185–89.

Tremblay, Marie-Ève, Martha L. Zettel, James R. Ison, Paul D. Allen, and Ania K. Majewska. 2012. "Effects of Aging and Sensory Loss on Glial Cells in Mouse Visual and Auditory Cortices." *Glia* 60 (4): 541–58.

Tustison, Nicholas J., Brian B. Avants, Philip A. Cook, and James C. Gee. 2010. "N4ITK: Improved N3 Bias Correction with Robust B-Spline Approximation." *2010 IEEE International Symposium on Biomedical Imaging: From Nano to Macro*. https://doi.org/10.1109/isbi.2010.5490078.

Versaevel, Marie, Jean-Baptiste Braquenier, Maryam Riaz, Thomas Grevesse, Joséphine Lantoine, and Sylvain Gabriele. 2014. "Super-Resolution Microscopy Reveals LINC Complex Recruitment at Nuclear Indentation Sites." *Scientific Reports* 4 (December): 7362.

Wu, Dan, and Jiangyang Zhang. 2016. "Recent Progress in Magnetic Resonance Imaging of the Embryonic and Neonatal Mouse Brain." *Frontiers in Neuroanatomy* 10 (March): 18.

Zeighami, Yashar, Seyed-Mohammad Fereshtehnejad, Mahsa Dadar, D. Louis Collins, Ronald B. Postuma, Bratislav Mišić, and Alain Dagher. 2019. "A Clinical-Anatomical Signature of Parkinson's Disease Identified with Partial Least Squares and Magnetic Resonance Imaging." *NeuroImage* 190 (April): 69–78.

Zhang, Zhi, Amar Jyoti, Bindu Balakrishnan, Monica Williams, Sarabdeep Singh, Diane C. Chugani, and Sujatha Kannan. 2018. "Trajectory of Inflammatory and Microglial Activation Markers in the Postnatal Rabbit Brain Following Intrauterine Endotoxin Exposure." *Neurobiology of Disease* 111 (March): 153–62.

CHAPTER 6: Investigating the synergistic effects of prenatal maternal immune activation and adolescent cannabis use

6.1 Preface

The previous chapter, **5**, built upon **Chapter 4** to better characterize the neurodevelopmental alterations elicited by prenatal MIA-exposure either early or late in gestation to cover development from gestation to adulthood. The findings from **Chapter 4**, highlighted striking, yet transient neuroanatomical and behavioural alterations in offspring prenatally exposed to MIA in early gestation, with only subtle neuroanatomical alterations in late exposed offspring. These observations suggest that early exposure may be a more potent risk factor than late exposure, but that even so, exposure to a single risk factor does not induce sustained neurodevelopmental changes, as those often observed in full blown psychiatric conditions. Indeed, a working theory in biological psychiatry is that multiple exposures, either environmental or genetic, are required for disease presentation.

The work presented in **Chapter 6** aims to extend upon these observations and determine whether the transient deviation observed in the adolescent/early adult period due to early MIA-exposure could reflect an emerging window of enhanced vulnerability to other risk factors, in line the multi-hit hypothesis associated with the onset of neuropsychiatric diseases (Rapoport, Giedd, and Gogtay 2012; van Os, Kenis, and Rutten 2010). Thus, mice prenatally exposed to MIA early in gestation were subsequently exposed to chronic adolescent cannabis (delta-9-tetrahydrocannabinol [THC]), another known risk factor for the onset of psychosis, amongst other psychiatric disorders. The adolescent period was selected as the brain is undergoing substantial remodeling, making it a potential vulnerable period to risk factor exposure, similar to the developing fetal brain. In addition, this is the maturational window in which significant deviations in development were identified in **Chapter 4**. Indeed, a cumulative effect of both risk factors is observed on neurodevelopmental trajectories at the level of anatomy, but not behaviour. This work

provides an initial framework in which to characterize the effects of exposure to multiple environmental risk factors and may be extended to investigate other combinations.

CHAPTER 6

Investigating the synergistic effects of prenatal maternal immune activation and adolescent cannabis use

Elisa Guma [1,2], Lani Cupo [1,2], Daniel Gallino [1], Luc Moquin [4], Joe Rochford [2,4], Alain Gratton [2,4], M Mallar Chakravarty [1,2,3,5]

[1] Computational Brain Anatomy Laboratory, Cerebral Imaging Center, Douglas Mental Health University Institute, Montreal, Quebec, Canada
[2] Integrated Program in Neuroscience, McGill University, Montreal, Quebec, Canada
[3] Department of Psychiatry, McGill University, Montreal Quebec, Canada
[4] Douglas Mental Health University Institute, Department of Psychiatry, McGill University, Montréal, Québec, Canada
[5] Department of Biological and Biomedical Engineering, McGill University, Montreal, Quebec, Canada

6.2 Abstract

Prenatal exposure to maternal immune activation (MIA) and adolescent cannabis use have both been identified as environmental risk factors for neuropsychiatric disorders. However, most individuals exposed to a single risk factor do not typically develop major mental illness, which indicates that multiple exposures may be required for illness onset. Here, we examine whether combined exposure to prenatal MIA and adolescent delta-9-tetrahydrocannabinol (THC), the main psychoactive component of cannabis, lead to enduring neuroanatomical and behavioural changes in adult offspring. Mice were prenatally exposed to a viral mimetic, poly I:C (5mg/kg), or vehicle at gestational day (GD) 9, and then postnatally exposed to chronic THC (5mg/kg) or vehicle during adolescent development (postnatal day [PND] 28-45). Longitudinal *in vivo* whole-brain magnetic resonance imaging (MRI) was performed pre-treatment, PND 25, post-treatment, PND 50, and in adulthood, PND 85, followed by a series of behavioural tests aimed at assessed anxiety-like and locomotor, social, and sensorimotor gating behaviour. Greater deviations in neurodevelopmental trajectory and subthreshold anxiety-like behaviours were observed in mice exposed to both risk factors than those exposed to a single risk factor, or none. Interestingly, sex-dependent effects were observed in patterns of shared brain-behaviour covariation, suggesting that exposure to MIA and THC may affect males and females separately. These findings suggest that there may be a cumulative effect of risk factor exposure on neurodevelopment.

6.3 Introduction

Multiple lines of evidence suggest that exposure to environmental risk factors during sensitive periods of brain development may alter neurodevelopmental trajectories, increasing risk for neuropsychiatric disorders (Shah et al. 2016; van Os, Kenis, and Rutten 2010). The fetal brain is highly plastic and sensitive to environmental perturbations (Monk, Lugo-Candelas, and Trumpff 2019). Exposure to maternal immune activation (MIA) during this developmental phase (*in utero*) has been identified as a strong risk factor for neuropsychiatric illness, wherein elevated levels of maternal pro-inflammatory cytokines interfere with fetal brain development (Estes and McAllister 2016; Meyer 2014). However, many maternal infections do not directly lead to full blown disorders in the offspring (Estes and McAllister 2016). Multiple exposures may be required for illness onset; MIA appears to act as a "disease primer" making individuals more susceptible to the effects of other risk factors later in life (Reisinger et al. 2015). In support of this hypothesis, previous work from our group found that mice prenatally exposed to MIA in early gestation (gestational day [GD] 9) exhibited altered neurodevelopment, characterized by the emergence of neuroanatomical and behavioural abnormalities in the adolescent period (Guma et al. 2021), a time in which many neuropsychiatric illnesses emerge (Keshavan et al. 2014). However, MIA-exposure alone was insufficient to create sustained brain and behavioural abnormalities as we observed that the adolescent alterations normalized in adulthood (Guma et al. 2021).

Exposure to drugs of abuse is considered a risk factor for mental illness, particularly when exposure occurs during adolescence, a time of critical neurobiological remodeling; further, effects of this risk factor may be enhanced by previous exposure to other risk factors (Keshavan et al. 2014). Prospective, longitudinal, epidemiological evidence suggests that adolescent cannabis use may increase risk for psychosis, amongst other neuropsychiatric disorders (Arseneault et al. 2002) (Volkow et al. 2016; Zammit et al. 2002). This is particularly evident if use begins before 16 years age (French et al. 2015). The age of sensitivity corresponds to the developmental period of the endocannabinoid system (specifically the cannabinol [CB]-1 receptor), which plays a fundamental role in brain development (Battistella et al. 2014). The main psychoactive component of cannabis is THC, which activates the endocannabinoid system via CB-1 (and to some extent CB-2) receptors in the brain (Campolongo and Trezza 2012). Activation of CB-1 receptors is thought to create an imbalance in excitatory-inhibitory signaling in the brain by influencing the GABA-ergic, glutamatergic, and dopaminergic systems (Keshavan et al. 2014). Even if cannabis exposure in

adolescence increases risk for psychosis, only a minority of cannabis users develop psychosis, suggesting that cannabis use may interact with pre-existing vulnerabilities. If exposure to early life risk factors such as maternal infection during gestation renders individuals susceptible, cannabis use may exacerbate this vulnerability, thereby increasing risk for neuropsychiatric illness (Dalton et al. 2012; Lecca et al. 2019).

In this study, as with our previous work (Guma et al. 2021), we perform longitudinal assays of brain development using repeated *in vivo* magnetic resonance imaging (MRI) throughout development, and multi-behavioural testing in adulthood to investigate the potential synergistic effects of prenatal MIA-exposure in early gestation (GD9), followed by chronic adolescent delta-9-tetrahydrocannabinol (THC) exposure (postnatal day [PND] 28-45) in mice. We hypothesized that exposure to each risk factor alone would result in subthreshold neuroanatomical and behavioural alterations in offspring, but that the combined exposure would lead to enduring neuroanatomical and behavioural changes in adult offspring. Indeed, we find that mice exposed to both risk factors have greater neuroanatomical deviations than those exposed to each risk factor alone, with some regions showing sustained deviation in adulthood, while others recover. Behaviourally, however, we did not uncover any significant differences, other than some subthreshold deviations in locomotor, and sensorimotor gating behaviours in the combined group. Finally, sex-dependent effects were observed in patterns of shared brain-behaviour covariation, suggesting that exposure to MIA and THC may affect males and females separately.

6.4 Methods

6.4.1 Animals

Timed-mating was used to generate pregnant dams (injected intraperitoneally with poly I:C (POL; P1530-25MG sodium salt TLR ligand tested; Sigma Aldrich; 5mg/kg) or vehicle (SAL; 0.9% NaCl) at GD 9 (10 POL and 8 SAL dams; breeding details in **Supplement 6.8.1**). Offspring were then randomly assigned to receive daily injections (intraperitoneal) of THC (1:1:18 THC 5mg/kg [Cayman Chemicals]:cremophor [Sigma Aldrich]:saline solution) or vehicle (1:18 cremophor:saline) throughout the adolescent period (PND 28-45). The dosage was chosen to model moderate daily use, with a potency roughly equivalent to one average-sized joint with 11%

THC content, scaled to the weight of the mouse (Campolongo et al. 2011; DiNieri and Hurd 2012; Harkany et al. 2007). This resulted in 4 groups: (SAL-SAL, SAL-THC, POL-SAL, POL-THC; **Figure 6.1**). All procedures were approved by McGill University's Animal Care Committee under the guidelines of the Canadian Council on Animal Care. In a separate group of dams, the immunostimulatory potential of our poly I:C was confirmed (n=4 GD9-POL, n=5 GD9-SAL; **Supplement 6.8.2** for methods, **Supplement 6.9.1** and **Supplementary table 6.S1** for results).

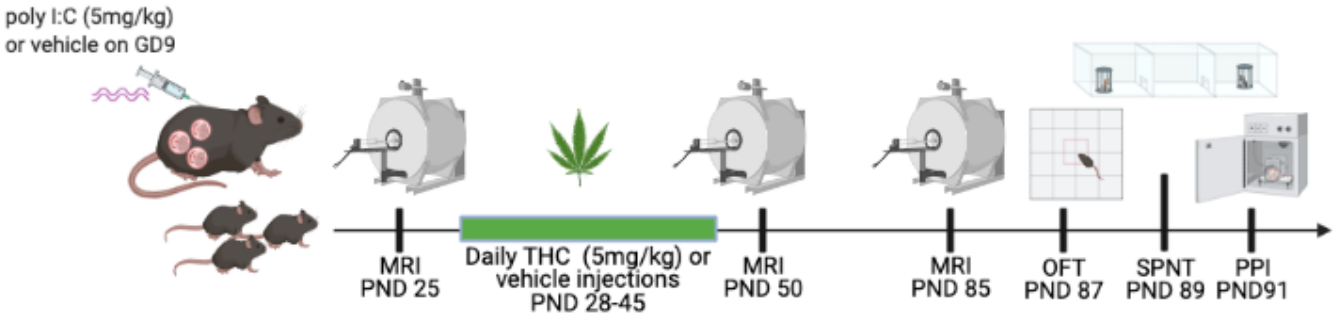


Figure 6.1. Experimental timeline. Pregnant dams were injected (i.p.) with poly I:C (5mg/kg) or vehicle (0.9% sterile NaCl solution) at gestational day (GD) 9. Offspring were weaned and sexed on postnatal day (PND) 21. Longitudinal structural magnetic resonance imaging (MRI) was performed at PND ~25, ~50, and ~85 (denoted by the MRI image). Following the first scan on PND 25, offspring were randomly assigned to receive daily injections (i.p.) of delta-9-tetrahydrocannabinol (THC; 5mg/kg) or vehicle (1:18 cremophor:saline) from PND 28-45. Two days following the final scan on PND 85, mice were assessed in the open field test (OFT), the social preference/novelty test (SNPT), and the prepulse inhibition task (PPI), with a 2-day rest between tests.

6.4.3 THC solution preparation and validation

A stock solution of 2 g of pure THC 10 mg/ml in ethanol was obtained (Cayman Chemicals, Ann Arbor, MI, USA). To prepare a physiologically compatible solution, vacuum drying was used to extract the THC from ethanol and prevent conformational changes to the compound. Pure THC was then dissolved in a 1:18 cremophor:saline solution, resulting in a 1:1:18 THC:cremophor:saline solution (the lipophilic cremophor ensures the THC remains in solution). To validate our THC solution, we used gas-chromatography mass spectrometry (GCMS) to measure plasma levels of THC and two THC metabolites (11-hydroxy-delta-9-THC and 11nor-

9carboxy delta-9-THC). These were measured in the plasma of a separate group of mice 1 hour following a single injection of either 2.5, 5, or 10mg/kg of THC (**Supplement 6.8.3** for methods**, 6.9.2** and **Supplementary table 6.S2** for results).

6.4.4 Magnetic resonance imaging

6.4.4.1 Acquisition

Longitudinal T1-weighted (100 μm^3) structural MRIs and functional MRIs were acquired *in vivo* at postnatal day (PND) 25±1 (24-26; ~childhood; pre-treatment), 50±3 (47-51; ~adolescence; post-treatment), 85±5 (80-86; ~adulthood) (Semple et al. 2013; Clancy et al. 2007) in anesthetized offspring (Kong et al. 2018; Gallino et al. 2019; Rollins et al. 2019; Guma et al. 2018)**.** Anesthesia was induced with 3% isoflurane in oxygen and a (0.075 mg/kg bolus) dexmedetomidine injection. Anesthesia was maintained during the scan between 1.5-0.5% isoflurane, and a constant infusion of dexmedetomidine (0.05 mg/kg/h). Scans were conducted in a 7 Tesla Bruker, 30 cm bore magnet with AVANCE electronics, using a cryogenically cooled surface coil. A 3D FLASH (Fast, Low Angle SHot) sequence was used with TE/TR of 4.5 ms/20 ms (2 averages, ~14 minutes). Following the acquisition of the structural scan, a resting state functional scan was acquired (EPI, TE/TR of 15/1000ms (500 repetitions) (data not included in this thesis).

6.4.4.2 Image processing

Structural images (n=245) were exported as DICOM, converted to MINC format, preprocessed, and visually inspected for quality control (QC). Three scans from one subject were excluded due to hydrocephalus and one scan was excluded due to motion (n=242).

Table 6.1. Sample per timepoint following quality control. Postnatal day (PND); poly I:C (POL); saline (SAL); delta-9-tetrahidrocannabinol (THC); male (M); female (F).

	PND ~25		PND ~50		PND ~85		
	Males	**Females**	**Males**	**Females**	**Males**	**Females**	**Litters**
SAL-SAL	10	9	10	9	10	9	7
POL-SAL	10	10	11	10	11	10	9
POL-THC	12	9	11	11	11	11	8
SAL-THC	10	8	10	8	9	9	7

Images were processed using a longitudinal two-level deformation-based morphometry technique using the ANTs toolkit (http://stnava.github.io/ANTs/) for linear and non-linear registration (Avants et al. 2011). All scans per subject were registered to create a subject average (first level). These were registered using a group-wise averaging technique to create a study average (second level). The final average provides voxel correspondence between subjects, which allows for comparison of local individual changes across subjects. Voxel-level volume changes were captured in the Jacobian determinant of the deformation field derived from the image registration. Both relative and absolute Jacobian determinants of the deformations fields of the first level were resampled into the second level average space in order to perform statistics. Relative Jacobians explicitly model only the non-linear part of the deformations to remove residual global linear transformation attributable to differences in total brain size and were used for subsequent statistical analysis. Absolute Jacobians include overall linear transformations. Jacobian determinants (Chung et al. 2001) of the first-level deformation fields were resampled into final average space and blurred (0.2 mm Gaussian kernel) prior to statistical analyses (Friedel et al. 2014; van Eede et al. 2013). All trajectory plots (**Figure 2-4**) were created using the Effect function in R to correctly reflect the statistical model, accounting for covariates (https://github.com/CoBrALab/documentation/wiki/Properly-plotting-a-lm-or-lmer-model-predicted-curve-in-R-with-ggplot).

6.4.4.3 Statistical analyses

A voxel-wise linear mixed-effects model (R-3.5.1, RMINC-1.5.2.2, lme4 1.1-21) was used to examine a group-by-age (age as a natural second order spline) interaction covarying for sex, with subject and litter as random intercepts. The False Discovery Rate (FDR) (Benjamini and Hochberg 1995) correction was applied to control for multiple comparisons. First, we examined whether each risk factor alone had significant effects on neurodevelopment. We examined the effect of prenatal MIA-exposure by comparing POL SAL to SAL SAL, and the effect of adolescent THC exposure by comparing SAL THC to SAL SAL. Finally, we examined the effects of the dual hit by comparing POL THC to SAL SAL (POL THC was also compared to SAL SAL described in **6.9.4**). Given evidence of sex-differences in response to MIA and THC, sex-by-group-by-age interaction was investigated post-hoc (**Supplement 6.8.5.1)**.

6.4.5 Behavioural testing

6.4.5.1 Behavioural tasks

Following the final scan at PND 85, testing was performed to assay a number of behaviours relevant to neurodevelopmental and neuropsychiatric disorders. These included first the open field test aimed at assessing exploratory and anxiety-like behaviours, wherein the distance traveled in the anxiogenic center zone was examined relative to the total distance traveled. Next, the three chambered social approach task were performed to assay both social preference (i.e. preference for a novel mouse relative to a non-social object), and social novelty (i.e. preference for a novel mouse relative to a familiar mouse) behaviours. Finally, sensorimotor gating to an acoustic startle was assessed with the prepulse inhibition (PPI) task, wherein a prepulse tone is presented (at various levels, across multiple trials) to determine whether it is successful at dampening the startle reaction to the acoustic stimulation. A 2-day resting phase was allowed between tests; details for all behavioural tests are provided in **Supplement 6.8.4**.

6.4.5.2 Statistical Analyses

We used linear mixed-effects models for adult behavioural data, with group and sex as fixed effects, and litter as a random effect. A Bonferroni correction was applied (4 tests: $\alpha = 0.05/4$

= 0.0125 set as significance threshold, uncorrected p-values, and corrected q-values reported). As with neuroanatomy, behavioural sex differences were investigated post-hoc (**Supplement 6.8.5.2)**.

6.4.6 Partial least squares analysis

In order to assess putative brain-behaviour relationships in our mice, we used a partial least squares (PLS) analysis in two ways. This multivariate technique allows us to relate two sets of variables by finding the optimal weighted linear combinations of variables that maximally covary with each other (Zeighami et al. 2019; McIntosh and Mišić 2013; McIntosh and Lobaugh 2004).

First, we assessed the relationship between brain anatomy at the final MRI timepoint (PND 85; brain matrix) and behaviour across the three tests performed following the PND 85 scan (behaviour matrix). Second, we assessed the relationship between the within-subject brain voxel-wise volume change from PND 50 to 85 (i.e., immediately post-treatment, to the final timepoint; brain matrix), and behaviour as in the first application (behaviour matrix). The within-subject volume change was computed by calculating the difference in voxel-wise Jacobian determinants for each subject between the two scans (https://github.com/CoBrALab/documentation/wiki/Create-a-nifti-of-within-subject-change-(2-timepoints,-output-from-dbm)). In both applications, the behaviour matrix was z-scored and correlated to the brain matrix to create a brain-behaviour covariance matrix. Singular value decomposition was applied to brain-behaviour matrix to generate a set of orthogonal latent variables (LVs), which describe linked patterns of covariation between the input brain and behaviour matrices. Permutation testing and bootstrap resampling (n=1000, each) were used to assess LV significance and reliability (**Supplement 6.8.6).**

6.5 Results

6.5.1 Alterations in neurodevelopmental trajectories

6.5.1.1 Effects of prenatal MIA-exposure on neurodevelopmental trajectories

Overall differences between all groups are summarized in **Supplementary figure 6.S1.** Effects of MIA exposure on offspring development were assessed by comparing offspring exposed to prenatal POL and adolescent SAL (POL SAL) relative to those exposed to prenatal and postnatal SAL (SAL SAL). The group by age (group : ns(age,2)1) interaction was significant in a limited number of voxels in the cerebellum, at a lenient 20% FDR threshold (t=4.662; **Figure 6.2**). In order to determine whether some of the changes we observed in our previous work (Guma et al. 2021) were recapitulated here at a subthreshold level, we investigated changes in trajectory at a threshold of uncorrected $p<0.01$. At this exploratory level, we were able to recapitulate some of the findings observed in our previous study (Guma et al. 2021), wherein POL SAL exposed offspring had a smaller volume at PND 25, relative to SAL SAL, which overshot and was larger at PND 50, and then normalized at PND 85. This was observed in regions such as the lateral septum, striatum, hippocampus, and somatosensory cortex. Post-hoc investigation of sex differences revealed no effects (<20%FDR).

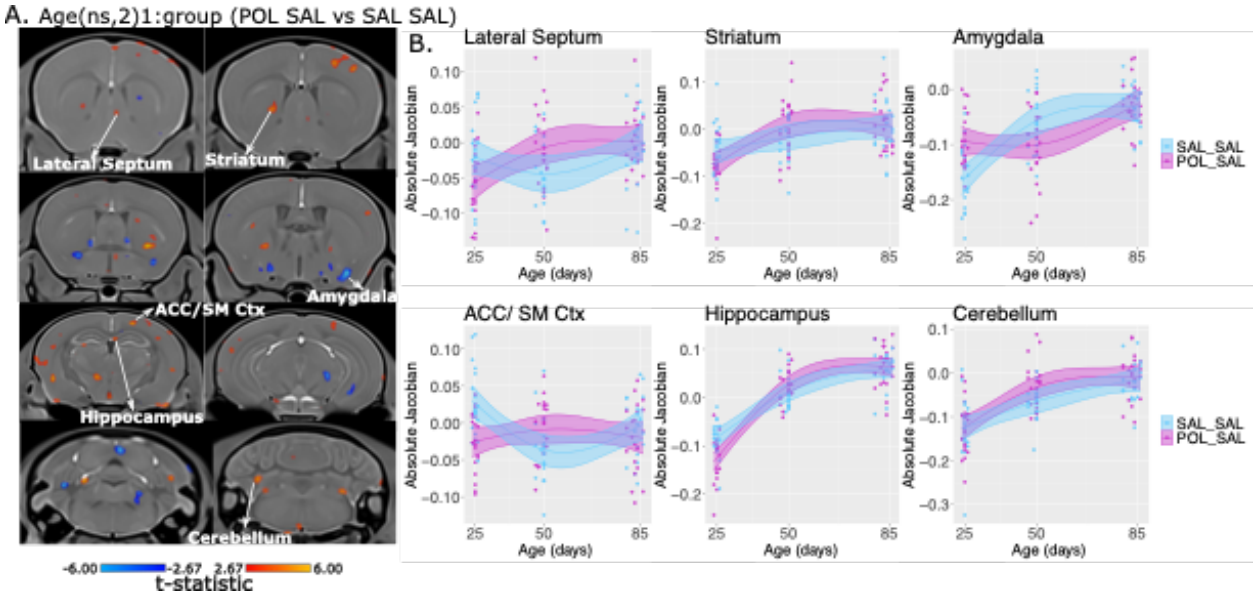


Figure 6.2. Exploration of neuroanatomical alteration due to prenatal MIA-exposure alone at an uncorrected threshold (p<0.01). **A**. t-statistic map of group (POL SAL vs SAL SAL) by age (first order natural spline of age) (t=2.67, p=0.01). **B**. Plot of peak voxels selected from regions of interest highlighted in **A**, wherein age is plotted on the x-axis, and the absolute Jacobian determinants plotted on the y-axis. Here a value of 1 means the voxel is no different than the average, anything above one is relatively larger, and blow 1 is relatively smaller. Trajectories reflect the statistical model used (see **6.4.4.2** & **6.8.5**).

6.5.1.2 Effects of chronic adolescent THC exposure on neurodevelopmental trajectories

Effects of adolescent THC were explored by comparing offspring prenatally exposed to SAL, and then postnatally exposed either to THC (SAL THC) or SAL (SAL SAL); we observed a significant group by age (group : ns(age,2)1) interaction (t=5.16, 5% FDR; **Figure 6.3)**. To better investigate the neuroanatomical changes, t-statistic brain maps were investigated at a more lenient threshold of 20% FDR. We observed the greatest deviations in SAL THC mouse trajectory relative to SAL SAL at the PND 50, or post-treatment timepoint. This suggests that the chronic THC exposure was inducing some brain remodeling, however, we see that the trajectories normalize in later adulthood (PND 85), which may indicate that the brain has had a chance to normalize following a washout period from THC. In the medial preoptic area, somatosensory cortex, globus pallidus, third ventricle, and reticular nucleus, the SAL THC offspring had larger volumes at PND 50, whereas in the CA1 region of the hippocampus, they had smaller volumes. Post-hoc investigation of sex differences revealed no significant sex-by-group-by-age interactions.

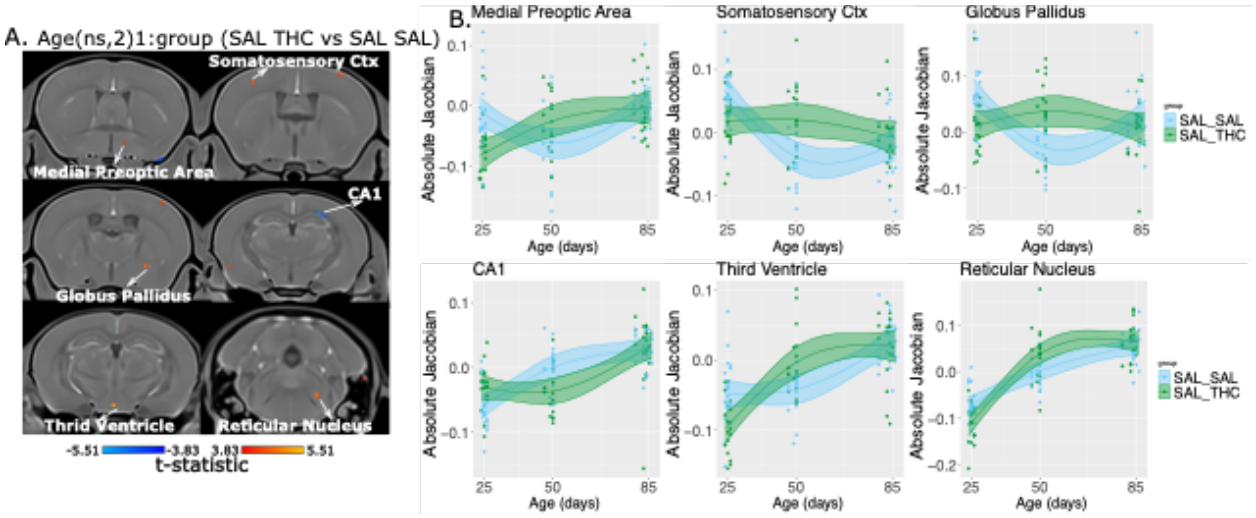


Figure 6.3. Neuroanatomical alteration due to adolescent THC-exposure. **A**. t-statistic map of group (SAL THC vs SAL SAL) by age (first order natural spline of age) thresholded between 5% FDR (top, t=5.51) and 20% FDR (bottom, t=3.83). **B**. Plot of peak voxels selected from regions of interest highlighted in **A**, wherein age is plotted on the x-axis, and the absolute Jacobian determinants plotted on the y-axis. Trajectories reflect the statistical model used (see **6.4.4.2** & **6.8.5**).

6.5.1.3 Effects of combined prenatal MIA and adolescent THC exposure on neurodevelopmental trajectories

Effects of the combined risk factors were explored by comparing offspring exposed prenatally to POL and postnatally to THC (POL THC) to those exposed both pre- and postnatally to SAL (SAL SAL). A significant group by age (group : ns(age,2)1) interaction (t=3.63, 5% FDR) was observed. Again, there was a significant deviation in trajectory in the post-treatment, PND 50 timepoint wherein increased volume in the POL THC group was detected in the nucleus accumbens, striatum, somatosensory, anterior cingulate, and entorhinal cortices, while decreases were observed in the subiculum. Interestingly, this deviation in trajectory normalized in a number of regions, including the nucleus accumbens, anterior cingulate and entorhinal cortices, while the deviation was sustained in later adulthood (PND 85) in the striatum, somatosensory cortex, and subiculum. This suggests that some regions may recover following THC exposure, while others remain affected throughout development (**Figure 6.4**). Investigation of the effects of POL THC relative to POL SAL are summarized in **Supplement 6.9.4 and Supplementary figure 6.S2.** Post-hoc investigation of sex differences in this group revealed a significant three-way interaction

(group : ns(age,2)1 : sex) (t=4.238, 5% FDR) (described in **Supplement 6.9.6.1, Supplementary figure 6.S4**).

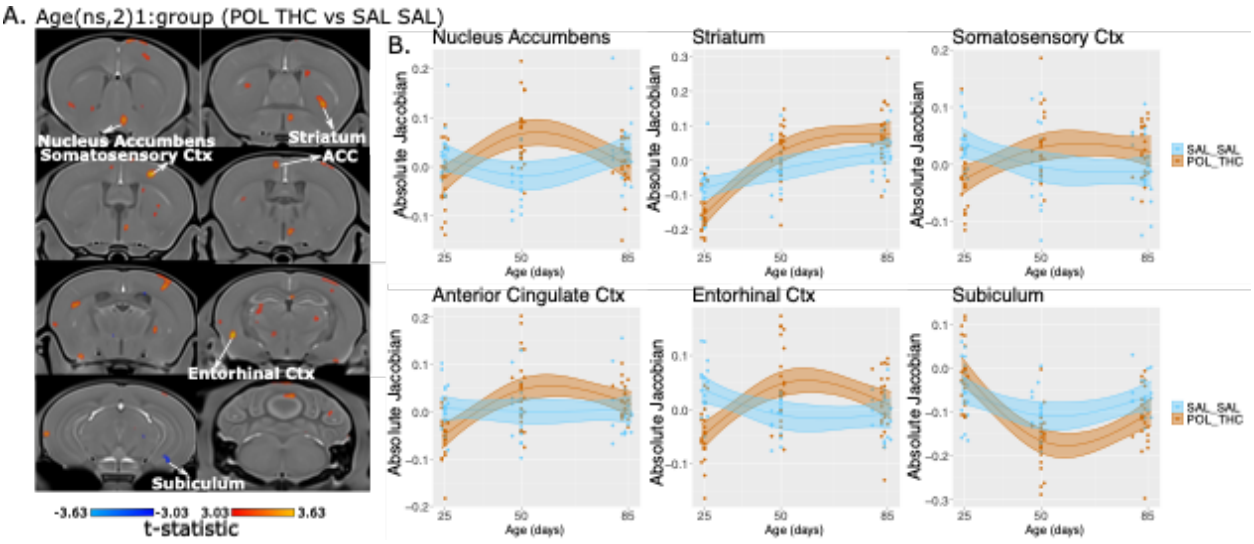


Figure 6.4. Neuroanatomical alteration due to combined prenatal MIA-exposure and adolescent THC-exposure. **A**. t-statistic map of group (SAL THC vs SAL SAL) by age (first order natural spline of age) thresholded between 5% FDR (top, t=3.63) and 10% FDR (bottom, t=3.03). **B**. Plot of peak voxels selected from regions of interest highlighted in **A**, wherein age is plotted on the x-axis, and the absolute Jacobian determinants plotted on the y-axis. Trajectories reflect the statistical model used (see **6.4.4.2** & **6.8.5**).

6.5.2 Effects of risk factor exposure on adult behaviour

Overall, none of the behavioural effects observed survived multiple comparisons correction. No significant effects due to prenatal MIA exposure (POL SAL vs. SAL SAL) were observed in the open field test, PPI, or social preference or novelty behaviours (q>0.0125). Similarly, no effects due to adolescent THC exposure alone were observed (SAL THC vs. SAL SAL) in any of the behavioural tests performed. Finally, for the combined exposure group (POL THC vs. SAL SAL), a subthreshold increase in the relative distance traveled in the center zone of the open field was observed (t=2.344, p=0.041, q=0.164). No significant effects were observed in sensorimotor gating overall, however, when investigating effects over increasing prepulse level, there was a subthreshold interaction (t=2.239, p=0.027, q=0.108) wherein POL THC offspring

were impaired at lower tones but not at higher prepulse tones relative to SAL SAL offspring. Again, no social deficits were observed for either preference or novelty. Finally, investigating THC effects within the prenatal POL groups (POL SAL vs. POL THC) are detailed in **Supplement 6.9.5**. Behavioural results (**Figure 6.5)** are summarized in **Supplementary table 6.S3**. Comparison between POL THC and POL SAL detailed in **Supplement 6.9.5.1, Supplementary figure 6.S3, Supplementary table 6.S3**). No significant sex-by-group interactions were observed (**Supplement 6.9.6, Supplementary figure 6.S5; Supplementary table 6.S4**).

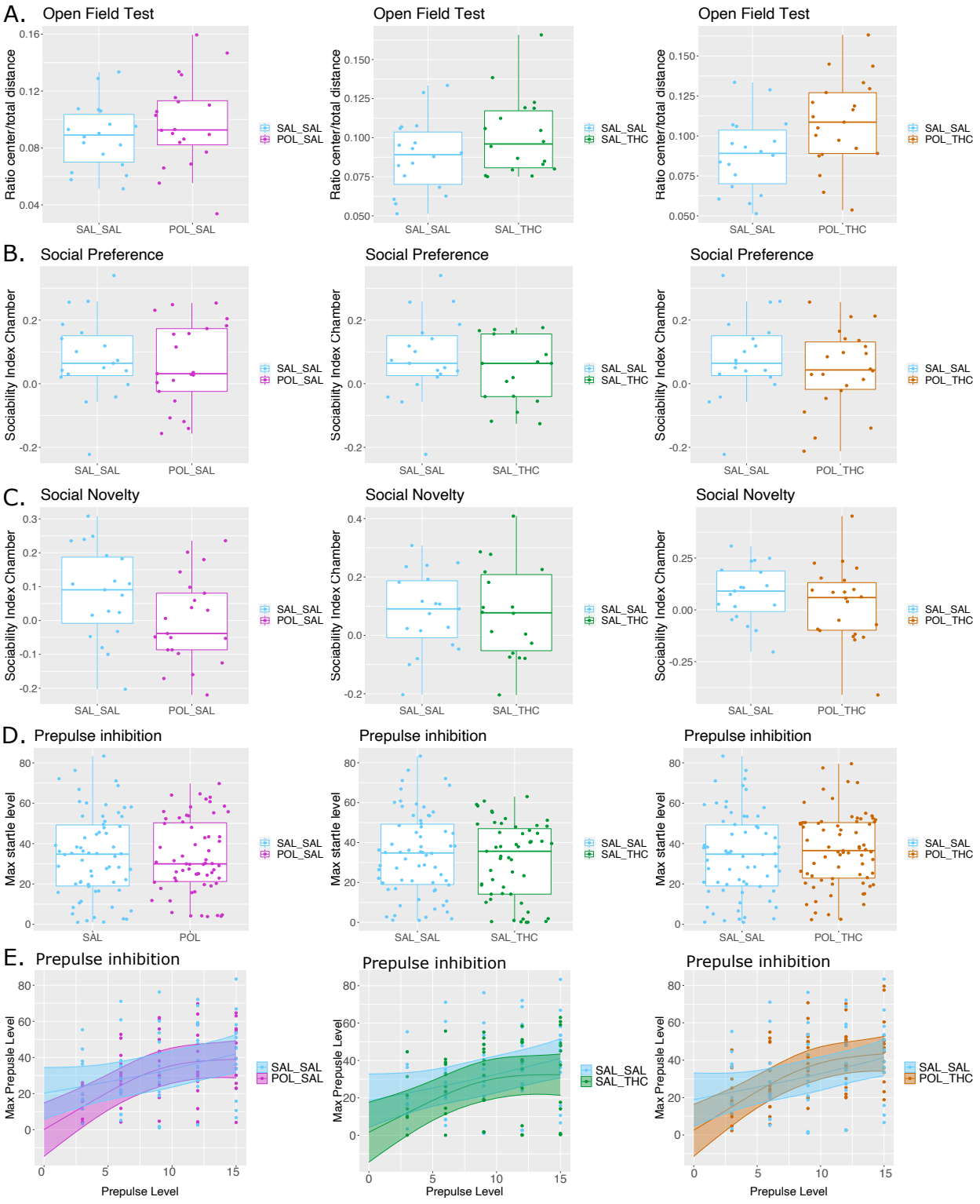


Figure 6.5 (caption on next page).

Figure 6.5 (continued). Prenatal MIA-exposure and/or adolescent THC exposure do not affect adult behaviour. Behavioural results for all treatment groups: SAL SAL (cyan), POL SAL (magenta), SAL THC (green), POL THC (orange). For all boxplots the midline represents the median of the data, the box represents the interquartile range, with whiskers denoting the full range of the data. **A**. No significant effects for any groups on the distance traveled in the center zone relative to the total distance traveled, although a subthreshold increase was observed in the POL THC group relative to SAL SAL (t=2.344, p=0.041, q=0.164). No statistically significant differences were observed in the social preference (**B**) or social novelty (**C**) tasks for any of the groups. Finally, no overall differences in prepulse inhibition, based on the maximum startle amplitude were observed (**D**), although a subthreshold interaction was observed between POL THC vs SAL SAL and increasing prepulse level (ns(level, 2)1:group; t=2.239, p=0.027, q=0.108) (**E**)**.**

6.5.3 Investigating brain-behaviour covariation

6.5.3.1 Covariation between adult (PND85) brain-behaviour

Using PLS to examine adult whole-brain voxel-wise volume differences and 18 behavioural metrics across our 3 main tests (including sex and litter size) we identified one significant LV ($p<0.00001$, %covariance=35%; **Figure 6.6A)**. The observed pattern identified increased pancortical, striatal, thalamic, and cerebellar volume to associate with decreased interactions in the social preference test, decreased sensorimotor gating abilities, highly expressed in females **(Figure 6.6BC)**. Correlations between brain-behaviour scores suggests that all female offspring strongly express the brain-behaviour pattern, as do the SAL SAL male offspring, but all male offspring exposed to either a single (prenatal MIA, adolescent THC) or combined risk factors (both MIA and THC) do not strongly express the pattern. This is indicative of potential sex differences in response to risk factor exposure, with potentially greater group differences in male offspring (**Figure 6.6D)**.

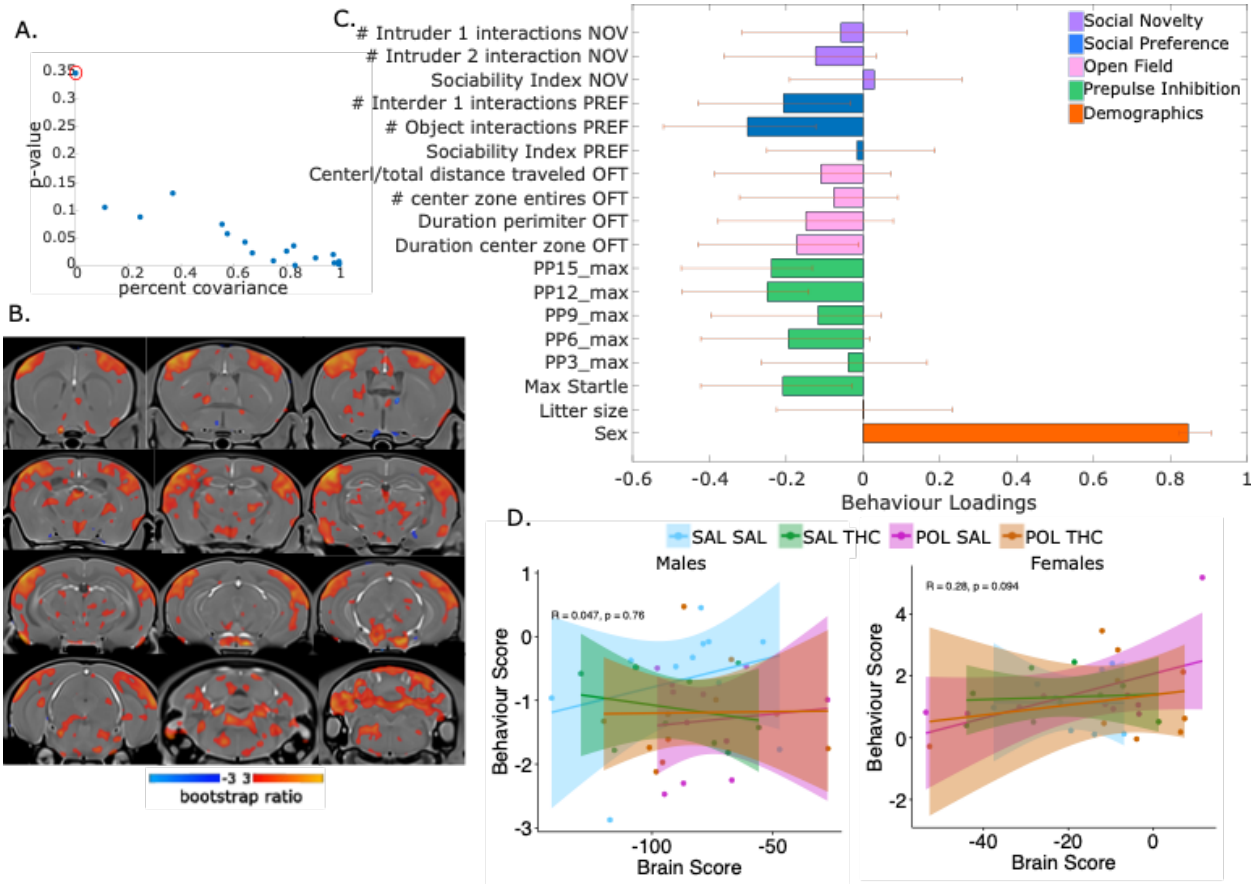


Figure 6.6. Partial least squares (PLS) analysis results for first latent variable (LV1) comparing adult (PND85) brain and behaviour. **A.** Covariance explained (y-axis) and permutation p-values (x-axis) for all 18 LVs in the PLS analysis. LV1 is circled in red ($p<0.00001$, %covariance=35%) and was chosen for subsequent investigation based on the covariance explained and behavioural relevance of results. **B**. Brain loading bootstrap ratios for the LV1 deformation pattern overlaid on the population average, with positive bootstrap ratios in orange-yellow (indicative or larger volume), and negative in blue (indicative of smaller volume). Colored voxels make significant contributions to LV1. **C.** Behaviour weight for each behavioural measure included in the analysis showing how much they contribute to the pattern of LV1. Singular value decomposition estimates the size of the bars whereas confidence intervals are estimated by bootstrapping. Bars with error bars that cross the 0 line should not be considered. **D**. Correlation of individual mouse brain and behaviour score, color coded by treatment group with a trend line per group split by sex. Male SAL SAL offspring, and all female offspring express the pattern more strongly; male offspring exposed to any of the risk factors express the pattern less strongly. SAL SAL (cyan), POL SAL (magenta), SAL THC (green), POL THC (orange).

6.5.3.2 Covariation between within subject volume change from post-treatment to adulthood and adult behaviour

Next, we used PLS to examine within subject voxel-wise volume change from the post-treatment timepoint (PND 50) to the adult timepoint (PND 85) and the same 18 behavioural and demographics metrics as in **6.5.3.1**. We identified two significant LV (LV1: $p<0.00001$, percent covariance=24%; LV2: $p=0.01$, percent covariance=19%; **Supplementary figure 6.S6)**. For LV1, the observed pattern identified increased somatomotor, striatal, hippocampal, and cerebellar within-subject volume change (i.e., larger from PND 50 to 85) to associate with fewer interaction in the social preference test, greater impairments in sensorimotor gating, and to be more expressed in females, similar to the pattern observed in **6.5.3.1 (Figure 6.7AB)**. Correlations between brain-behaviour scores show that females express this pattern, while male show some group differences, with greater loading in SAL SAL and POL THC groups (left two plots **Figure 6.7C)**. Removal of outliers (right two plots **Figure 7.6C)** suggests that male offspring express this pattern, as do females, except for the SAL THC female offspring, who seem to express this pattern less, indicative of potential sex differences.

The pattern captured by LV2 reflects decreased within-subject cortical (somatomotor) and cerebellar volume and increased thalamic volume to associate with increased social novelty interactions, increased locomotion in the open field test, and to be more associated with male mice from smaller litters (**Figure 6.7EF)**. Brain-behaviour score correlations suggest that all offspring express this pattern, with a potential difference in the POL THC exposed female offspring (left two plots **Figure 6.7G)**. Removal of outliers (right two plots **Figure 6.7G**) confirms this association.

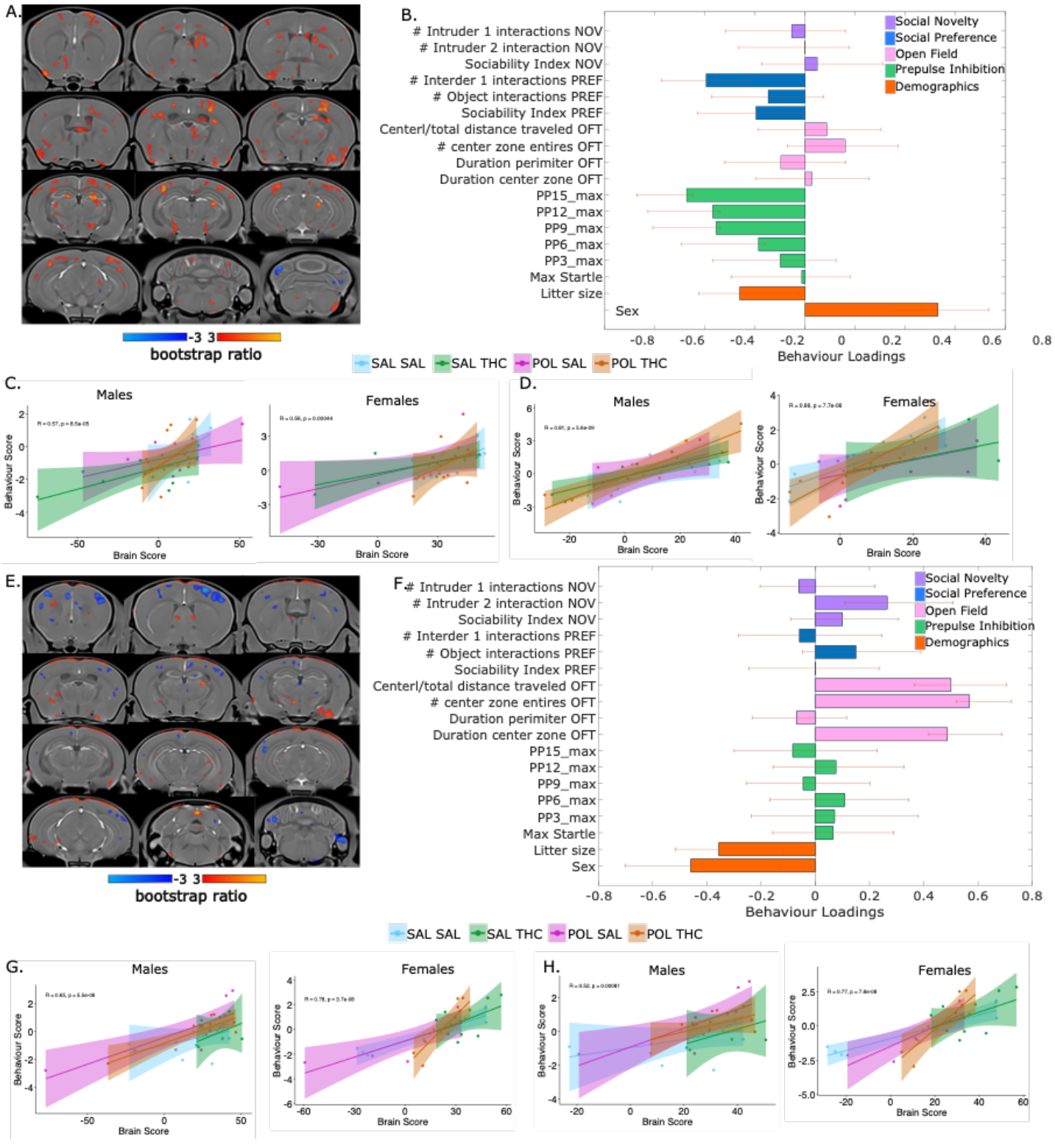


Figure 6.7 (caption on next page).

Figure 6.7 (continued). Partial least squares (PLS) analysis results for the first and second latent variable (LV) comparing the difference between PND 50 and PND 85 voxel-wise brain volume, and behaviour. **A** Brain loading bootstrap ratios for the LV1 deformation pattern overlaid on the population average, with positive bootstrap ratios in orange-yellow (indicative or larger volume), and negative in blue (indicative of smaller volume). Coloured voxels make significant contributions to LV1. **B**. Behaviour weight for each behavioural measure included in the analysis showing how much they contribute to the pattern of LV1. Singular value decomposition estimates the size of the bars whereas confidence intervals are estimated by bootstrapping. Bars with error bars that cross the 0 line should not be considered. **C.** Correlation of individual mouse brain and behaviour score, color coded by treatment group with a trend line per group split by sex. **D**. Correlation of individual brain-behaviour scores with the exclusion of outliers. **E.** Brain loadings for LV2 (as in **A**). **F**. Behaviour loadings for LV2. **G.** Correlation for individual mouse brain and behaviour score, coloured by treatment and split by sex. **H.** Correlation of individual brain-behaviour scores with the exclusion of outliers. SAL SAL (cyan), POL SAL (magenta), SAL THC (green), POL THC (orange).

6.6 Discussion

Converging lines of evidence across epidemiological and experimental studies have identified prenatal MIA-exposure and adolescent THC exposure as risk factors for neuropsychiatric illness. However, most individuals exposed to either risk factor alone do not develop psychiatric or neurodevelopmental disorders, which suggests that multiple exposures within an individual are required for disease onset (Rapoport et al. 2005; Estes and McAllister 2016). The work presented here is aimed at experimentally testing the multiple exposure hypothesis, by exposing MIA-primed offspring to a second hit: chronic adolescent THC exposure. Our results suggest that exposure to either prenatal MIA or adolescent THC were not sufficient to induce enduring neuroanatomical or behavioural alterations in adulthood, while combined exposure is, in some regions but not all. Behaviourally, only subtle, subthreshold decreases in anxiety-like and impairments in sensorimotor gating behaviours were observed in the group exposed to both risk factors. Interestingly, sex-differences were observed in patterns of brain-behaviour covariation, both for adult brain volume and for the within-subject change in neuroanatomy post-treatment to adulthood, and adult behaviour. Our results show that a single risk factor may not be sufficient to cause enduring changes in neuroanatomy and behaviour, but that a combined exposure may be.

Preclinical investigation of MIA-exposure has been critical to our understanding of neurodevelopmental disruptions due to this risk factor (Brown and Meyer 2018; Reisinger et al. 2015; Gumusoglu and Stevens 2019), identifying behavioural, neuroanatomical, and transcriptional alterations in exposed offspring relevant to ASD and schizophrenia pathology (Boksa 2010; Guma, Plitman, and Chakravarty 2019; Gumusoglu and Stevens 2019; Haddad, Patel, and Schmid 2020; Kreitz et al. 2020; Piontkewitz, Arad, and Weiner 2011). Here, mice prenatally exposed to MIA had no statistically significant deviations in developmental trajectories, nor in anxiety-like, social, or sensorimotor gating behaviours. The lack of statistically significant effects was somewhat surprising, and not consistent with previous reports of significantly altered brain development from our group (Guma et al. 2021) and others (Crum et al. 2017; Piontkewitz, Arad, and Weiner 2011). However, exploration of the findings at a lenient, uncorrected threshold identified altered trajectories in the striatum, hippocampus, lateral septum, and somatosensory cortex, wherein the MIA-exposed mice had an overshoot in the adolescent/early-adult period followed by a normalization in later adulthood (relative to SAL SAL), in line with our previously published findings (Guma et al. 2021). The lack of behavioural alterations in adulthood is consistent with our previous findings; however, there are many reports of altered behaviour in adult MIA-exposed offspring across multiple domains (Gumusoglu and Stevens 2019). Some methodological differences may explain why we do not recapitulate our findings at the same level of statistical significance between this study and our previous one (Guma et al. 2021); most importantly, mice were scanned only three times (compared to four in our previous work), and at slightly lower sample sizes, which may have reduced our sensitivity to detect alterations in trajectory (Lerch et al. 2012). Additionally, the ages selected for MRI in this study did not perfectly match those of our previous work, which may have decreased our ability to capture the stages of greatest change across development. Finally, structural imaging was performed slightly differently between studies; here we used a cryogenically cooled surface coil with no contrast enhancement (manganese chloride administration 24 hours prior to scan), whereas in the previous study we used a quadrature volumetric coil, with the use of manganese chloride for contrast enhancement; perhaps differences in structural MRI acquisition could contribute to the lack of consistency in our findings.

Mice chronically exposed to THC in adolescence similarly had transient deviations in neurodevelopmental trajectories, with a peak difference in the adolescent/early-adult period. In

various regions including the somatosensory cortex, medial preoptic area, globus pallidus, third ventricle, and reticular nucleus, THC exposed mice experience a volume increase at the post-treatment timepoint (PND 50), followed by a normalization at the later adult timepoint. In contrast, hippocampal volume decreased at the post-treatment timepoint, but also normalized. These results indicate that the chronic THC treatment may have induced neuroanatomical remodeling, but that given a few weeks without THC exposure, the brain normalizes relative to the control (SAL SAL) exposed offspring. In agreement with the lack of neuroanatomical differences by the adult timepoint (PND 85), we did not detect any behavioural alterations; had we tested these mice earlier in development or on other behavioural domains, we may have detected differences.

To our knowledge, no longitudinal rodent MRI studies exist examining the effects of chronic adolescent THC exposure on brain structure. However, studies using other modalities do report neuroanatomical, neurochemical, and behavioural abnormalities relevant to the psychosis spectrum (Rubino et al. 2009; Renard et al. 2017; Miller et al. 2019). Positron emission tomography studies of rats exposed to chronic THC report increased D2-like receptor availability in the dorsal striatum (Ginovart et al. 2012). Furthermore, chronic THC exposure in adolescence has been associated with decreased synaptic arborization in the rat hippocampus (Rubino et al. 2009), and the prefrontal cortex (Miller et al. 2019). In contrast with our findings, impairments in cognitive flexibility, sensorimotor gating, and memory have all been identified in rodents chronically exposed to THC in adolescence (Verrico et al. 2014; Gomes, Guimarães, and Grace 2014; Gleason et al. 2012).

Mice who were exposed to both prenatal MIA and chronic adolescent THC exhibited the greatest neurodevelopmental alterations. In the nucleus accumbens, anterior cingulate cortex, and entorhinal cortex significant deviations in trajectory were observed, defined by enlarged volume in the POL THC group relative to SAL SAL at the post-treatment (PND 50) scan. Similar to the SAL THC group, these brain regions normalized in absence of continued THC exposure by later adulthood (PND 85). However, in the striatum and somatosensory cortex, the volume increase observed following the post-treatment scan was sustained into later adulthood indicative of a more lasting neuroanatomical change. Similarly, in the subiculum, a volume decrease was observed following treatment, which was sustained into later adulthood. These regions may be more sensitive to the combined risk factor exposure; interestingly, they have been previously implicated in neurodevelopmental and neuropsychiatric disorders (Piontkewitz, Arad, and Weiner 2012;

Langen et al. 2014; Grace et al. 2010; Schobel et al. 2013; Khan et al. 2015; Daskalakis et al. 2020). Finally, investigation of the effects of THC effects above and beyond those of MIA (POL THC vs POL SAL groups) revealed that mice exposed to both risk factors experience sustained growth of the bed nucleus of the stria terminalis, ventromedial thalamus, reticular nucleus and CA1, relative to mice exposed to MIA and adolescent SAL.

Behaviourally, we only observed some subthreshold decrease in anxiety-like behaviours in our combined POL THC group relative to SAL SAL, potentially reflective of increased risk-taking behaviours. A subthreshold difference was also observed in sensorimotor gating behaviour, wherein the POL THC mice performed worse at lower prepulse tones, but better at louder tones. Thus, although the combined risk factors were sufficient to induce neuroanatomical changes, they may not be sufficient to affect the behaviours we tested; of course, it is possible that behavioural impairments exist in other domains not tested.

Alterations to the endocannabinoid system, i.e., decreased CB1 receptor availability, due to MIA-exposure have been previously observed in the adult rat hypothalamus, as well as the sensory cortex, a region where we detect sustained alterations due to MIA and THC exposure in this study (Verdurand et al. 2014). Few studies exist investigating the combined effects of the two risk factors studied here on offspring development. Changes to small non-coding microRNAs in the entorhinal cortex, associated with neurotransmission, cellular signaling, and schizophrenia, have been identified in rats exposed to both risk factors (S. L. Hollins et al. 2014; Sharon L. Hollins et al. 2016). Finally, alterations to the serotonergic (Dalton et al. 2012) and dopaminergic systems (Lecca et al. 2019) have both been identified in rats exposed to both risk factors; these neurotransmitter systems are relevant to the disease pathology of a number of neuropsychiatric disorders (Daubert and Condron 2010; Ali and Pereira 2017). These findings indicate that exposure to the combined risk factors may cause some neurochemical and cellular changes in some of the regions where we observe volume alterations; this relationship should be further investigated. In addition the putative synergistic effects of MIA with cannabis, MIA-exposure has been shown to potentiate the effects of other environmental risk factors such as adolescent stress (Giovanoli et al. 2013) or exposure to drugs of abuse (Dalton et al. 2012), as well as genetic risk factors such as *DISC1* (Abazyan et al. 2010; Lipina et al. 2013), *NRG1* (Vuillermot et al. 2012), *NR4A2*, *TSC2* (Crawley 2007) causing greater deficits than either exposure alone (Reisinger et al. 2015; Meyer 2014).

Finally, our investigation of brain-behaviour covariation identified some underlying sex differences, not clearly uncovered in our univariate analyses. The pattern identified in adulthood differentiated the male SAL SAL offspring from all other male offspring exposed to either a single or a combined risk factor, whereas it did not differentiate the female offspring. This was defined by increased cortical, thalamic and cerebellar volume associated with decreased social interactions and sensorimotor gating. The assessment of within-subject volume changes post-treatment to adulthood with behaviour identified a similar behavioural pattern, associated with the somatosensory cortex striatum, hippocampus, and cerebellum; in this case, male mice were more similar in their expression of the pattern, whereas female SAL THC offspring different from the other three groups. Overall, this analysis suggests that there may be interesting sex differences underlying the neurodevelopmental abnormalities associated MIA and THC exposure.

Sex differences in response to MIA-exposure have been identified in human birth cohorts, showing that males are more likely to develop neurodevelopmental disorders following MIA-exposure to bacterial infection (Lee et al. 2020). Rodent studies also provide evidence for increased susceptibility in males, with reports of earlier deviations in neuroanatomy and behaviour based on longitudinal studies (Piontkewitz, Arad, and Weiner 2011), as well as male-biased deficits in spatial working memory, PPI, and locomotion (Gogos et al. 2020). Finally, altered synaptic function in the hippocampus and abnormal microglia activation have been reported in male MIA-exposed offspring at a greater level than in females (Haida et al. 2019; Hui et al. 2020). Sex-biases have also been observed in response to cannabinoids; some evidence from human studies suggest that males may be more susceptible to the effects of cannabis use if they carry genetic risk for schizophrenia (French et al. 2015), and are also more likely to initiate use earlier than females (Kohn, Kittel, and Piette 2004). Rodent studies suggest that females may be more likely to develop behavioural despair, anhedonia, and catalepsy in response to THC treatment (Rubino et al. 2008; Biscaia et al. 2003). These behavioural differences may, in part, be explained by sex differences in THC metabolism, with higher rates observed in males (Tseng and Craft 2004), and sex differences in CB1 receptor density, with elevated density in the striatum, limbic system, and pituitary in males (González et al. 2000). A better understanding of how MIA-induced sex-differences in microglia and synaptic function may interact with CB1 receptor density and THC metabolism could help us disentangle some of the sex-differences observed in the brain-behaviour alterations.

Based on our multivariate results, we believe that future studies should be performed to specifically investigate sex differences in response to both risk factors, ensuring sufficient power to detect these complex statistical interactions. Further, acquiring MRI and behavioural data more frequently throughout the development of exposed offspring may allow us to uncover more subtle changes. This may, in part, explain why we did not detect significant alteration in the MIA-exposed mice, and only detected subtle changes due to THC exposure. Furthermore, performing behavioural assay more proximal to the chronic THC exposure would allow us to uncover more acute behavioral alteration in response to treatment.

We find that exposure to a single risk factor, either prenatal MIA-exposure or chronic adolescent THC exposure are not sufficient to induce lasting neuroanatomical or behavioural changes in adulthood, although transient alterations were observed in adolescent/early-adult neuroanatomy. In contrast, exposure to both risk factors may induce lasting neuroanatomical deviations in some regions, but not others, while only inducing subthreshold behavioural alterations. In conclusions, our findings show that exposure to multiple risk factors may have more severe effects on offspring development than exposure to a single risk factor, which may further our understanding of how exposure to multiple risk factors could increase the likelihood of developing neuropsychiatric illnesses.

6.7 Additional manuscript information

6.7.1 Acknowledgements

The authors would like to acknowledge their funding bodies, including the Canadian Institute of Health Research and Healthy Brains for Healthy Lives for providing support for this research. Additionally, we would like to thank the Fonds de Recherche du Québec en Santé for providing salary support for EG and MMC, as well as the Kappa Kappa Gamma Foundation of Canada for supporting EG's salary.

6.7.2 Conflict of interest statement

The authors report no conflicts of interest.

6.8 Supplementary methods

6.8.1 Animals and time mating protocol

C57BL/6J mice were bred in our facility under a 12-hour light cycle (8am-8pm), with food and water access *ad libitum*. Females and males of breeding age (8-12 weeks) were placed in new cages (1:1 ratio) for up to 2 days until seminal plug was observed. This was considered gestational day (GD) 0. Each female was weighed and moved to a new cage. Animals were weighed again on injection day (GD 9) to confirm pregnancy as identified by a weight gain of at least 2 grams from GD 0.

6.8.2 Assessment of maternal cytokines levels

In a separate group of dams, poly I:C or saline was injected as described above (n=4 GD9-POL, n=5 GD9-SAL). Three hours following injection, dams were sacrificed by decapitation without euthanasia, and trunk blood was collected in a 1.5mL Eppendorf tube. The blood was allowed to coagulate at room temperature for 30 minutes, and then centrifuged for 10 minutes at 4 ℃, with 2000 revolutions per minute. Serum was collected and stored at -80 ℃ until ready for analysis. Serum samples were shipped to the University of Maryland Core Cytokine Facility (http://www.cytokines.com/) for multiplex ELISA to measure levels of IL-6, TNF-α, IL-1β, IL-10. We chose to use a separate group of dams to ensure we could collect enough blood for analysis, and to ensure we were not introducing additional stress to the experimental dams, which may have confounding effects on the neurodevelopmental trajectory of offspring. Detection ranges were as follows IL-6 (1.95-8000 pg/ml), TNF-alpha (0.64-3500 pg/ml), IL-1β (0.64-15000 pg/ml), IL-10 (0.64-20000 pg/ml).

6.8.3 Validation of THC solution

Plasma levels of THC were measured in a separate cohort of mice with gas-chromatography mass spectrometry (GCMS) (Tynon, Porto, and Logan 2015). Adult C57BL/6J mice were injected with dosages of either 2.5 mg/kg (N=5, 2F/3M), 5 mg/kg (N=5, 2F/3M), 10 mg/kg (N = 5, 3F/2M), or cremophor:saline control (N=5, 3F/2M). One hour post injection, mice were euthanized by live decapitation and trunk blood was collected for analysis in 1.5 mL EDTA

Eppendorf tubes containing chilled EDTA (final concentration of 5mM). Samples were centrifuged for 15 minutes (1500 rotations) at 4 ℃. Plasma was collected in separated Eppendorf tubes and stored at -80 ℃ until samples were ready for analysis. 1 mg/mL THC in methanol was purchased from Millipore Sigma-Canada (Product Number T4764) to serve as the standard for the GCMS. Samples were tested for the presence of delta-9-THC, as well as two THC metabolites, 11-hydroxy-delta-9-THC and 11-nor-9carboxy delta-9-THC. Results indicated for all dosages THC and its metabolites were present at values within the expected bounds of error across the groups (**Supplementary Table 6.1**).

6.8.4 Behavioural tests

6.8.4.1 Open field test

This test was used to assess exploratory and anxiety-like behaviours. Mice were gently placed in the center of a 45 x 45 cm^2 rectangular light grey arena and allowed to explore for 15 minutes, while being video recorded. Distance traveled and time spent in conceptually partitioned arenas was measured: a center zone (40% of the total area), and the perimeter (corners and edges). Distance traveled in the center zone relative to total distance traveled was used as the main assay.

6.8.4.2 Three chambered social preference and social novelty task

Mice were habituated (10 minutes) under red light to a three-chamber plastic box (26 (l) x 21.6 (w) x 21.6 (h) cm) with divider panels that have open doors, with a wire container (9.5 (h) 7.6 (d) cm) in each of the two extreme chambers. To measure social preference (10 minutes), time spent interacting with a stranger mouse was compared to that with a non-social object using the following social preference index formula 1:

([time spent sniffing intruder 1 zone] / [time spent sniffing object zone + time spent sniffing intruder 1 zone]) - 0.5. (1)

Similarly, to measure social novelty (10 minutes), the non-social object was replaced with another stranger mouse, and a social novelty index was calculated as formula 2:

([time spent sniffing intruder 2 zone] / [time spent sniffing intruder 1 zone + time spent sniffing intruder 2 zone]) - 0.5 (2)

Stranger mice were the same strain, sex, and similar age (within 2 weeks) of the test mice and were habituated to the wire containers (20 minutes twice a day) for two days prior to the test.

6.8.4.3 Prepulse inhibition task

Prepulse inhibition (PPI) to acoustic startle was measured using commercially available startle chambers (San Diego Instruments, San Diego, CA) consisting of a Plexiglass chamber (8 cm diameter, 16 cm long) mounted on a Plexiglass base with a sound-attenuating chamber, and a speaker located in the ceiling of the chamber (24 cm above the animal) to provide the background noise (70dB) and the acoustic stimuli. A piezoelectric accelerometer fixed to the animal enclosure frame was used to detect and transduce motion resulting from the animal's startle response. A microcomputer using a commercial software package by SR-LAB was used to control pulse parameters, and digitize (0-4095), rectify, and record the stabilimeter readings. Animals were placed in the Plexiglass restrainers, and after 5 minutes of acclimatization. Mice underwent a total of 50 trials (5-30 s intertrial duration).

Startle magnitude to a 50 ms 120 dB stimulus, in absence of prepulse, was measured in the first 8 and final 7 trials. For the middle 35 trials, the startle tone was either presented alone, or preceded by a 30 ms prepulse stimulus ranging from 3-15 dB above background noise (73-85 dB) and varying randomly between trials in 3 dB increments (5 trials per prepulse stimulus). A measure of maximum and average startle response was derived from the 100 1 ms readings taken starting from the beginning of the startle stimulus onset. Percent PPI from each prepulse intensity formula 3:

(averaged over trials) was calculated using the formula: [(startle response - prepulse response) / startle response] x 100 (3)

Differences in maximum startle amplitude per trial were investigated as the main assay.

6.8.5 Statistical modeling

6.8.5.1 Neuroimaging data

A voxel-wise linear mixed-effects model was run at every voxel in the brain with the following two models, the first for assessing overall group-by-age interactions, and the second for investigating sex-by-group-by-age interactions:

Group-by-age models:

MIA model: $Y_{subject,j} = \beta_0 + \beta_1 sexF_{subject,j} + \beta_2 groupPOL_{subject,j} + \beta_4 age(ns,2)1_{subject,j} + \beta_5 age(ns,2)2_{subject,j} + \beta_6 ns(age,2)1{:}groupPOL_{subject,j} + \beta_7 ns(age,2)2{:}groupPOL_{subject,j} + \mathbf{b_1} subject + \mathbf{b_2} litter + \epsilon_{subject,j}$

THC model: $Y_{subject,j} = \beta_0 + \beta_1 sexF_{subject,j} + \beta_2 groupTHC_{subject,j} + \beta_4 age(ns,2)1_{subject,j} + \beta_5 age(ns,2)2_{subject,j} + \beta_6 ns(age,2)1{:}groupTHC_{subject,j} + \beta_7 ns(age,2)2{:}groupTHC_{subject,j} + + \mathbf{b_1} subject + \mathbf{b_2} litter + \epsilon_{subject,j}$

POL THC model: $Y_{subject,j} = \beta_0 + \beta_1 sexF_{subject,j} + \beta_2 groupPOL\text{-}THC_{subject,j} + \beta_4 age(ns,2)1_{subject,j} + \beta_5 age(ns,2)2_{subject,j} + \beta_6 ns(age,2)1{:}groupPOL\text{-}THC_{subject,j} + \beta_7 ns(age,2)2{:}groupPOL\text{-}THC_{subject,j} + + \mathbf{b_1} subject + \mathbf{b_2} litter + \epsilon_{subject,j}$

Sex-by-group-by-age models:

MIA model: $Y_{subject,j} = \beta_0 + \beta_1 sexF_{subject,j} + \beta_2 groupPOL_{subject,j} + \beta_4 age(ns,2)1_{subject,j} + \beta_5 age(ns,2)2_{subject,j} + \beta_6 ns(age,2)1{:}groupPOL_{subject,j} + \beta_8 ns(age,2)2{:}groupPOL_{subject,j} + \beta_9 groupPOL_{subject,j}{:}sexF_{subject,j} + \beta_{10} ns(age,2)1{:}groupPOL_{subject,j}{:}sexF_{subject,j} + \beta_{11} ns(age,2)2{:}groupPOL_{subject,j}{:}sexF_{subject,j} + \mathbf{b_1} subject + \mathbf{b_2} litter + \epsilon_{subject,j}$

THC model: $Y_{subject,j} = \beta_0 + \beta_1 sexF_{subject,j} + \beta_2 groupTHC_{subject,j} + \beta_4 age(ns,2)1_{subject,j} + \beta_5 age(ns,2)2_{subject,j} + \beta_6 ns(age,2)1{:}groupTHC_{subject,j} + \beta_7 ns(age,2)2{:}groupTHC_{subject,j} + \beta_8 groupTHC_{subject,}{:}sexF_{subject,j} + \beta_9 ns(age,2)1{:}groupTHC_{subject,}{:}sexF_{subject,j} + \beta_{10} ns(age,2)2{:}groupTHC_{subject,j}{:}sexF_{subject,j} + \mathbf{b_1} subject + \mathbf{b_2} litter + \epsilon_{subject,j}$

POL THC model: $Y_{subject,j} = \beta_0 + \beta_1 sexF_{subject,j} + \beta_2 groupPOL\text{-}THC_{subject,j} + \beta_4 age(ns,2)1_{subject,j} + \beta_5 age(ns,2)2_{subject,j} + \beta_6 ns(age,2)1{:}sexF_{subject,j} + \beta_7 ns(age,2)2{:}sexF_{subject,j} + \beta_8 ns(age,2)1{:}groupPOL\text{-}THC_{subject,j} + \beta_9 ns(age,2)2{:}groupPOL\text{-}THC_{subject,j} + \beta_{10} groupPOL\text{-}THC_{subject,j}{:}sexF_{subject,j} + \beta_{11} ns(age,2)1{:}groupPOL\text{-}THC_{subject,j}{:}sexF_{subject,j} + \beta_{12} ns(age,2)2{:}groupPOL\text{-}THC_{subject,j}{:}sexF_{subject,j} + \mathbf{b_1} subject + \mathbf{b_2} litter + \epsilon_{subject,j}$

Y= outcome measures (i.e. blurred absolute Jacobian determinants); β_i= fixed effect coefficient; β_0 = equation intercept; **b** = random predictor; **ϵ** = random error; **j** = repeated measure per subject; **:** = interaction; POL= early polyI:C group relative to SAL SAL as reference; THC = adolescent THC group relative to SAL SAL as reference (or POL SAL as reference); POL THC = POL THC group relative to SAL SAL as reference; SexF = female sex relative to male as reference

6.8.5.2 Behavioral data

For PPI data across increasing decibel levels, differences in trajectory of sensorimotor gating changed over increasing prepulse level were modeled with a second order natural spline, both to test for group-by-level interactions and for sex-by-group-by-level interactions, similar to the way in which age was modeled above for the MRI data above.

6.8.6 Partial least squares significance and reliability assessment

Permutation testing: was used to assess the statistical significance of each LV wherein the rows (subjects) of the brain data matrix were randomly shuffled to 1) nullify dependencies between brain and behaviour (n=1000 repetitions) and 2) generate a null distribution of possible brain-behaviour correlations. SVD was applied to these "null" correlations, generating a distribution of singular values under the null hypothesis. The probability that a permuted singular value exceeds the original, non-permuted singular value allows us to generate the p-value (Zeighami et al. 2019; Patel et al. 2020). A threshold of $p<0.05$ was used (95% or greater chance that the singular value of the non-permuted data exceeds that of a permuted singular value).

Bootstrap resampling: was applied to assess the contribution of individual brain and behaviour variables to each LV. Subjects (rows for both X and Y matrices) were randomly sampled and replaced (n=1000) to generate a set of resampled correlation matrices to which SVD was applied to generate a sampling distribution for each weight of the singular vectors. The ratio of each singular vector weight and its bootstrap-estimated standard error were used to calculate a "bootstrap ratio" for each voxel. Voxels that made large contributions to the identified patterns were therefore identified by large bootstrap ratios.

6.9 Supplementary results

6.9.1 Poly I:C injection does increase pro-inflammatory cytokines in the dam

We observed an increase in levels of all three pro-inflammatory cytokines measured, IL-6, IL-1β, and TNF-α, as well as in the anti-inflammatory cytokine IL-10 in a separate cohort of pregnant dams 3-hours post poly I:C injection on GD 9 relative to saline control on GD 9. Values are summarized below (**Supplementary Table 6.S1**).

Supplementary Table 6.S1. Maternal serum cytokine levels for our 2 treatment groups, mean [range]

	IL-1β (pg/ml)	**IL-6 (pg/ml)**	**IL-10 (pg/ml)**	**TNF-α (pg/ml)**
SAL (n=5)	21.578 [0.64-96.62]	45.544 [30.58-56.59]	21.862 [0.64-56.22]	14.296 [0.64-26.05]
POL (n=4)	36.3325 [0.62-142.81]	5819.96 [4552.18-6512.84]	135.845 [56.73-166.50]	75.095 [45.72-90.51]

6.9.2 THC plasma concentrations in blood reflected injected dose

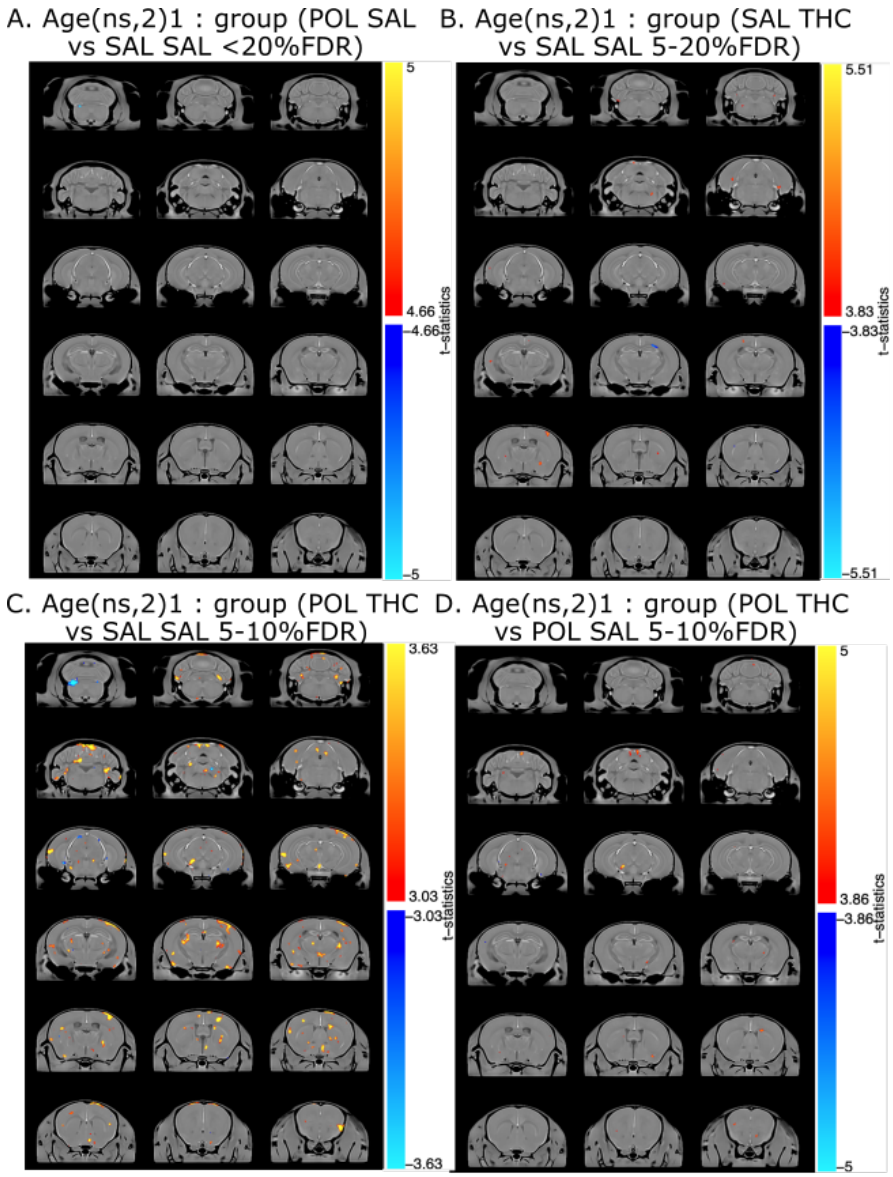
GCMS analysis of blood plasma THC concentrations confirmed that our doses were delivering a comparable amount in the mouse bloodstream 1-hour post-injection. Concentrations of other metabolites were below detection threshold, as expected (**Supplementary Table 6.S2**).

Supplementary Table 6.S2. Maternal serum cytokine levels for our 2 treatment groups, mean [range], n/d = not detected

	delta-9THC concentration (ng/ml)	**11OH delta-9THC (ng/ml)**	**11-nor-9carboxy d-9THC (ng/ml)**
10 mg/kg solution injection	9.6 mg/kg	n/d	n/d
2.5 mg/kg solution injection	2.8 mg/kg	n/d	n/d
5 mg/kg solution injection	5.8 mg/kg	n/d	n/d

6.9.3 Overview of neuroanatomical results

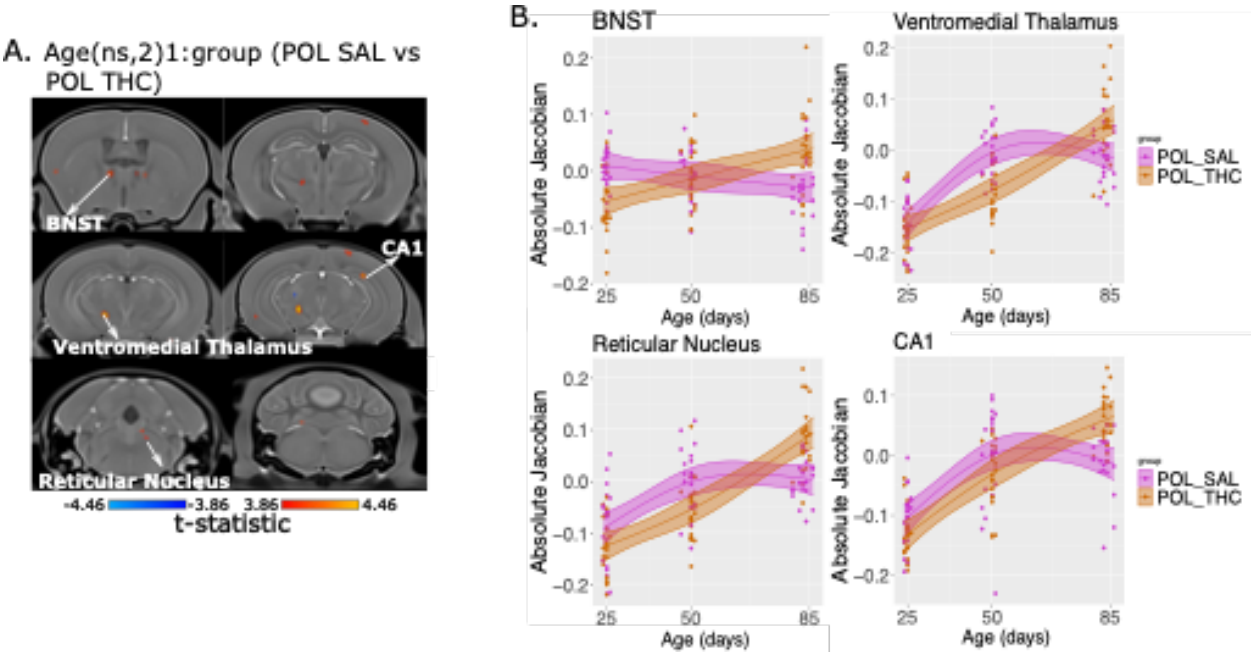
Pairwise comparisons for groups exposed to either one or both risk factors relative to control offspring revealed greater neuroanatomical differences in the combined risk factor group, POL THC (**Supplementary figure 6.S1**).



Supplementary figure 6.S1. Overview of neuroanatomical changes in each group. T-statistic maps for the interaction between groups and the first order natural spline of age (age(ns,2)1:group) are displayed on the population average for comparison between POL SAL and SAL SAL (**A**), SAL THC and SAL SAL (**B**), POL THC and SAL SAL (**C**), and POL SAL and POL THC (**D**). False discovery rate (FDR) correction thresholds highlighted for each panel.

6.9.4. Neuroanatomical alterations due to THC for POL offspring

For completeness, effects of the THC above and beyond those of MIA were explored by comparing offspring exposed both prenatally to POL and postnatally to THC (POL THC), to those exposed prenatally to POL and postnatally to SAL (POL SAL). A significant (group : ns(age,2)1) interaction (t=4.766, 10%FDR) was observed in the bed nucleus of the stria terminalis (BNST), the ventromedial thalamus, CA1 of the hippocampus, and the reticular nucleus. In all regions, the POL THC group started with a larger volume than POL SAL between PND 25 and 50, which decreased at PND 85 (**Supplementary figure 6.S2)**. No sex differences were observed.



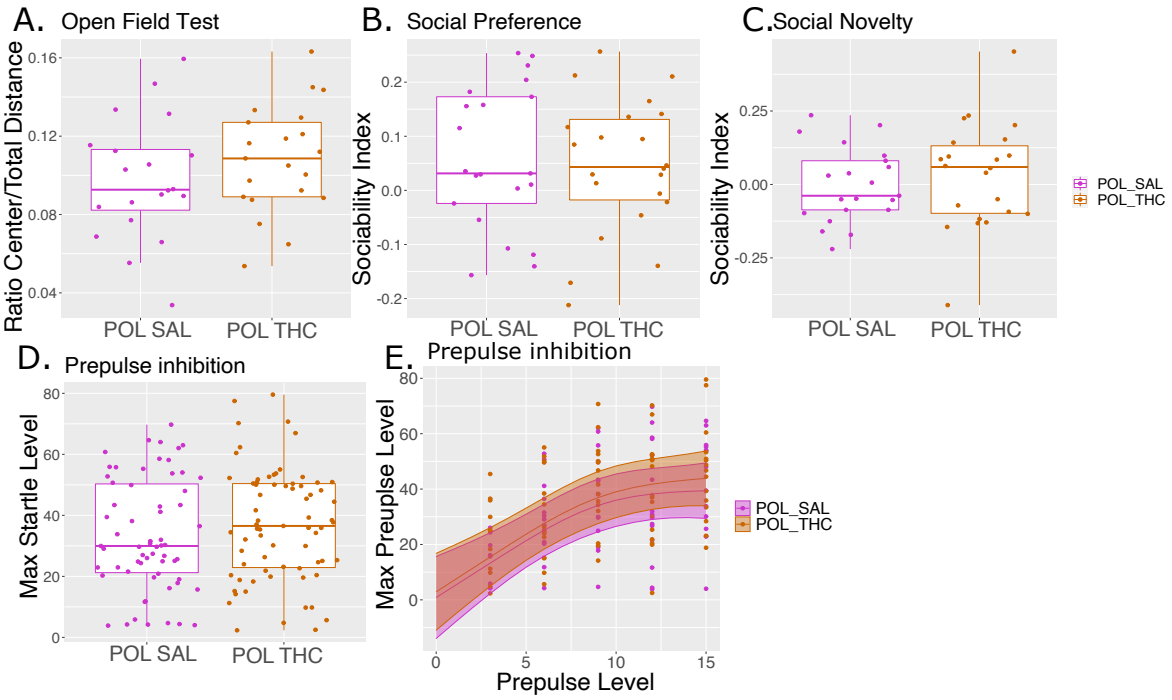
Supplementary Figure 6.S2. Neuroanatomical alteration due to adolescent THC-exposure in MIA-exposed offspring exclusively. **A**. t-statistic map of group (POL THC vs POL SAL) by age (first order natural spline of age) thresholded between 5% FDR (top, t=4.46) and 10% FDR (bottom, t=3.86). **B**. Plot of peak voxels selected from regions of interest highlighted in **A**, wherein age is plotted on the x-axis, and the absolute Jacobian determinants plotted on the y-axis. Trajectories reflect the statistical model used.

6.9.5 Behavioural results in the POL THC relative to POL SAL offspring

Overall, no statistically significant results were observed in our behavioural tests, however a few subthreshold effects were observed, as described in the main text (**3.4**) and in **supplementary table 6.S4** below. No significant differences were observed between POL THC and POL SAL (**Supplementary Figure 6.S3 & table 6.S4**).

Supplementary Table 6.S3. Summary of all behavioural results for all group comparisons. T-values, p-values (uncorrected) and q-values (corrected) are bolded if they survive Bonferroni correction (q-value derived from Bonferroni corrected p<0.0125).

	POL SAL vs. SAL SAL	**SAL THC vs. SAL SAL**	**POL THC vs. SAL SAL**	**POL THC vs. POL SAL**
OFT	t=0.943, p=0.361, q=1.000	t=1.559, p=0.129, q=0.516	t=2.344, p=0.041, q=0.164	t=1.070, p=0.292, q=1.000
SOPT	t=-0.496, p=0.632, q=1.000	t=-1.217, p=0.233, q=0.932	t=-0.977, p=0.335, q=1.000	t=-0.345, p=0.732, q=1.000
SONT	t=-1.990, p=0.054, q=0.216	t=-0.017, p=0.9866, q=1.000	t=-0.937, p=0.355, q=1.000	t=0.733, p=0.468, q=1.000
PPI	**Overall:** t=-0.148, p= 0.885, q=1.000 **By PP tone (ns(level, 2)1:group)** t=-0.560, p=0.577, q=1.000	**Overall:** t=-0.013, p=0.990, q=1.000 **By PP tone (ns(level, 2)1:group)** t=1.268, p=0.208, q=0.832	**Overall:** t=0.091, p=0.930, q=1.000 **By PP tone (ns(level, 2)1:group)** t=2.239, p=0.027, q=0.108	**Overall:** t=-0.370 , p=0.714, q=1.000 **By PP tone (ns(level, 2)1:group)** t=0.177, p=0.860, q=1.000

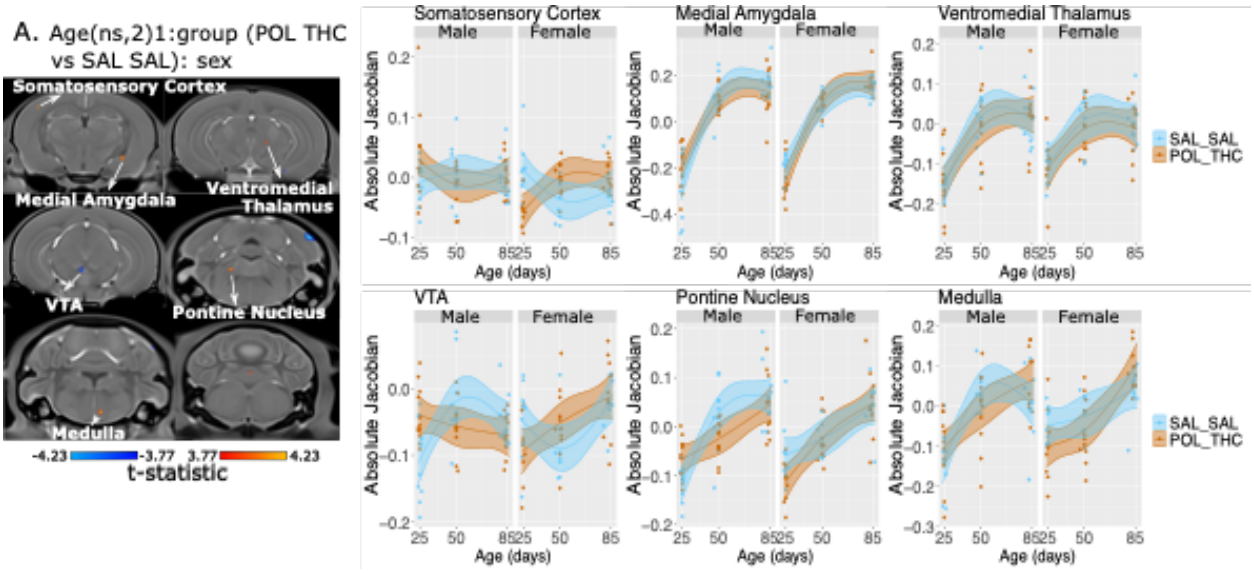


Supplementary Figure 6.S3. No behavioural alterations due to adolescent THC-exposure in MIA-exposed offspring exclusively. Behavioural results for treatment groups: POL SAL (magenta), POL THC (orange). For all boxplots the midline represents the median of the data, the box represents the interquartile range, with whiskers denoting the full range of the data. No significant effects for any groups on the distance traveled in the center zone relative to the total distance traveled (**A**), social preference (**B**) or social novelty tasks (**C**), nor in prepulse inhibition, based on the maximum startle amplitude overall (**D**), nor for increasing prepulse level (**E**)**.**

6.9.6. Investigating sex differences

6.9.6.1 Sex differences in response to both prenatal MIA-exposure and adolescent THC exposure

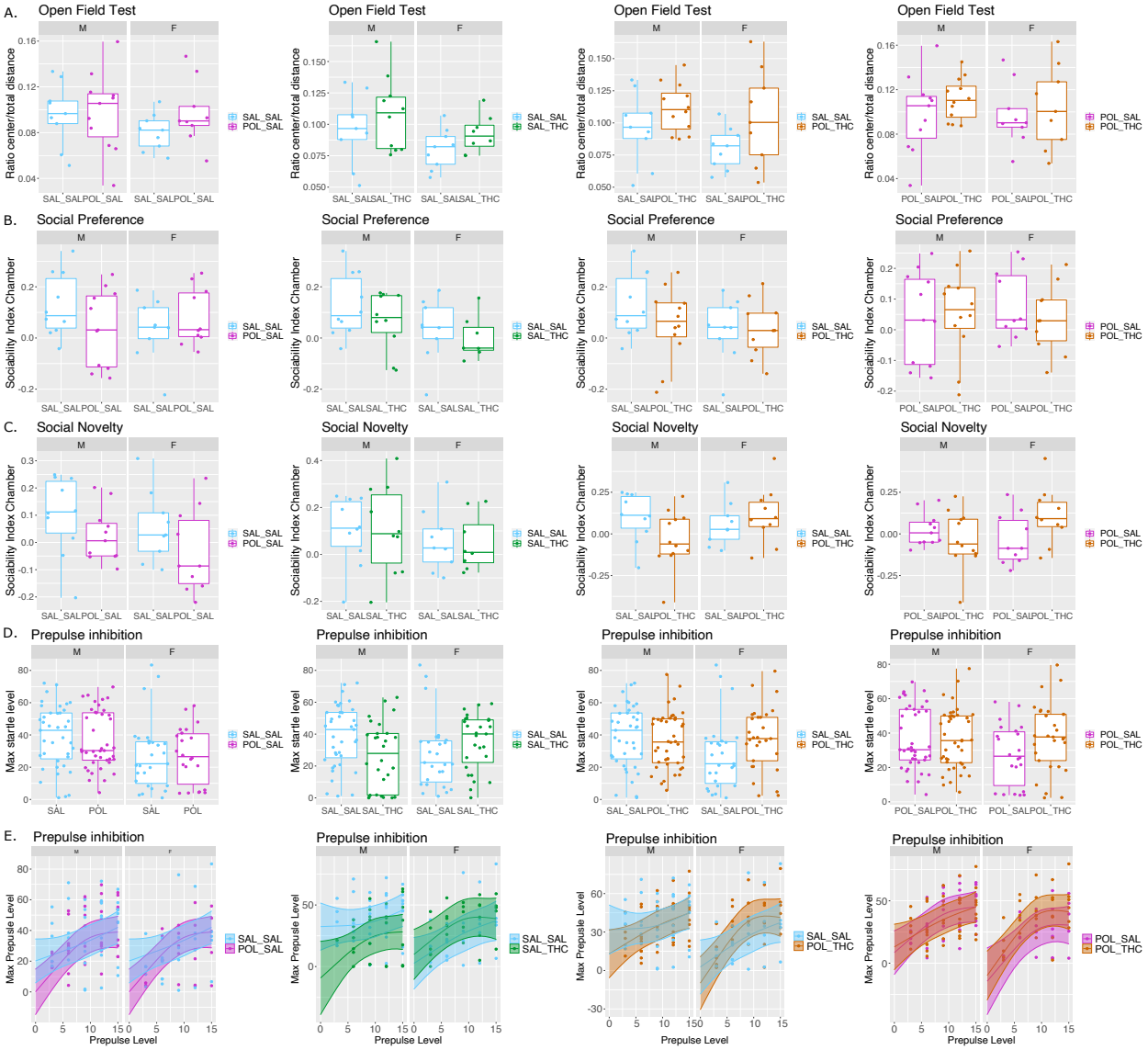
Post-hoc investigation of sex differences in the POL THC group relative to the SAL SAL group revealed a significant three way interaction (group : ns(age,2)1 : sex) (t=4.238, 5%FDR) (**supplementary figure 6.S4**). In the somatosensory cortex, medial amygdala, ventromedial thalamus, ventral tegmental area (VTA), pontine nucleus, and medulla, male POL THC offspring had a flatter trajectory, with smaller volumes in the PND 50-85 period relative to SAL SAL, whereas the opposite pattern was observed in females in all regions except for the medial amygdala and pontine nucleus, where female curves between POL THC and SAL SAL offspring were very similar.



Supplementary Figure 6.S4. Sex differences in neuroanatomical alteration due to combined prenatal MIA-exposure and adolescent THC-exposure. **A**. t-statistic map of group (SAL THC vs SAL SAL) by age (first order natural spline of age) by sex thresholded between 5% FDR (top, t=4.23) and 10% FDR (bottom, t=3.77). **B**. Plot of peak voxels selected from regions of interest highlighted in **A**, wherein age is plotted on the x-axis, and the absolute Jacobian determinants plotted on the y-axis. Trajectories reflect the statistical model used.

6.9.6.2 No significant sex differences in behaviour following prenatal MIA-exposure and adolescent THC exposure

Post-hoc investigation of sex differences in this group revealed no significant sex-by-group interactions surviving multiple comparisons correction. A subthreshold sex-by-group interaction (SAL THC vs SAL SAL) was observed in PPI, over increasing prepulse tone (t=2.332, p=0.022, q=0.088), wherein SAL THC males showed impairment relative to SAL SAL males, while SAL THC females performed very slightly better than SAL SAL females (**Supplementary Figure 6.S5 & Supplementary Table 6.S4**).



Supplementary Figure 6.S5 (caption continues on next page)**.** Sex differences in behaviour due to prenatal MIA-exposure and/or adolescent THC exposure do not affect adult behaviour. Behavioural results for all treatment groups: SAL SAL (cyan), POL SAL (magenta), SAL THC (green), POL THC (orange). For each boxplot, males (M) are on the left, and females (F) on the right; the midline represents the median of the data, the box represents the interquartile range, with whiskers denoting the full range of the data. No significant effects for any groups on the distance traveled in the center zone relative to the total distance traveled **(A)**. No statistically significant differences were observed in the social preference (**B**) or social novelty (**C**) tasks for any of the groups, although a subthreshold sex-by-group (POL THC vs POL SAL) interaction was observed in social novelty (t= 2.194, p=0.034, q=0.136) wherein POL SAL females showed less preference for the novel mouse. No overall differences in prepulse inhibition, based on the maximum startle amplitude were observed (**D**) apart from a subthreshold sex-by-group (POL THC vs POL SAL) interaction (t= 2.194, p=0.034, q=0.136) wherein POL SAL females showed impairment in PPI.

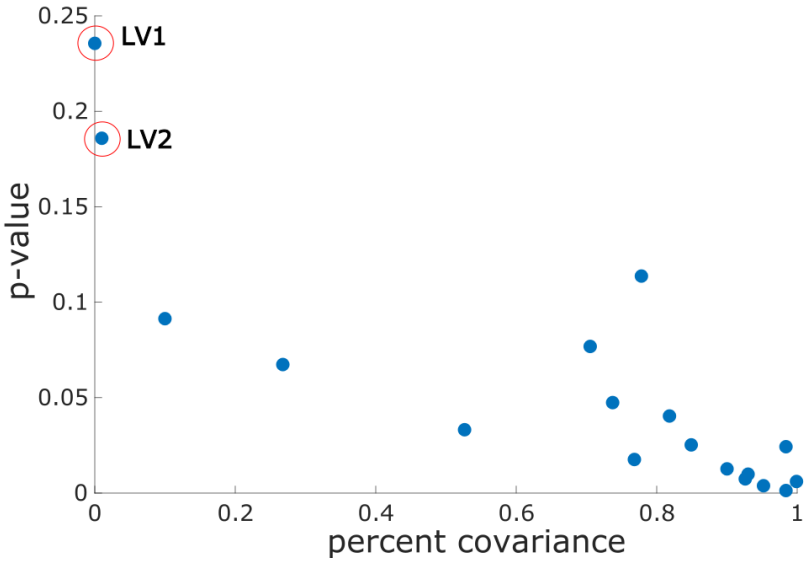
Supplementary Figure 6.S5 (continued). Similarly, no group differences were observed over increasing prepulse told, apart from a subthreshold group-by-sex-by-prepulse tone interaction (POL THC vs SAL SAL: sex : ns(level, 2)1:group:sex; t=2.332, p=0.022, q=0.088) wherein females showed improvement in PPI for louder prepulse tones (**E**).

Supplementary Table 6.S4. Summary of all behavioural results for all group by sex interactions. T-values, p-values (uncorrected) and q-values (corrected) are bolded if they survive Bonferroni correction (q-value=p<0.0125).

	POL SAL vs. SAL SAL : sex	**SAL THC vs. SAL SAL : sex**	**POL THC vs. SAL SAL : sex**	**POL SAL vs. POL THC : sex**
OFT	t=0.919, p=0.366, q=1.000	t=-0.005, p=0.996, q=1.000	t=0.529, p=0.601, q=1.000	t=-0.400, p=0.692, q=1.000
SOPT	t=1.678, p=0.105, q=0.42	t=0.370, p=0.714, q=1.000	t=0.952, p=0.347, q=1.000	t=-0.781, p=0.440, q=1.000
SONT	t=-0.201, p=0.842, q=1.000	t=-0.133, p=0.895, q=1.000	t=1.886, p=0.067, q=0.268	t= 2.194, p=0.034, q=0.136
PPI	**Overall:** t=0.169, p=0.867, q=1.000 **By PP tone (ns(level, 2)1:group:sex)** t=-0.560, p=0.577, q=1.000	**Overall:** t= 1.841, p=0.079, q=0.316 **By PP tone (ns(level, 2)1:group:sex)** t=-1.331, p=0.187, q=0.748	**Overall:** t=1.458, p=0.162, q=0.648 **By PP tone (ns(level, 2)1:group:sex)** t=2.332, p=0.022, q=0.088	**Overall:** t=1.751, p=0.092, q=0.368 **By PP tone (ns(level, 2)1:group:sex)** t=0.723, p=0.471, q=1.000

6.9.7 PLS of within subject volume change - behaviour

PLS was used to examine within subject brain-wide voxel-wise volume change from the post-treatment timepoint (PND 50) to the adult timepoint (PND 85) and the same 18 behavioural and demographics metrics as described in **6.4.6**. We identified two significant LV (LV1: p<0.00001, %covariance=24%; LV2: p=0.01, %covariance=19%; **supplementary figure 6.S6)**.



Supplementary Figure 6.S6. Covariance explained (y-axis) and permutation p-values (x-axis) for all 18 LVs in the PLS analysis based on the difference in volume from PND 50 to 85, and behaviour. LV1 and LV2 are circled in red (LV1: $p<0.00001$, %covariance=24%; LV2: $p=0.01$, %covariance=19%).

References

Abazyan, Bagrat, Jun Nomura, Geetha Kannan, Koko Ishizuka, Kellie L. Tamashiro, Frederick Nucifora, Vladimir Pogorelov, et al. 2010. "Prenatal Interaction of Mutant DISC1 and Immune Activation Produces Adult Psychopathology." *Biological Psychiatry* 68 (12): 1172–81.

Ali, Syed, and Frederico Pereira. 2017. "Dopamine: Neuropsychiatric Disorders and Neurotoxicity." *Toxicology Letters*. https://doi.org/10.1016/j.toxlet.2017.07.156.

Arseneault, Louise, Mary Cannon, Richie Poulton, Robin Murray, Avshalom Caspi, and Terrie E. Moffitt. 2002. "Cannabis Use in Adolescence and Risk for Adult Psychosis: Longitudinal Prospective Study." *BMJ* 325 (7374): 1212–13.

Avants, Brian B., Nicholas J. Tustison, Gang Song, Philip A. Cook, Arno Klein, and James C. Gee. 2011. "A Reproducible Evaluation of ANTs Similarity Metric Performance in Brain Image Registration." *NeuroImage* 54 (3): 2033–44.

Battistella, Giovanni, Eleonora Fornari, Jean-Marie Annoni, Haithem Chtioui, Kim Dao, Marie Fabritius, Bernard Favrat, Jean-Frédéric Mall, Philippe Maeder, and Christian Giroud. 2014. "Long-Term Effects of Cannabis on Brain Structure." *Neuropsychopharmacology: Official Publication of the American College of Neuropsychopharmacology* 39 (9): 2041–48.

Benjamini, Yoav, and Yosef Hochberg. 1995. "Controlling the False Discovery Rate: A Practical and Powerful Approach to Multiple Testing." *Journal of the Royal Statistical Society. Series B, Statistical Methodology* 57 (1): 289–300.

Biscaia, Miguel, Susana Marín, Beatriz Fernández, Eva M. Marco, Marina Rubio, Carmen Guaza, Emilio Ambrosio, and Maria Paz Viveros. 2003. "Chronic Treatment with CP 55,940 during the Peri-Adolescent Period Differentially Affects the Behavioural Responses of Male and Female Rats in Adulthood." *Psychopharmacology* 170 (3): 301–8.

Boksa, Patricia. 2010. "Effects of Prenatal Infection on Brain Development and Behavior: A Review of Findings from Animal Models." *Brain, Behavior, and Immunity* 24 (6): 881–97.

Brown, Alan S., and Urs Meyer. 2018. "Maternal Immune Activation and Neuropsychiatric Illness: A Translational Research Perspective." *The American Journal of Psychiatry* 175 (11): 1073–83.

Campolongo, Patrizia, and Viviana Trezza. 2012. "The Endocannabinoid System: A Key Modulator of Emotions and Cognition." *Frontiers in Behavioral Neuroscience* 6 (November): 73.

Campolongo, Patrizia, Viviana Trezza, Patrizia Ratano, Maura Palmery, and Vincenzo Cuomo. 2011. "Developmental Consequences of Perinatal Cannabis Exposure: Behavioral and Neuroendocrine Effects in Adult Rodents." *Psychopharmacology* 214 (1): 5–15.

Chung, M. K., K. J. Worsley, T. Paus, C. Cherif, D. L. Collins, J. N. Giedd, J. L. Rapoport, and A. C. Evans. 2001. "A Unified Statistical Approach to Deformation-Based Morphometry." *NeuroImage* 14 (3): 595–606.

Clancy, Barbara, Barbara L. Finlay, Richard B. Darlington, and K. J. S. Anand. 2007. "Extrapolating Brain Development from Experimental Species to Humans." *Neurotoxicology* 28 (5): 931–37.

Crawley, Jacqueline N. 2007. *What's Wrong With My Mouse?: Behavioral Phenotyping of Transgenic and Knockout Mice*. John Wiley & Sons.

Crum, William R., Stephen J. Sawiak, Winfred Chege, Jonathan D. Cooper, Steven C. R.

Williams, and Anthony C. Vernon. 2017. "Evolution of Structural Abnormalities in the Rat Brain Following in Utero Exposure to Maternal Immune Activation: A Longitudinal in Vivo MRI Study." *Brain, Behavior, and Immunity* 63 (July): 50–59.

Dalton, Victoria S., Mathieu Verdurand, Adam Walker, Deborah M. Hodgson, and Katerina Zavitsanou. 2012. "Synergistic Effect between Maternal Infection and Adolescent Cannabinoid Exposure on Serotonin 5HT1A Receptor Binding in the Hippocampus: Testing the 'Two Hit' Hypothesis for the Development of Schizophrenia." *ISRN Psychiatry*. https://doi.org/10.5402/2012/451865.

Daskalakis, Anastasios A., Reza Zomorrodi, Daniel M. Blumberger, and Tarek K. Rajji. 2020. "Evidence for Prefrontal Cortex Hypofunctioning in Schizophrenia through Somatosensory Evoked Potentials." *Schizophrenia Research* 215 (January): 197–203.

Daubert, Elizabeth A., and Barry G. Condron. 2010. "Serotonin: A Regulator of Neuronal Morphology and Circuitry." *Trends in Neurosciences* 33 (9): 424–34.

DiNieri, Jennifer A., and Yasmin L. Hurd. 2012. "Rat Models of Prenatal and Adolescent Cannabis Exposure." In *Psychiatric Disorders: Methods and Protocols*, edited by Firas H. Kobeissy, 231–42. Totowa, NJ: Humana Press.

Eede, Matthijs C. van, Jan Scholz, M. Mallar Chakravarty, R. Mark Henkelman, and Jason P. Lerch. 2013. "Mapping Registration Sensitivity in MR Mouse Brain Images." *NeuroImage* 82 (November): 226–36.

Estes, Myka L., and A. Kimberley McAllister. 2016. "Maternal Immune Activation: Implications for Neuropsychiatric Disorders." *Science* 353 (6301): 772–77.

French, Leon, Courtney Gray, Gabriel Leonard, Michel Perron, G. Bruce Pike, Louis Richer, Jean R. Séguin, et al. 2015. "Early Cannabis Use, Polygenic Risk Score for Schizophrenia and Brain Maturation in Adolescence." *JAMA Psychiatry* 72 (10): 1002–11.

Friedel, Miriam, Matthijs C. van Eede, Jon Pipitone, M. Mallar Chakravarty, and Jason P. Lerch. 2014. "Pydpiper: A Flexible Toolkit for Constructing Novel Registration Pipelines." *Frontiers in Neuroinformatics* 8 (July): 67.

Gallino, Daniel, Gabriel A. Devenyi, Jürgen Germann, Elisa Guma, Chloe Anastassiadis, and M. Mallar Chakravarty. 2019. "Longitudinal Assessment of the Neuroanatomical Consequences of Deep Brain Stimulation: Application of Fornical DBS in an Alzheimer's Mouse Model." *Brain Research* 1715 (July): 213–23.

Ginovart, Nathalie, Benjamin B. Tournier, Marcelle Moulin-Sallanon, Thierry Steimer, Vicente Ibanez, and Philippe Millet. 2012. "Chronic Δ9-Tetrahydrocannabinol Exposure Induces a Sensitization of Dopamine D2/3 Receptors in the Mesoaccumbens and Nigrostriatal Systems." *Neuropsychopharmacology*. https://doi.org/10.1038/npp.2012.91.

Giovanoli, S., H. Engler, A. Engler, J. Richetto, M. Voget, R. Willi, C. Winter, et al. 2013. "Stress in Puberty Unmasks Latent Neuropathological Consequences of Prenatal Immune Activation in Mice." *Science*. https://doi.org/10.1126/science.1228261.

Gleason, K. A., S. G. Birnbaum, A. Shukla, and S. Ghose. 2012. "Susceptibility of the Adolescent Brain to Cannabinoids: Long-Term Hippocampal Effects and Relevance to Schizophrenia." *Translational Psychiatry* 2 (November): e199.

Gogos, Andrea, Alyssa Sbisa, Diede Witkamp, and Maarten van den Buuse. 2020. "Sex Differences in the Effect of Maternal Immune Activation on Cognitive and Psychosis-like Behaviour in Long Evans Rats." *The European Journal of Neuroscience* 52 (1): 2614–26.

Gomes, Felipe V., Francisco S. Guimarães, and Anthony A. Grace. 2014. "Effects of Pubertal Cannabinoid Administration on Attentional Set-Shifting and Dopaminergic Hyper-

Responsivity in a Developmental Disruption Model of Schizophrenia." *The International Journal of Neuropsychopharmacology / Official Scientific Journal of the Collegium Internationale Neuropsychopharmacologicum* 18 (2). https://doi.org/10.1093/ijnp/pyu018.

González, S., T. Bisogno, T. Wenger, J. Manzanares, A. Milone, F. Berrendero, V. Di Marzo, J. A. Ramos, and J. J. Fernández-Ruiz. 2000. "Sex Steroid Influence on Cannabinoid CB1 Receptor mRNA and Endocannabinoid Levels in the Anterior Pituitary Gland." *Biochemical and Biophysical Research Communications*. https://doi.org/10.1006/bbrc.2000.2406.

Grace, Anthony A., K. M. Fox, W. J. Lipski, O. Valenti, M. M. Behrens, and D. J. Lodge. 2010. "STRESS AND THE HIPPOCAMPUS SUBICULUM: KEY SITE FOR INTERVENTION IN THE PREVENTION AND TREATMENT OF DOPAMINE HYPER-RESPONSIVITY IN PSYCHOSIS." *Schizophrenia Research*. https://doi.org/10.1016/j.schres.2010.02.158.

Guma, Elisa, Pedro do Couto Bordignon, Gabriel A. Devenyi, Daniel Gallino, Chloe Anastassiadis, Vedrana Cvetkovska, Amadou Barry, et al. 2021. "Early or Late Gestational Exposure to Maternal Immune Activation Alters Neurodevelopmental Trajectories in Mice: An Integrated Neuroimaging, Behavioural, and Transcriptional Study." *Biological Psychiatry*, March. https://doi.org/10.1016/j.biopsych.2021.03.017.

Guma, Elisa, Eric Plitman, and M. Mallar Chakravarty. 2019. "The Role of Maternal Immune Activation in Altering the Neurodevelopmental Trajectories of Offspring: A Translational Review of Neuroimaging Studies with Implications for Autism Spectrum Disorder and Schizophrenia." *Neuroscience and Biobehavioral Reviews*. https://www.sciencedirect.com/science/article/pii/S0149763419302088.

Guma, Elisa, Jill Rocchetti, Gabriel A. Devenyi, Arnaud Tanti, Axel Mathieu, Jason P. Lerch, Guillaume Elgbeili, et al. 2018. "Regional Brain Volume Changes Following Chronic Antipsychotic Administration Are Mediated by the Dopamine D2 Receptor." *NeuroImage* 176 (April): 226–38.

Gumusoglu, Serena B., and Hanna E. Stevens. 2019. "Maternal Inflammation and Neurodevelopmental Programming: A Review of Preclinical Outcomes and Implications for Translational Psychiatry." *Biological Psychiatry* 85 (2): 107–21.

Haddad, Faraj L., Salonee V. Patel, and Susanne Schmid. 2020. "Maternal Immune Activation by Poly I:C as a Preclinical Model for Neurodevelopmental Disorders: A Focus on Autism and Schizophrenia." *Neuroscience and Biobehavioral Reviews* 113 (June): 546–67.

Haida, Obelia, Tareq Al Sagheer, Anais Balbous, Maureen Francheteau, Emmanuel Matas, Federico Soria, Pierre Olivier Fernagut, and Mohamed Jaber. 2019. "Sex-Dependent Behavioral Deficits and Neuropathology in a Maternal Immune Activation Model of Autism." *Translational Psychiatry* 9 (1): 124.

Harkany, Tibor, Manuel Guzmán, Ismael Galve-Roperh, Paul Berghuis, Lakshmi A. Devi, and Ken Mackie. 2007. "The Emerging Functions of Endocannabinoid Signaling during CNS Development." *Trends in Pharmacological Sciences* 28 (2): 83–92.

Hollins, Sharon L., Katerina Zavitsanou, Frederick Rohan Walker, and Murray J. Cairns. 2016. "Alteration of Transcriptional Networks in the Entorhinal Cortex after Maternal Immune Activation and Adolescent Cannabinoid Exposure." *Brain, Behavior, and Immunity* 56 (August): 187–96.

Hollins, S. L., K. Zavitsanou, F. R. Walker, and M. J. Cairns. 2014. "Alteration of Imprinted Dlk1-Dio3 miRNA Cluster Expression in the Entorhinal Cortex Induced by Maternal Immune Activation and Adolescent Cannabinoid Exposure." *Translational Psychiatry* 4

(September): e452.
Hui, Chin Wai, Haley A. Vecchiarelli, Étienne Gervais, Xiao Luo, Félix Michaud, Lisa Scheefhals, Kanchan Bisht, Kaushik Sharma, Lisa Topolnik, and Marie-Ève Tremblay. 2020. "Sex Differences of Microglia and Synapses in the Hippocampal Dentate Gyrus of Adult Mouse Offspring Exposed to Maternal Immune Activation." *Frontiers in Cellular Neuroscience* 14 (October): 558181.
Keshavan, Matcheri S., Jay Giedd, Jennifer Y. F. Lau, David A. Lewis, and Tomáš Paus. 2014. "Changes in the Adolescent Brain and the Pathophysiology of Psychotic Disorders." *The Lancet. Psychiatry* 1 (7): 549–58.
Khan, Sheraz, Konstantinos Michmizos, Mark Tommerdahl, Santosh Ganesan, Manfred G. Kitzbichler, Manuel Zetino, Keri-Lee A. Garel, Martha R. Herbert, Matti S. Hämäläinen, and Tal Kenet. 2015. "Somatosensory Cortex Functional Connectivity Abnormalities in Autism Show Opposite Trends, Depending on Direction and Spatial Scale." *Brain: A Journal of Neurology* 138 (Pt 5): 1394–1409.
Kohn, Laurence, France Kittel, and Danielle Piette. 2004. "Peer, Family Integration and Other Determinants of Cannabis Use among Teenagers." *International Journal of Adolescent Medicine and Health* 16 (4): 359–70.
Kong, Vincent, Gabriel A. Devenyi, Daniel Gallino, Gülebru Ayranci, Jürgen Germann, Colleen Rollins, and M. Mallar Chakravarty. 2018. "Early-in-Life Neuroanatomical and Behavioural Trajectories in a Triple Transgenic Model of Alzheimer's Disease." *Brain Structure & Function*, June. https://doi.org/10.1007/s00429-018-1691-4.
Kreitz, Silke, Alice Zambon, Marianne Ronovsky, Lubos Budinsky, Thomas H. Helbich, Spyros Sideromenos, Claudiu Ivan, et al. 2020. "Maternal Immune Activation during Pregnancy Impacts on Brain Structure and Function in the Adult Offspring." *Brain, Behavior, and Immunity* 83 (January): 56–67.
Langen, Marieke, Dienke Bos, Siri D. S. Noordermeer, Hilde Nederveen, Herman van Engeland, and Sarah Durston. 2014. "Changes in the Development of Striatum Are Involved in Repetitive Behavior in Autism." *Biological Psychiatry* 76 (5): 405–11.
Lecca, Salvatore, Antonio Luchicchi, Maria Scherma, Paola Fadda, Anna Lisa Muntoni, and Marco Pistis. 2019. "Δ9-Tetrahydrocannabinol During Adolescence Attenuates Disruption of Dopamine Function Induced in Rats by Maternal Immune Activation." *Frontiers in Behavioral Neuroscience* 13 (September): 202.
Lee, Younga H., Sara Cherkerzian, Larry J. Seidman, George D. Papandonatos, David A. Savitz, Ming T. Tsuang, Jill M. Goldstein, and Stephen L. Buka. 2020. "Maternal Bacterial Infection During Pregnancy and Offspring Risk of Psychotic Disorders: Variation by Severity of Infection and Offspring Sex." *The American Journal of Psychiatry* 177 (1): 66–75.
Lerch, Jason P., Lisa Gazdzinski, Jürgen Germann, John G. Sled, R. Mark Henkelman, and Brian J. Nieman. 2012. "Wanted Dead or Alive? The Tradeoff between in-Vivo versus Ex-Vivo MR Brain Imaging in the Mouse." *Frontiers in Neuroinformatics* 6 (March): 6.
Lipina, Tatiana V., Clement Zai, Daniela Hlousek, John C. Roder, and Albert H. C. Wong. 2013. "Maternal Immune Activation during Gestation Interacts with Disc1 Point Mutation to Exacerbate Schizophrenia-Related Behaviors in Mice." *The Journal of Neuroscience: The Official Journal of the Society for Neuroscience* 33 (18): 7654–66.
McIntosh, Anthony Randal, and Nancy J. Lobaugh. 2004. "Partial Least Squares Analysis of Neuroimaging Data: Applications and Advances." *NeuroImage* 23 Suppl 1: S250–63.

McIntosh, Anthony R., and Bratislav Mišić. 2013. "Multivariate Statistical Analyses for Neuroimaging Data." *Annual Review of Psychology* 64: 499–525.

Meyer, Urs. 2014. "Prenatal poly(i:C) Exposure and Other Developmental Immune Activation Models in Rodent Systems." *Biological Psychiatry* 75 (4): 307–15.

Miller, Michael L., Benjamin Chadwick, Dara L. Dickstein, Immanuel Purushothaman, Gabor Egervari, Tanni Rahman, Chloe Tessereau, et al. 2019. "Adolescent Exposure to Δ9-Tetrahydrocannabinol Alters the Transcriptional Trajectory and Dendritic Architecture of Prefrontal Pyramidal Neurons." *Molecular Psychiatry*. https://doi.org/10.1038/s41380-018-0243-x.

Monk, Catherine, Claudia Lugo-Candelas, and Caroline Trumpff. 2019. "Prenatal Developmental Origins of Future Psychopathology: Mechanisms and Pathways." *Annual Review of Clinical Psychology* 15 (May): 317–44.

Os, Jim van, Gunter Kenis, and Bart P. F. Rutten. 2010. "The Environment and Schizophrenia." *Nature* 468 (7321): 203–12.

Patel, Raihaan, Christopher J. Steele, Anthony G. X. Chen, Sejal Patel, Gabriel A. Devenyi, Jürgen Germann, Christine L. Tardif, and M. Mallar Chakravarty. 2020. "Investigating Microstructural Variation in the Human Hippocampus Using Non-Negative Matrix Factorization." *NeuroImage* 207 (February): 116348.

Piontkewitz, Yael, Michal Arad, and Ina Weiner. 2011. "Abnormal Trajectories of Neurodevelopment and Behavior Following in Utero Insult in the Rat." *Biological Psychiatry* 70 (9): 842–51.

Yael Piontkewitz, Michal Arad, Ina Weiner. 2012. "Tracing the Development of Psychosis and Its Prevention: What Can Be Learned from Animal Models." *Neuropharmacology* 62 (3): 1273–89.

Rapoport, J. L., A. M. Addington, S. Frangou, and M. R. C. Psych. 2005. "The Neurodevelopmental Model of Schizophrenia: Update 2005." *Molecular Psychiatry* 10 (5): 434–49.

Rapoport, J. L., J. N. Giedd, and N. Gogtay. 2012. "Neurodevelopmental Model of Schizophrenia: Update 2012." *Molecular Psychiatry* 17 (12): 1228–38.

Reisinger, Sonali, Deeba Khan, Eryan Kong, Angelika Berger, Arnold Pollak, and Daniela D. Pollak. 2015. "The Poly(I:C)-Induced Maternal Immune Activation Model in Preclinical Neuropsychiatric Drug Discovery." *Pharmacology & Therapeutics* 149 (May): 213–26.

Renard, Justine, Hanna J. Szkudlarek, Cecilia P. Kramar, Christina E. L. Jobson, Kyra Moura, Walter J. Rushlow, and Steven R. Laviolette. 2017. "Adolescent THC Exposure Causes Enduring Prefrontal Cortical Disruption of GABAergic Inhibition and Dysregulation of Sub-Cortical Dopamine Function." *Scientific Reports* 7 (1): 11420.

Rollins, Colleen P. E., Daniel Gallino, Vincent Kong, Gülebru Ayranci, Gabriel A. Devenyi, Jürgen Germann, and M. Mallar Chakravarty. 2019. "Contributions of a High-Fat Diet to Alzheimer's Disease-Related Decline: A Longitudinal Behavioural and Structural Neuroimaging Study in Mouse Models." *NeuroImage: Clinical* 21 (January): 101606.

Rubino, Tiziana, Natalia Realini, Daniela Braida, Sandra Guidi, Valeria Capurro, Daniela Viganò, Cinzia Guidali, et al. 2009. "Changes in Hippocampal Morphology and Neuroplasticity Induced by Adolescent THC Treatment Are Associated with Cognitive Impairment in Adulthood." *Hippocampus* 19 (8): 763–72.

Rubino, Tiziana, Daniela Vigano', Natalia Realini, Cinzia Guidali, Daniela Braida, Valeria Capurro, Chiara Castiglioni, et al. 2008. "Chronic Delta 9-Tetrahydrocannabinol during

Adolescence Provokes Sex-Dependent Changes in the Emotional Profile in Adult Rats: Behavioral and Biochemical Correlates." *Neuropsychopharmacology: Official Publication of the American College of Neuropsychopharmacology* 33 (11): 2760–71.

Schobel, Scott A., Nashid H. Chaudhury, Usman A. Khan, Beatriz Paniagua, Martin A. Styner, Iris Asllani, Benjamin P. Inbar, et al. 2013. "Imaging Patients with Psychosis and a Mouse Model Establishes a Spreading Pattern of Hippocampal Dysfunction and Implicates Glutamate as a Driver." *Neuron* 78 (1): 81–93.

Semple, Bridgette D., Klas Blomgren, Kayleen Gimlin, Donna M. Ferriero, and Linda J. Noble-Haeusslein. 2013. "Brain Development in Rodents and Humans: Identifying Benchmarks of Maturation and Vulnerability to Injury across Species." *Progress in Neurobiology*. https://doi.org/10.1016/j.pneurobio.2013.04.001.

Shah, Jai L., M. Mallar Chakravarty, Ridha Joober, and Martin Lepage. 2016. "Dynamic Endophenotypes and Longitudinal Trajectories: Capturing Changing Aspects of Development in Early Psychosis." *Journal of Psychiatry & Neuroscience: JPN* 41 (3): 148–51.

Tseng, Alan H., and Rebecca M. Craft. 2004. "CB(1) Receptor Mediation of Cannabinoid Behavioral Effects in Male and Female Rats." *Psychopharmacology* 172 (1): 25–30.

Tynon, Marykathryn, Marcellino Porto, and Barry K. Logan. 2015. "Simplified Analysis of 11-Hydroxy-Delta-9-Tetrahydrocannabinol and 11-Carboxy-Delta-9-Tetrahydrocannabinol in Human Meconium: Method Development and Validation." *Journal of Analytical Toxicology* 39 (1): 35–40.

Verdurand, Mathieu, Victoria S. Dalton, Vu Nguyen, Marie-Claude Grégoire, David Zahra, Naomi Wyatt, Leena Burgess, Ivan Greguric, and Katerina Zavitsanou. 2014. "Prenatal Poly I:C Age-Dependently Alters Cannabinoid Type 1 Receptors in Offspring: A Longitudinal Small Animal PET Study Using [(18)F]MK-9470." *Experimental Neurology* 257 (July): 162–69.

Verrico, Christopher D., Hong Gu, Melanie L. Peterson, Allan R. Sampson, and David A. Lewis. 2014. "Repeated Δ9-Tetrahydrocannabinol Exposure in Adolescent Monkeys: Persistent Effects Selective for Spatial Working Memory." *The American Journal of Psychiatry* 171 (4): 416–25.

Volkow, Nora D., James M. Swanson, A. Eden Evins, Lynn E. DeLisi, Madeline H. Meier, Raul Gonzalez, Michael A. P. Bloomfield, H. Valerie Curran, and Ruben Baler. 2016. "Effects of Cannabis Use on Human Behavior, Including Cognition, Motivation, and Psychosis: A Review." *JAMA Psychiatry* 73 (3): 292–97.

Vuillermot, Stéphanie, Eliza Joodmardi, Thomas Perlmann, Sven Ove Ögren, Joram Feldon, and Urs Meyer. 2012. "Prenatal Immune Activation Interacts with Genetic Nurr1 Deficiency in the Development of Attentional Impairments." *The Journal of Neuroscience: The Official Journal of the Society for Neuroscience* 32 (2): 436–51.

Zammit, Stanley, Peter Allebeck, Sven Andreasson, Ingvar Lundberg, and Glyn Lewis. 2002. "Self Reported Cannabis Use as a Risk Factor for Schizophrenia in Swedish Conscripts of 1969: Historical Cohort Study." *BMJ* 325 (7374): 1199.

Zeighami, Yashar, Seyed-Mohammad Fereshtehnejad, Mahsa Dadar, D. Louis Collins, Ronald B. Postuma, Bratislav Mišić, and Alain Dagher. 2019. "A Clinical-Anatomical Signature of Parkinson's Disease Identified with Partial Least Squares and Magnetic Resonance Imaging." *NeuroImage* 190 (April): 69–78.

Chapter 7: Discussion and conclusions

7.1 Summary of results

Exposure to MIA *in utero* is a well-established risk factor associated with a number of neurodevelopmental and neuropsychiatric disorders in offspring. Significant evidence from epidemiological, clinical, and preclinical experimental research confirms this association is due to the increase in maternal pro-inflammatory cytokines, rather than the pathogen itself.

In **Chapter 3**, a systematic review was conducted to evaluate studies of the effects of MIA-exposure on offspring outcomes using neuroimaging methods at the primary assay. This was performed across 5 different species and allowed us to identify a number of gaps in our knowledge. The critical appraisal of the state of the field of this review allowed for the identification of important gaps in our knowledge. These gaps were assessed in the following three chapters. Specifically, they include:

1) Very few studies investigating the effects of MIA exposure longitudinally. Longitudinal studies are critical in determining alterations in neurodevelopmental trajectories and the specific ages at which alterations emerge.
2) There were limited investigations directly comparing the differential effects of exposures at different gestational timepoints. The association between the timing of MIA exposure may indeed be critical given that the maternal immune system goes through large changes during gestation, and that offspring neurodevelopment varies greatly depending on the stage of gestation.
3) There were few studies examining offspring outcomes early in the lifespan and most focused on the adolescent and adult periods, typically with cross-sectional assays.
4) Finally, a shocking minority of studies, particularly in the preclinical research, included female subjects in their analyses. The body of work presented in this thesis aims to address many of these gaps.

In **Chapter 4**, the effects of MIA-exposure in early (GD 9) or late (GD 17) gestation were investigated throughout offspring development, using longitudinal *in vivo* MRI and behavioural

phenotyping. From these univariate analyses, we identified a deviation in neuroanatomical trajectory (relative to controls), which normalized in adulthood. Behavioural development followed the same pattern, where adolescent sensorimotor gating deficits, and increased anxiety-like, and stereotypic behaviours normalized in adulthood. In contrast, offspring exposed to MIA late in gestation had subtle deviations in brain development, with no significant behavioural impairments. These two modalities were integrated in a data-driven multivariate analyses at the age of greatest change (adolescence), which yielded comprehensive brain-behaviour maps describing patterns of brain-behaviour covariation across our groups. Based on these results, we investigated transcriptional phenotypes at the age (adolescence), and in the group where greatest deviations were observed (offspring exposed to MIA early in gestation). These analyses allowed us to link transcriptional alterations of genes involved in FGF signaling, microRNA regulation, autistic behaviours, apoptosis, and inflammatory signaling to patterns of altered brain-behaviour covariation. This work highlights the differential effects of early and late maternal immune activation on brain and behavioural development and shows that early exposure may be a more potent modulator of brain development. Further, we provide a thorough characterization of brain-behaviour covariation and describe how it may relate to a transcriptional phenotype.

The questions addressed in **Chapter 4** were extended in **Chapter 5**, where a detailed characterization of the effects of GD 9 or 17 MIA-exposure was performed on embryo brain anatomy, and neonatal brain anatomy and communicative behaviour. First, we show that the effects of both early and late MIA-exposure on brain volume are already detectable at GD 18. Early exposure resulted in more focal changes in anatomy, tending more towards volume decreases (albeit volume increases were also observed). In contrast, late exposure resulted in very striking volume increases throughout the brain, potentially capturing an acute response to inflammation, or an acceleration in brain development. To gain more mechanistic insight into the volumetric changes, the presence of dark neurons and glial cells was assessed in the dorsal hippocampus (a region in which MIA timing had a differential effect on brain volume, and a region showing neuroanatomical and transcriptional alterations in **Chapter 4**). We found an increase in the presence of these cells in females and a decrease in males exposed to MIA late in gestation. These dark glia are thought to be present in response to an immune stimulus (Bisht et al. 2016). In contrast, both early and late MIA-exposure caused subtle changes in neonate brain anatomy, and a subtle decrease in communication in the early exposed group (as measured by the ultrasonic

vocalization task). Thus, it is possible that the neonatal brain recovers from the effects of MIA-exposure, and that perhaps the changes observed in the embryo brain reflect latent alterations that manifest again later in development, i.e., in adolescence.

Finally, **Chapter 6** addressed how exposure to a second risk factor in the adolescent period might interact with the putative disease priming effects of MIA-exposure, building on the popular two-hit hypothesis proposed to lead to many neuropsychiatric illnesses. We found that either risk factor alone (MIA or adolescent THC exposure) led to subtle alterations in neurodevelopment in offspring. However, more striking deviations were observed in the group of mice exposed to both risk factors indicating that exposure to MIA may potentiate the effects of exposure to risk factors later in life. A similar pattern was observed in anxiety-like behaviours, with the mice exposed to both MIA and THC exhibiting more risk-taking behaviour relative to the other two groups, however, no deficits in sensorimotor gating or social behaviours were observed. Finally, investigation of the association between brain and behaviour yielded two interesting observations. First, the covariance between neuroanatomy and behaviour in the final timepoint revealed an association with cortical size and subtle alterations in sensorimotor gating and social behaviour, more expressed in female mice. Further, the change in brain volume form the post-treatment to the final adult scan revealed more focal patterns of brain behaviour covariation with differential effects in males and females.

In summary, this thesis proposes that exposure to MIA *in utero*, particularly if this exposure occurs in early gestation, leads to a window of altered neuroanatomical trajectories and behaviour in the adolescent and early adult period, which normalize in later adulthood. Further, we identify that neuroanatomical alterations may already be present in the embryo brain, and linked to the presence of highly active, or dark, neurons and glia. The neonatal period appeared to be less vulnerable to the effects of MIA; MIA-exposure did not seem to induce many changes, other than a few subtle neuroanatomical changes and communicative anomalies. Finally, MIA does seem to interact with a second risk factor, adolescent cannabis use, to induce greater alterations in offspring brain anatomy and behaviour, however, only some of the changes persist in later adulthood, which suggests that these are both subtle risk factors for aberrant neurodevelopment.

7.2 Limitations

The MIA model in animals, leveraged in this thesis, has been an essential tool for testing causality and identifying molecular mechanisms (Kentner et al. 2019). However, its utility, and the work presented in this thesis, needs to be considered in the context of a few limitations. The use of any animal model to study human neuropsychiatric disorders, or associated risk factors, is extremely challenging due to the subjective nature of many core symptoms currently used to diagnose the disorders. To further complicate the problem, there are no objective biomarkers that map onto specific symptoms used for diagnosis (Nestler and Hyman 2010). As discussed in the section below, however, the MIA model does meet the current standards of validity, and although there has been significant variability in findings associated with the model (discussed in **7.3**), we propose some ways in which to leverage this in a positive and potentially informative way (section **7.4**).

Another important consideration is that the use of immunogenic substances to mimic maternal infection, such as the double-stranded RNA, poly I:C, used in this work, and the bacterial endotoxin, LPS, activate distinct and specific molecular pathways (covered in detail in section **2.2.3**). Poly I:C mainly targets the TLR3 pathway, and LPS, the TLR4 pathway, and, although they have both been successfully used to model many features of neuropsychiatric illness, they may not recapitulate the full spectrum immune response elicited by exposure to a live pathogen (Reisinger et al. 2015). The first animal models used to investigate MIA directly applied live viruses (influenza virus H1N1); this, arguably, most closely replicates the human situation, although it encompasses its own limitations regarding biosafety and dose control.

Finally, the stages of *in utero* and postnatal brain development differ between humans and mice; most importantly, mice are born at the equivalent of the beginning of the human third trimester (Gumusoglu and Stevens 2019). Thus, the mouse may not be an ideal species for studying the effects of MIA-exposure *in utero* in certain processes such as myelination and synaptogenesis, as they occur postnatally in mice (and prenatally in humans). Furthermore, although there are also differences in parturition biology, such as the type of uterus, litter size, and length of pregnancy (Ratajczak, Fay, and Muglia 2010), there are still many similarities between the two species. Due to these developmental differences, it may be beneficial to rely on key benchmarks of brain development rather than chronological age in order to compare across timelines of development (Semple et al. 2013). Developing better methods for attaining cross species homology may an

important next step; this may be attained by integrating information on gene expression patterns along with the already identified gross morphology or cellular process benchmarks. The importance of cross-spices homology is further discussed in **section 7.6.**

Some methodological limitations should be considered as well. Although the use of multivariate techniques is both useful and promising, as discussed in **Chapters 4, 5, 6**, and below in **section 7.5**, they must be used with some degree of caution as they have a tendency to overfit the data. Some ways in which to evaluate whether overfitting is occurring include attempting to replicate results in a separate dataset or cohort with comparative data and determining whether similar patterns emerge. Additionally, one can perform a cross-validation analysis within the same dataset to determine whether brain-behaviour scores calculated in a training set (i.e., 75% of the data) are correlated with a test set (i.e., the remaining 25% of the data). However, this would require a large sample, and may be a more exploratory approach, as the PLS is not designed to be a predictive analysis.

Additionally, we have focused on voxel-based techniques throughout this thesis, however there are other surface based approaches, which are very commonly used when analyzing human MRI data. These are particularly informative when investigating cortical development, or the shape of specific structures. However, in the mouse brain, the cortex in lissencephalic, which does not lend itself as well to the use of surface-based techniques. The use of voxel-based techniques allows us to evaluate the entire brain and determine both focal and localized changes, as well as more global changes in brain anatomy. In some cases, however, voxel-based approaches may detect differences that are perhaps too focal or localized for strong interpretation. Cluster level or topological inference may be one method to threshold for such small changes.

7.3 Reproducibility of the MIA-model

Developing animal models to study complex human brain diseases, such as those associated with MIA-exposure, poses a major challenge. The MIA model is emerging as a powerful translational tool to explore the effects of prenatal MIA on the developing brain, though the need for a more consistent model is paramount. It is well accepted that no animal model can fully recapitulate the human condition, however, the validity of animal models has historically been determined by: (1) construct validity, i.e. whether there are mechanistic similarities between

the model and clinical condition, (2) face validity, i.e. whether there are similarities in outcomes (ex: behaviours, brain volume) between the model and human disease, and (3) predictive validity, i.e. whether the model response to therapeutic agents used to treat the human disease (Willner 1984; Tordjman et al. 2007; Careaga, Murai, and Bauman 2017; Kentner et al. 2019). The MIA model does meet all three criteria. It has most often been used to model deficits associated with ASD or schizophrenia (given the initial epidemiological evidence linking MIA to these specific disorders); however, evidence suggests that MIA-exposure may be relevant to other neuropsychiatric or CNS disorders (Knuesel et al. 2014). Restricting the scope to a small subset of disorders may limit the utility and relevance of the MIA model. Rather, MIA-exposure should be conceptualized as a "disease primer" which alters the trajectory of fetal brain development and may combine with other risk factors to result in the emergence of full-blown disorders (Estes and McAllister 2016). Further, MIA-models can be used to test numerous behaviours related to neurodevelopmental disorders, which will be discussed more deeply in this chapter (**7.5**).

Given the popularity of the model, and increased use globally, increased variability across different research laboratories has been identified. There is significant heterogeneity of the literature (surveyed in **Chapter 3**) across species, gestational windows, infections/immunogens used, and techniques used. Systematic reviews are an important approach that can help us parse some of the heterogeneity; meta-analyses may also be an informative next step. However, any type of meta-analysis would be difficult to conduct without enforcing a harmonization in methodologies across laboratories. Further, this concept is not necessarily isolated to the MIA model, and may be extended to other environmental or genetic models (Weber-Stadlbauer and Meyer 2019). Many of the factors contributing to increased variability in the outcomes of MIA-exposed offspring include the choice of animal species and associated genetic background, the type of immunogen used, the dose and timing of exposure, molecular weight and batch of immunogen used (particularly relevant to poly I:C (Kowash et al. 2019)), hygiene of the animal facility, and differences in the postnatal environment of the offspring (Weber-Stadlbauer and Meyer 2019; Kentner et al. 2019).

7.4 Harnessing variability

While this increased variability may pose problems for reproducibility, it may also be a source of important information, and potentially a key feature that needs to be better understood. In many models of brain development and models used in psychiatric disorders, clean group and treatment effects are difficult to ascertain. Thus, using this variability as a feature in analyses may be a more useful way to move forward as a means to better understand environmental exposures such as MIA.

Variance in the type of immunogen used allows researchers to explore possible commonalities in pathological effects due to these agents, leading to a better understanding of common and dissimilar effects of MIA relative to specific pathogens (Meyer 2014). Between-litter and within-litter variability for multiparous species has been observed in the MIA literature. Rather than attempting to nullify differences between groups, it may be interesting to investigate this variability by determining which factors make some pregnancies more susceptible than others, such as the intensity of pro-inflammatory cytokine secretion or of maternal temperature decrease due to poly I:C (Mueller et al. 2019) or increase due to fever in the mother (Weber-Stadlbauer and Meyer 2019). Investigating the variability within a litter could provide avenues for understanding factors that may be associated with a differential response MIA-exposure, including placement of the offspring within the womb, litter size, male/female ratio, and more. Additionally, by leveraging multivariate methods, such as those used in this thesis, and data from richly phenotype individuals or mice, one can capture the shared variability across different modalities to better understand individual differences in response to risk factors for neurodevelopmental disorders.

Phenotypic variability is commonly observed across mental illnesses such as schizophrenia and autism, amongst other neurodevelopmental disorders, attributable to contributions for various genetic and environmental risk factors. Previous work by Ellegood and colleagues has leveraged the heterogeneity associated with ASD by characterizing the neuroanatomical phenotypes of 26 genetic mouse models related to the disorder (Ellegood et al. 2015). This allowed the authors to investigate the heterogeneity in brain structure associated with various genetic risk factors and led to the identification of no single unifying overlapping pattern, but rather, a number of distinct neuroanatomical patterns. This approach may be useful in the context of MIA-exposure. The existence of variability in MIA models can provide some insight into the sources of variability observed in human pathology, particularly if it is investigated rather than ignored. Perhaps a

comprehensive analysis within the same study of the overlap or divergence in neuroanatomical or behavioural phenotypes due to gestational timing, immunogen type, dose, and other important factors, may be an exciting avenue for future work.

7.5 The need for cross-modality integration

In order to fully leverage these subtle differences within the MIA models, taking a more integrative approach, such as those proposed throughout this thesis, may be of value. In order to harness this variability, researchers may need to use new methodological approaches and statistical analyses that allow for the identification of differences along dimensions rather than discrete categories. In order to achieve this level of characterization, one cannot rely on one or two modalities, but should integrate across a range of data types to generate translational phenotypes. This is in line with the National Institute of Health-led Research Domain Criteria (RDoC) initiative, which provides a novel framework for psychiatric disorder research that focuses on dimensional classification of genes, neural circuits, and behaviours, rather than on more traditional diagnostic classifications (Cuthbert and Insel 2013). Indeed, receiving a diagnosis for schizophrenia, for example, is highly predictive of all other Axis I and II psychiatric disorders based on diagnostic categories assigned by the Diagnostic Category Manual (DSM) (McMillan et al. 2009), indicating that there is a high degree of overlap between symptoms. Additionally, there is a high degree of overlap across risk factors for psychiatric illness, such as MIA, which indicates that focusing on dimensions rather than categories may be more useful.

Some groups have started to do this; for example, recent work by Muller and colleagues used clustering approaches on a large sample of behaviourally phenotype MIA-offspring and identified subgroups of MIA-offspring that were more or less behaviourally impaired. These subgroups were further found to differ based on transcriptional profiles and structural covariance, possibly linked to differences in inflammatory cytokine profiles (Mueller et al. 2021).

While limited in the preclinical literature, a more integrative approach has been successful in the human literature. Mapping neurobiological abnormalities to specific psychiatric conditions has not been successful given high co-morbidity and variability between disorders. Thus, researchers are moving away from clinical diagnostic categories in order to identify more nuanced biomarkers in psychiatry. Work by Xia and colleagues identified associations between brain

connectivity and four dimensions of psychopathology, mood, psychosis, fear, and externalizing behaviour using sparse canonical correlations analysis, a similar multivariate approach to the PLS analysis that we used (Xia et al. 2018). In doing so, they were able to identify putative neural biomarkers for symptom dimensions, rather than specific psychopathology. Using these types of strategies to identify linked dimensions of brain and behavioural phenotypes may be critical to improving translation from animal models to the clinic.

7.6 The need for better cross-species homology

The majority of MIA models have been developed in rats or mice, however, some have been extended to other species, such as the rabbit, pig, sheep, and rhesus monkey; the latter is both evolutionarily and ethologically more similar to humans (Careaga, Murai, and Bauman 2017). Relying on a single species to gain insight into complex human brain disorders is dangerous, however, each species provides unique advantages. Mice have been favoured in biomedical research for the ability to manipulate their genetics and their low cost. Work in this species has laid the foundation for our understanding of how MIA-exposure affects brain development (Brown and Meyer 2018). Rats provide many of the benefits of mice, however they have more complex brains and social behaviours, which allow for more nuanced investigation of potential MIA-induced alterations (Careaga, Murai, and Bauman 2017).

Modeling MIA in nonhuman primates provides more similarities for comparison of MIA-induced pathology across placental physiology, gestational timelines, brain development pre- and postnatally, and brain architecture (Bauman and Schumann 2018). Furthermore, they live in large groups, engaging in more complex social behaviours, which may be lacking and harder to study in rodent models. This may provide a better bridge between the rodent models and human neurodevelopmental disorders, enhancing cross-species translation. Identifying homologies across species may also provide guidance for preclinical research in order to focus efforts on investigating behaviours and biological outcomes that may be more evolutionarily conserved (Stewart and Kalueff 2015). However, one must consider the costs and ethical constraints associated with nonhuman primate research (Careaga, Murai, and Bauman 2017).

In order to appropriately translate between animal models and humans, some quantitative strategies should be implemented. *In vivo* neuroimaging is one of few non-invasive methods for

studying the living human brain. Several hallmark features of neurodevelopmental disorders have been identified using MRI in humans (Shaw et al. 2008; Gogtay et al. 2004; Lerch et al. 2017). However, these tools are often limited to coarse macroscopic measures (Barron et al. 2021). Non-invasive MRI techniques provide an exciting avenue through which to achieve cross-species homology. In addition to its utility in studying the living human brain, recent advances in ultra-high-field imaging, and the use of cryogenically-cooled coils allow for this modality to be applied to the study of the living rodent and nonhuman primate brains ensuring sufficient signal-to-noise and resolution (Ratering et al. 2008). Comparable signals can be obtained across species, which can allow for the identification of both preserved homology across dramatic differences in size, as well as unique connections and regions within each species (Rilling 2014; Reid et al. 2016). Furthermore, genetic manipulations and invasive methods can be applied to the rodents to study circuit function, as can post-mortem investigation of underlying transcriptional and histological changes. The work presented in this thesis provides some evidence for integration of MRI data with other modalities such as behaviour, transcription, and electron microscopy. However, some other frameworks have been proposed, wherein data collected across voxels and, for example electrophysiology or calcium imaging data, are assessed using correlation or distance metrics yielding a similarity or dissimilarity between data types (Barron et al. 2021). Finding ways to do so across species and across modalities remains an active and exciting area of research.

7.7 Future directions

7.7.1 Investigating the maternal immune response at varying scales

In this thesis we focused on the effects of acute inflammation induced by a viral mimetic on offspring neurodevelopment in two different gestational windows and observed different downstream effects on offspring. Another factor that may modulate offspring outcomes, other than gestational timing, is the dose of immunogen used. Typical doses of poly I:C range between 1 and 20 mg/kg; in our studies we used 5mg/kg which may be considered a milder dose. Higher doses may impart greater alterations to fetal neurobiology.

Although we carefully examined the effects due to MIA-exposure either early or late in gestation, we did not cover the mid-gestation period. Rodent mid-gestation corresponds to the

early-middle second trimester of primate gestation, during which sex determination occurs, immune and neuronal cell migration, neurogenesis is occurring in the midbrain and subcortical regions, and the formation of the blood brain barrier are all underway (Clancy et al. 2007; Semple et al. 2013; Selemon and Zecevic 2015). There is a large body of literature identifying several deficits relevant to neurodevelopmental disorders in rodent offspring (Guma, Plitman, and Chakravarty 2019). The only other MRI-based longitudinal investigations of MIA-exposed offspring have focused on this stage of (mid) gestation (rats, GD 15), and have identified significant deviations in developmental trajectories across adulthood (Crum et al. 2017), with earlier and more severe deviations in male offspring trajectories (Piontkewitz, Arad, and Weiner 2011). These studies have focused on the adolescent and adult periods, however, the early life period has yet to be investigated. In order to better understand how MIA-exposure affects offspring outcomes, a detailed analysis of exposure across all gestational stages, and all sensitive developmental stages is critical.

Although initial observations between aberrant neurodevelopment and maternal inflammation were made in the context of acute inflammation due to illness such as influenza or a bacterial infection, substantial evidence suggests that chronic inflammation may be harmful as well. Common causes of chronic inflammation include obesity, stress, gestational diabetes, pre-eclampsia, smoking, depression, exposure to pollution, low socioeconomic status, autoimmune diseases, or asthma (many of which are discussed in **Chapter 2**) (Han et al. 2021). Some of these maternal risk factors, such as obesity (Bilbo and Tsang 2010), stress, exposure to pollution (Bilbo et al. 2018), and asthma (Vogel Ciernia et al. 2018) have been modeled in animals. Behavioural and transcriptional alterations in exposed offspring have been associated with placental-mediated altered cytokine signaling and alterations in microglia function (Vogel Ciernia et al. 2018).

In humans, chronic inflammation as measured by either IL-6 or CRP have both been associated with subtle alterations in neonate brain structure and function, as well as toddler behaviours (Graham et al. 2018; Rudolph et al. 2018; Spann et al. 2018). Further, a recent birth cohort study from Finland associated increased maternal inflammation (confirmed via collection of inflammatory biomarkers throughout pregnancy) due to preeclampsia, obesity, gestational diabetes, hypertension, depression, and anxiety with a two-fold higher risk for childhood neurodevelopmental delays (Girchenko et al. 2020). A recent meta-analysis investigating the association between maternal inflammatory states and neurodevelopmental disorders identified a

significant association increased chronic maternal inflammation due to lifestyle, disease, and environmental factors with risk for ASD and ADHD, as well Tourette's Syndrome in the offspring (Han et al. 2021). Thus, it is possible that some of the structural and functional alterations identified in the early MRI studies reflect deviations in neurodevelopmental trajectory that may lead to the development of neuropsychiatric disorders in the offspring. Understanding how chronic versus acute maternal inflammation affects offspring brain development may be an interesting avenue through which to better understand the mechanisms by which MIA-exposure alters brain development. Furthermore, it may provide new perspectives for understanding, and possibly preventing these effects in high-risk pregnancies (Han et al. 2021).

7.7.2 Identifying possible windows for treatments and interventions

Several groups have investigated potential interventions for attenuation or prevention of neurodevelopmental impairments due to MIA-exposure during pregnancy. Investigation of putative therapeutic interventions is of interest as they may be applied to high-risk pregnancies, or to offspring in prodromal or high-risk phases as preventative measures. Previous studies have shown that administration of anti-inflammatory medications in pregnant mothers may mitigate the effects of high fevers associated with contraction of the influenza virus and thereby decreased the risk for ASD related phenotypes in offspring (odds-ratio=1.3) compared to those who did not receive anti-inflammatories (Zerbo et al. 2013; Careaga, Murai, and Bauman 2017). As discussed in **Chapter 3**, a number of preclinical studies successfully normalized offspring deficits (as measured by MRI) by treating mothers with either anti-inflammatory compounds or antioxidant compounds in combinations with the MIA-challenge (Sharabi et al. 2018; Ginsberg et al. 2017; Girard et al. 2010). For example, blocking specific pro-inflammatory pathways following MIA-exposure, such as IL-17a (Gloria B. Choi et al. 2016) or IL-1β (Crampton et al. 2012) has been shown to minimize neurodevelopmental abnormalities in offspring. Administration of magnesium sulfate, an anti-inflammatory compound, has also been investigated as a possible therapeutic agent, and shown to decrease neuroanatomical abnormalities in MIA-exposed animals in a recent cross-sectional study (Ginsberg et al. 2017).

In addition to modulating inflammation via medicinal compounds, several groups have investigated a nutritional approach. For example, positive evidence for diets rich in omega-3

polyunsaturated fatty acids have been shown to normalize neurochemical alterations in mouse offspring exposed to MIA (Li et al. 2015). Further, several human studies have found positive effects of dietary interventions in individuals with neurodevelopmental disorders such as ASD or ADHD (Gow and Hibbeln 2014), however the findings are mixed and require further research. Probiotics (Hsiao et al. 2013), maternal zinc (Chua et al. 2012), vitamin D (Vuillermot et al. 2017), and iron (Aguilar-Valles, Rodrigue, and Matta-Camacho 2020) supplementation have also shown promise in reducing negative behavioural and neurochemical outcomes in offspring, and targeting nutrient deficiencies associated with (or predating) maternal infection. Thus, lifestyle interventions via supplementation in the diet, or anti-inflammatory or antioxidant supplementation may be favourable avenues for potentially well tolerated intervention during pregnancy and for future research. Finally, a number of rodent studies have also found that administration of antipsychotic medications, such as risperidone or clozapine, to rats prenatally exposed to MIA led to a preservation of hippocampal and ventricular volume only if administration occurred in the early adolescent period; treatment later in life (early adulthood) did not prevent hippocampal volume decrease and ventricular enlargement, observed in their non-treated littermates (Piontkewitz et al. 2012; Piontkewitz, Assaf, and Weiner 2009). This may be akin to treating adolescents and young adults in prodromal or pre-symptomatic phases of illness. These findings suggest that early intervention both in the mother and affected offspring may be more successful at mitigating effects of risk factor exposure, and potentially at preventing the onset of a full-blown psychiatric illness.

7.7.3 Further probing the molecular underpinnings associated with maternal immune activation: fibroblast growth signaling and beyond

Our integrative investigation of MIA-exposure in **Chapter 4** identified enrichment for FGF signaling in differentially upregulated genes due to MIA-exposure at GD 9. Further investigation of these signaling proteins in the context of brain development may provide interesting mechanistic insight into the effects of MIA-exposure on brain development, or possible treatment avenues. Growth factors such as nerve growth factor and brain derived neurotrophic factor have been heavily studied for their roles in modulating brain development and remodeling throughout the lifespan (Turner et al. 2016). Furthermore, altered levels of these growth factors have been observed in neuropsychiatric disorders in both rodent and human studies (Lin and Wang 2014;

Ricci et al. 2013). FGF signaling has received much less attention in the context of neuroscience, although its role in supporting the health of other tissues such as the skin have been well studied (Turner et al. 2016).

In mammals, 18 different FGF ligands have been identified, many of which are involved in cortical patterning, such as intracortical wiring, size, and gyrification (FGF 3, 8, 15, 17, 18), with particular regulatory control from FGF 8 (Rubenstein 2011). FGF 8 is also critical in midbrain, hindbrain, and forebrain patterning (Shimamura and Rubenstein 1997), as well as synaptic differentiation in the hippocampus (as are FGF 7 & 22) (Mott et al. 2010). Their receptors are expressed across neurons (FGFR 1 & 4) as well as oligodendrocytes and astrocytes (FGFR 2 & 3). Given the regulatory role of FGF 8 in midbrain, hindbrain, and forebrain patterning, and cortical refinement, and the relevance of possible cortical pathology in ASD and schizophrenia this may be an interesting target. Interestingly, previous work investigating the effects of MIA-exposure at GD 9 has identified alterations in FGF8 mRNA in the fetal brain (Meyer et al., 2008). Based on the FGF signaling abnormalities we identified in adolescence MIA-exposed (GD 9) offspring, it would be important to understand if or how these genes are affected more proximal to the MIA-exposure to determine whether these observed alterations are a direct effect of MIA-exposure, or more of a downstream alteration due to general disruptions in brain development.

FGF 2 and FGFR 2 have previously been associated with both schizophrenia, autism, (Vaccarino et al. 2009; Terwisscha van Scheltinga, Bakker, and Kahn 2010), bipolar disorder (Wang et al. 2012), while FGF 1 and FGFR3 have been associated with (Evans et al. 2004). As stated above, the FGF 2 receptors are expressed on glial, rather than neuronal cells, thus it may be interesting to investigate whether altered FGF 2 signaling is associated with some of the glial dysfunction associated with neurodevelopmental disorders and in MIA-exposed offspring. One could also take the opposite approach, and experimentally silence or downregulate expression of FGF 2 and FGFR 2 to determine whether this causes similar neurodevelopmental delays to those induced by MIA-exposure.

Given the wide range of functions of the FGF system throughout development, and within psychiatric disorders, finding strategies to modify its actions as a means to ameliorate outcomes may be of interest. Some researchers have developed FGF ligand antibodies, which have been helpful in cancer therapy (Herbert, Lassalle, and Alcouffe 2014). Perhaps an FGFR 2 ligand may be of interest in the context of neuropsychiatric disorders either as a treatment strategy, or in a

more preventative approach. The use of other peptides, such as neural cell adhesion molecules, a transmembrane protein that can interact with FGF receptors, and regulate synaptic plasticity (Turner et al. 2016) may provide another avenue for intervention. The FGFR 2 ligand can cross the blood brain barrier and has been shown to reduce inflammation and improve deficits in individuals with Alzheimer's disease and those with multiple sclerosis (Enevoldsen et al. 2012). Its ability to reduce inflammation may make this strategy more promising in the context of MIA.

7.7.4 Investigating genetic risk

Neuropsychiatric illnesses are complex conditions thought to arise due to a combination of risk factors (Rapoport et al. 2005). The work presented in this thesis has focused on environmental risk factors, however, there is substantial evidence for the role of genetic risk factors in neurodevelopmental and neuropsychiatric illness (Barešić et al. 2020). Understanding the genetic risk associated with the onset of mental illness is a critical piece to our understanding of the underlying biological mechanisms. Twin studies have provided evidence for the role of genetics in the pathology of neuropsychiatric illness, with heritability estimates ranging between 40-80% (Martin, Taylor, and Lichtenstein 2018). Copy number variation in a few genes have been clearly associated with ASD and thought to account for about 20% of cases (B. S. Abrahams and Geschwind 2008); these include, *TSC1* and *TSC2* (the tuberous sclerosis–associated genes), *MECP2* (the Rett syndrome–associated gene) and *FMR1* (the fragile X–associated gene) (*NLGN3* and *NLGN4*), neurexins and *SHANK3* (SH3 and multiple ankyrin repeat domains-3 gene) (Abrahams and Geschwind 2008; Durand et al. 2006; Jamain et al. 2003). Similarly, in schizophrenia, mutation in the DISC-1 (disrupted-in-schizophrenia 1) gene has been specifically associated with schizophrenia, causing alterations in the dopaminergic system (Dahoun et al. 2017).

In addition to these identified genes, genome-wide association studies (GWAS) have served an important role in identifying some of the biological mechanisms associated with neuropsychiatric disease. This approach attempts to identify the statistically significant overrepresentation of specific genetic polymorphisms (single-nucleotide polymorphisms [SNPs]) in groups of affected individuals relative to healthy controls (de Vries 2009). Using these tools, numerous robust and replicable genetic findings have been reported for psychiatric disorders.

Rather than distinguishing across diagnostic categories (i.e., case vs. control), one can think of the genetic vulnerability due to GWAS-identified SNPs as being associated with continuous phenotypes, converging on disruption of specific biological pathways associated with numerous neurodevelopmental and neuropsychiatric disorders (Martin, Taylor, and Lichtenstein 2018). For example, genes that regulate transcriptional activity have been strongly implicated in early onset neurodevelopmental disorders such as ASD, and in schizophrenia, such as *CHD8* and *SETD1A*, involved in chromatin remodeling (Werling et al. 2020).

However, each genetic risk (or altered SNP) only contributes a small effect to the overall phenotype, except for in the case of rare genetic syndromes. Rather, the current model of thinking suggests that a single gene mutation is not sufficient to affect neurodevelopment, but rather, a number of affected genes need to interact in order to have a cumulative effect (de Vries 2009). The genetic architecture of psychiatric disorders is highly polygenic, with hundreds of risk alleles spread throughout the genome (Rees and Owen 2020). This is a similar conceptualization of the way in which environmental risk factors are thought to interact with each other and with genetic risk.

Availability of large open datasets including the Adolescent Brain Cognitive Development (ABCD) study (Volkow et al. 2018), UK Biobank (Alfaro-Almagro et al. 2018), and IMAGEN (Schumann et al. 2010) provide opportunities to examine the association between various risk factors and neurodevelopment. In addition, harnessing animal models as investigative tools have been useful in this context. Classical transgenic rodent models have contributed to our understanding of the loss of function associated with specific genes associated with psychiatric disorders (Baker et al. 2020). Advances in tissue-specific Cre-loxP system have enabled researchers to induce genetic deletion or overexpression with high temporal and regional specificity (Baker et al. 2020). Finally, the CRISPR-associated protein 9 system has emerged as a powerful tool, allowing researchers to edit the genome of any organism with precision, which again, opens many doors for examining the effects of genetic manipulations on an entire organism (Doudna and Charpentier 2014). Of course, translating the findings in animal studies back to humans remains an open area of investigation.

The ability to model interactions between multiple genetic risk factors and environmental risk factors is a current challenge facing the field. In humans, enhanced integration of information regarding the timing and severity of experienced environmental risk factors in combination with

genetic phenotyping, or by inclusion of first-, second-, and/or third-degree relatives could provide a framework in which to probe these complex interactions (van Os, Kenis, and Rutten 2010). A better understanding of interactions between different molecular genetic variations, such as SNPs or copy number variants, with environmental measures would be an important avenue to further explore; animal models may be useful tools for investigating these interactions. Some studies have already identified, for example, that carriers of the Val158Met polymorphism of the COMT gene are more susceptible to cannabis induced psychosis (Vaessen et al. 2018), while the individuals with the BDNF Val66Met polymorphism are more susceptible to developing anxiety (Montag et al. 2010). Additionally, gaining a better understanding of the ways in which genes interact with each other and with non-coding sequences and microRNAs may help us understand how environmental exposures perturb entire pathways of genes, rather than single genes, and how that may influence entire biological systems.

7.8 Main conclusions of thesis

A few consistent themes emerged across the three studies presented in this thesis. First, the differential effects of MIA-timing on brain anatomy and neurodevelopmental trajectory were observed across development. In early life, both in the embryo and neonate brains, late exposure induced greater structural remodeling than early exposure, with a tendency for volume increases, as discussed in **Chapter 5**. However, trajectories of neurodevelopment were only subtly affected in these mice in later development as discussed in **Chapter 4**. Early exposure did lead to some structural and behavioural changes in the early life period discussed in **Chapter 5**. However, the effects of this exposure on development became pronounced later in development, based on altered neurodevelopmental, behavioural, and transcriptional phenotypes identified in the adolescent and early adult period, discussed in **Chapter 4**. These adolescent perturbations were thought to reflect a window of vulnerability to additional risk exposure which closed in later adulthood. Indeed, chronic THC exposure during this developmental window did worsen some of the transient deficits induced by early MIA-exposure, as discussed in **Chapter 6**. The original work presented in this thesis composes a thorough investigation of the effects of MIA-exposure on offspring development throughout the lifespan, from gestation to adulthood. It thoroughly characterizes differences in the type and evolution of neurodevelopmental abnormalities induced by MIA-exposure at two gestational timepoints. Finally, it employs an integrative approach, bridging whole-brain neuroimaging data, to behavioural and transcriptional phenotypes and cellular anatomy, providing a translational framework with which to study risk factors for neurodevelopmental disorders.

Bibliography

Aaltonen, Riikka, Tuija Heikkinen, Kristo Hakala, Kari Laine, and Anna Alanen. 2005. "Transfer of Pro-inflammatory Cytokines across Term Placenta." *Obstetrics and Gynecology* 106 (4): 802–7.

Aavani, Tooka, Shadna A. Rana, Richard Hawkes, and Quentin J. Pittman. 2015. "Maternal Immune Activation Produces Cerebellar Hyperplasia and Alterations in Motor and Social Behaviors in Male and Female Mice." *Cerebellum* 14 (5): 491–505.

Abazyan, Bagrat, Jun Nomura, Geetha Kannan, Koko Ishizuka, Kellie L. Tamashiro, Frederick Nucifora, Vladimir Pogorelov, et al. 2010. "Prenatal Interaction of Mutant DISC1 and Immune Activation Produces Adult Psychopathology." *Biological Psychiatry* 68 (12): 1172–81.

Abdallah, Morsi W., Nanna Larsen, Jakob Grove, Bent Nørgaard-Pedersen, Poul Thorsen, Erik L. Mortensen, and David M. Hougaard. 2013. "Amniotic Fluid Inflammatory Cytokines: Potential Markers of Immunologic Dysfunction in Autism Spectrum Disorders." *The World Journal of Biological Psychiatry: The Official Journal of the World Federation of Societies of Biological Psychiatry* 14 (7): 528–38.

Abrahams, Brett S., and Daniel H. Geschwind. 2008. "Advances in Autism Genetics: On the Threshold of a New Neurobiology." *Nature Reviews. Genetics* 9 (5): 341–55.

Abrahams, Vikki M., Todd M. Schaefer, John V. Fahey, Irene Visintin, Jacqueline A. Wright, Paulomi B. Aldo, Roberto Romero, Charles R. Wira, and Gil Mor. 2006. "Expression and Secretion of Antiviral Factors by Trophoblast Cells Following Stimulation by the TLR-3 Agonist, Poly (I: C)." *Human Reproduction* 21 (9): 2432–39.

Adachi, M., S. Matsukura, H. Tokunaga, and F. Kokubu. 1997. "Expression of Cytokines on Human Bronchial Epithelial Cells Induced by Influenza Virus A." *International Archives of Allergy and Immunology* 113 (1-3): 307–11.

Adams, I. B., and B. R. Martin. 1996. "Cannabis: Pharmacology and Toxicology in Animals and Humans." *Addiction* 91 (11): 1585–1614.

Aguilar-Valles, Argel, Brandon Rodrigue, and Edna Matta-Camacho. 2020. "Maternal Immune Activation and the Development of Dopaminergic Neurotransmission of the Offspring: Relevance for Schizophrenia and Other Psychoses." *Frontiers in Psychiatry / Frontiers Research Foundation* 11 (August): 852.

Agurell, S., M. Halldin, J. E. Lindgren, A. Ohlsson, M. Widman, H. Gillespie, and L. Hollister. 1986. "Pharmacokinetics and Metabolism of Delta 1-Tetrahydrocannabinol and Other Cannabinoids with Emphasis on Man." *Pharmacological Reviews* 38 (1): 21–43.

Alfaro-Almagro, Fidel, Mark Jenkinson, Neal K. Bangerter, Jesper L. R. Andersson, Ludovica Griffanti, Gwenaëlle Douaud, Stamatios N. Sotiropoulos, et al. 2018. "Image Processing and Quality Control for the First 10,000 Brain Imaging Datasets from UK Biobank." *NeuroImage* 166 (February): 400–424.

Allswede, Dana M., Robert H. Yolken, Stephen L. Buka, and Tyrone D. Cannon. 2020. "Cytokine Concentrations throughout Pregnancy and Risk for Psychosis in Adult Offspring: A Longitudinal Case-Control Study." *The Lancet. Psychiatry* 7 (3): 254–61.

Ames, F. 1958. "A Clinical and Metabolic Study of Acute Intoxication with Cannabis Sativa and Its Role in the Model Psychoses." *The Journal of Mental Science* 104 (437): 972–99.

Amodeo, Dionisio A., Chi-Yu Lai, Omron Hassan, Eran A. Mukamel, M. Margarita Behrens,

and Susan B. Powell. 2019. “Maternal Immune Activation Impairs Cognitive Flexibility and Alters Transcription in Frontal Cortex.” *Neurobiology of Disease* 125 (May): 211–18.
Andersen, Susan L., and Martin H. Teicher. 2008. “Stress, Sensitive Periods and Maturational Events in Adolescent Depression.” *Trends in Neurosciences* 31 (4): 183–91.
Anderson, S. A., O. Marín, C. Horn, K. Jennings, and J. L. Rubenstein. 2001. “Distinct Cortical Migrations from the Medial and Lateral Ganglionic Eminences.” *Development* 128 (3): 353–63.
Andréasson, S., P. Allebeck, A. Engström, and U. Rydberg. 1987. “Cannabis and Schizophrenia. A Longitudinal Study of Swedish Conscripts.” *The Lancet* 2 (8574): 1483–86.
Arseneault, Louise, Mary Cannon, Richie Poulton, Robin Murray, Avshalom Caspi, and Terrie E. Moffitt. 2002. “Cannabis Use in Adolescence and Risk for Adult Psychosis: Longitudinal Prospective Study.” *BMJ* 325 (7374): 1212–13.
Ashburner, John, and Karl Friston. 1998. “High-Dimensional Nonlinear Image Registration.” *NeuroImage*. https://doi.org/10.1016/s1053-8119(18)31570-2.
Ashdown, H., Y. Dumont, M. Ng, S. Poole, P. Boksa, and G. N. Luheshi. 2006. “The Role of Cytokines in Mediating Effects of Prenatal Infection on the Fetus: Implications for Schizophrenia.” *Molecular Psychiatry* 11 (1): 47–55.
Astrup, Arne, and Susanne Bügel. 2019. “Overfed but Undernourished: Recognizing Nutritional Inadequacies/deficiencies in Patients with Overweight or Obesity.” *International Journal of Obesity* 43 (2): 219–32.
Atakan, Z., S. Bhattacharyya, P. Allen, R. Martín-Santos, J. A. Crippa, S. J. Borgwardt, P. Fusar-Poli, et al. 2013. “Cannabis Affects People Differently: Inter-Subject Variation in the Psychotogenic Effects of Δ9-Tetrahydrocannabinol: A Functional Magnetic Resonance Imaging Study with Healthy Volunteers.” *Psychological Medicine*. https://doi.org/10.1017/s0033291712001924.
Atladóttir, Hjördis Ó., Poul Thorsen, Lars Østergaard, Diana E. Schendel, Sanne Lemcke, Morsi Abdallah, and Erik T. Parner. 2010. “Maternal Infection Requiring Hospitalization During Pregnancy and Autism Spectrum Disorders.” *Journal of Autism and Developmental Disorders*. https://doi.org/10.1007/s10803-010-1006-y.
Atladottir, H. O., T. B. Henriksen, D. E. Schendel, and E. T. Parner. 2012. “Autism After Infection, Febrile Episodes, and Antibiotic Use During Pregnancy: An Exploratory Study.” *PEDIATRICS*. https://doi.org/10.1542/peds.2012-1107.
Babri, Shirin, Mohammad-Hossein Doosti, and Ali-Akbar Salari. 2014. “Corrigendum to ‘Tumor Necrosis Factor-Alpha during Neonatal Brain Development Affects Anxiety- and Depression-Related Behaviors in Adult Male and Female Mice’ [Behav. Brain Res. 261 (2014) 305–314].” *Behavioural Brain Research*. https://doi.org/10.1016/j.bbr.2014.05.037.
Babulas, Vicki, Pam Factor-Litvak, Raymond Goetz, Catherine A. Schaefer, and Alan S. Brown. 2006. “Prenatal Exposure to Maternal Genital and Reproductive Infections and Adult Schizophrenia.” *The American Journal of Psychiatry* 163 (5): 927–29.
Baggiolini, M. 1998. “Chemokines and Leukocyte Traffic.” *Nature* 392 (6676): 565–68.
Baharnoori, Moogeh, Sanjeev K. Bhardwaj, and Lalit K. Srivastava. 2010. “Neonatal Behavioral Changes in Rats with Gestational Exposure to Lipopolysaccharide: A Prenatal Infection Model for Developmental Neuropsychiatric Disorders.” *Schizophrenia Bulletin* 38 (3): 444–56.
Baharnoori, Moogeh, Wayne G. Brake, and Lalit K. Srivastava. 2009. “Prenatal Immune Challenge Induces Developmental Changes in the Morphology of Pyramidal Neurons of the

Prefrontal Cortex and Hippocampus in Rats." *Schizophrenia Research* 107 (1): 99–109.
Baker, Matthew, Sa-Ik Hong, Seungwoo Kang, and Doo-Sup Choi. 2020. "Rodent Models for Psychiatric Disorders: Problems and Promises." *Laboratory Animal Research* 36 (April): 9.
Bao, Ai-Min, and Dick F. Swaab. 2010. "Sex Differences in the Brain, Behavior, and Neuropsychiatric Disorders." *The Neuroscientist: A Review Journal Bringing Neurobiology, Neurology and Psychiatry* 16 (5): 550–65.
Barešić, Anja, Alexander Jolyon Nash, Tarik Dahoun, Oliver Howes, and Boris Lenhard. 2020. "Understanding the Genetics of Neuropsychiatric Disorders: The Potential Role of Genomic Regulatory Blocks." *Molecular Psychiatry* 25 (1): 6–18.
Barnes, P. J. 1998. "Anti-Inflammatory Actions of Glucocorticoids: Molecular Mechanisms." *Clinical Science* 94 (6): 557–72.
Baron-Cohen, Simon. 1988. "Social and Pragmatic Deficits in Autism: Cognitive or Affective?" *Journal of Autism and Developmental Disorders*. https://doi.org/10.1007/bf02212194.
Barr, C. E., S. A. Mednick, and P. Munk-Jorgensen. 1990. "Exposure to Influenza Epidemics during Gestation and Adult Schizophrenia. A 40-Year Study." *Archives of General Psychiatry* 47 (9): 869–74.
Barron, Helen C., Rogier B. Mars, David Dupret, Jason P. Lerch, and Cassandra Sampaio-Baptista. 2021. "Cross-Species Neuroscience: Closing the Explanatory Gap." *Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences* 376 (1815): 20190633.
Bartzokis, G., M. Beckson, P. H. Lu, K. H. Nuechterlein, N. Edwards, and J. Mintz. 2001. "Age-Related Changes in Frontal and Temporal Lobe Volumes in Men: A Magnetic Resonance Imaging Study." *Archives of General Psychiatry* 58 (5): 461–65.
Batinić, Bojan, Anja Santrač, Branka Divović, Tamara Timić, Tamara Stanković, Aleksandar Lj Obradović, Srđan Joksimović, and Miroslav M. Savić. 2016. "Lipopolysaccharide Exposure during Late Embryogenesis Results in Diminished Locomotor Activity and Amphetamine Response in Females and Spatial Cognition Impairment in Males in Adult, but Not Adolescent Rat Offspring." *Behavioural Brain Research*. https://doi.org/10.1016/j.bbr.2015.11.025.
Battistella, Giovanni, Eleonora Fornari, Jean-Marie Annoni, Haithem Chtioui, Kim Dao, Marie Fabritius, Bernard Favrat, Jean-Frédéric Mall, Philippe Maeder, and Christian Giroud. 2014. "Long-Term Effects of Cannabis on Brain Structure." *Neuropsychopharmacology: Official Publication of the American College of Neuropsychopharmacology* 39 (9): 2041–48.
Battisti, Robert A., Steven Roodenrys, Stuart J. Johnstone, Colleen Respondek, Daniel F. Hermens, and Nadia Solowij. 2010. "Chronic Use of Cannabis and Poor Neural Efficiency in Verbal Memory Ability." *Psychopharmacology* 209 (4): 319–30.
Bauer, Sylvian, Bradley J. Kerr, and Paul H. Patterson. 2007. "The Neuropoietic Cytokine Family in Development, Plasticity, Disease and Injury." *Nature Reviews. Neuroscience* 8 (3): 221–32.
Bauman, M. D., and C. M. Schumann. 2018. "Advances in Nonhuman Primate Models of Autism: Integrating Neuroscience and Behavior." *Experimental Neurology* 299 (Pt A): 252–65.
Bauman, Melissa D., Ana-Maria Iosif, Stephen E. P. Smith, Catherine Bregere, David G. Amaral, and Paul H. Patterson. 2014. "Activation of the Maternal Immune System during Pregnancy Alters Behavioral Development of Rhesus Monkey Offspring." *Biological Psychiatry* 75 (4): 332–41.

Beattie, Eric C., David Stellwagen, Wade Morishita, Jacqueline C. Bresnahan, Byeong Keun Ha, Mark Von Zastrow, Michael S. Beattie, and Robert C. Malenka. 2002. “Control of Synaptic Strength by Glial TNFα.” *Science* 295 (5563): 2282–85.

Beckman, D. A., T. R. Koszalka, M. Jensen, and R. L. Brent. 1990. “Experimental Manipulation of the Rodent Visceral Yolk Sac.” *Teratology* 41 (4): 395–404.

Bedford, Saashi A., Min Tae M. Park, Gabriel A. Devenyi, Stephanie Tullo, Jurgen Germann, Raihaan Patel, Evdokia Anagnostou, et al. 2020. “Large-Scale Analyses of the Relationship between Sex, Age and Intelligence Quotient Heterogeneity and Cortical Morphometry in Autism Spectrum Disorder.” *Molecular Psychiatry* 25 (3): 614–28.

Benveniste, Helene, Hedok Lee, Fengfei Ding, Qian Sun, Ehab Al-Bizri, Rany Makaryus, Stephen Probst, Maiken Nedergaard, Elliot A. Stein, and Hanbing Lu. 2017. “Anesthesia with Dexmedetomidine and Low-Dose Isoflurane Increases Solute Transport via the Glymphatic Pathway in Rat Brain When Compared with High-Dose Isoflurane.” *Anesthesiology* 127 (6): 976–88.

Berg, B. J. van den, R. E. Christianson, and F. W. Oechsli. 1988. “The California Child Health and Development Studies of the School of Public Health, University of California at Berkeley.” *Paediatric and Perinatal Epidemiology* 2 (3): 265–82.

Berger, Abi. 2002. “Magnetic Resonance Imaging.” *BMJ* 324 (7328): 35.

Berghuis, Paul, Marton B. Dobszay, Xinyu Wang, Sabrina Spano, Fernanda Ledda, Kyle M. Sousa, Gunnar Schulte, et al. 2005. “Endocannabinoids Regulate Interneuron Migration and Morphogenesis by Transactivating the TrkB Receptor.” *Proceedings of the National Academy of Sciences of the United States of America* 102 (52): 19115–20.

Bernal-Rusiel, Jorge L., Douglas N. Greve, Martin Reuter, Bruce Fischl, Mert R. Sabuncu, and Alzheimer’s Disease Neuroimaging Initiative. 2013. “Statistical Analysis of Longitudinal Neuroimage Data with Linear Mixed Effects Models.” *NeuroImage* 66 (February): 249–60.

Bilbo, Staci D., Carina L. Block, Jessica L. Bolton, Richa Hanamsagar, and Phuong K. Tran. 2018. “Beyond Infection - Maternal Immune Activation by Environmental Factors, Microglial Development, and Relevance for Autism Spectrum Disorders.” *Experimental Neurology* 299 (January): 241–51.

Bilbo, Staci D., and Verne Tsang. 2010. “Enduring Consequences of Maternal Obesity for Brain Inflammation and Behavior of Offspring.” *FASEB Journal: Official Publication of the Federation of American Societies for Experimental Biology* 24 (6): 2104–15.

Biscaia, Miguel, Susana Marín, Beatriz Fernández, Eva M. Marco, Marina Rubio, Carmen Guaza, Emilio Ambrosio, and Maria Paz Viveros. 2003. “Chronic Treatment with CP 55,940 during the Peri-Adolescent Period Differentially Affects the Behavioural Responses of Male and Female Rats in Adulthood.” *Psychopharmacology* 170 (3): 301–8.

Bisht, Kanchan, Kaushik P. Sharma, Cynthia Lecours, Maria Gabriela Sánchez, Hassan El Hajj, Giampaolo Milior, Adrián Olmos-Alonso, et al. 2016. “Dark Microglia: A New Phenotype Predominantly Associated with Pathological States.” *Glia* 64 (5): 826–39.

Bitanihirwe, Byron K. Y., Daria Peleg-Raibstein, Forouhar Mouttet, Joram Feldon, and Urs Meyer. 2010. “Late Prenatal Immune Activation in Mice Leads to Behavioral and Neurochemical Abnormalities Relevant to the Negative Symptoms of Schizophrenia.” *Neuropsychopharmacology*. https://doi.org/10.1038/npp.2010.129.

Bloomfield, Michael A. P., Abhishekh H. Ashok, Nora D. Volkow, and Oliver D. Howes. 2016. “The Effects of Δ9-Tetrahydrocannabinol on the Dopamine System.” *Nature* 539 (7629): 369–77.

Bock, Nicholas A., Brian J. Nieman, Johnathan B. Bishop, and R. Mark Henkelman. 2005. “In Vivo Multiple-Mouse MRI at 7 Tesla.” *Magnetic Resonance in Medicine*. https://doi.org/10.1002/mrm.20683.

Boksa, Patricia. 2010. “Effects of Prenatal Infection on Brain Development and Behavior: A Review of Findings from Animal Models.” *Brain, Behavior, and Immunity* 24 (6): 881–97.

Borish, Larry C., and John W. Steinke. 2003. “2. Cytokines and Chemokines.” *The Journal of Allergy and Clinical Immunology* 111 (2 Suppl): S460–75.

Borrell, José, José Miguel Vela, Angel Arévalo-Martin, Eduardo Molina-Holgado, and Carmen Guaza. 2002. “Prenatal Immune Challenge Disrupts Sensorimotor Gating in Adult Rats. Implications for the Etiopathogenesis of Schizophrenia.” *Neuropsychopharmacology: Official Publication of the American College of Neuropsychopharmacology* 26 (2): 204–15.

Boulos, Peter K., Manish S. Dalwani, Jody Tanabe, Susan K. Mikulich-Gilbertson, Marie T. Banich, Thomas J. Crowley, and Joseph T. Sakai. 2016. “Brain Cortical Thickness Differences in Adolescent Females with Substance Use Disorders.” *PLOS ONE*. https://doi.org/10.1371/journal.pone.0152983.

Brauner, A., M. Söderhäll, S. H. Jacobson, J. Lundahl, U. Andersson, and J. Andersson. 2001. “Escherichia Coli-Induced Expression of IL-1α, IL-1β, IL-6 and IL-8 in Normal Human Renal Tubular Epithelial Cells.” *Clinical & Experimental Immunology* 124 (3): 423–28.

Braunschweig, Daniel, Paul Duncanson, Robert Boyce, Robin Hansen, Paul Ashwood, Isaac N. Pessah, Irva Hertz-Picciotto, and Judy Van de Water. 2012. “Behavioral Correlates of Maternal Antibody Status among Children with Autism.” *Journal of Autism and Developmental Disorders* 42 (7): 1435–45.

Braunschweig, Daniel, Mari S. Golub, Claire M. Koenig, Lihong Qi, Isaac N. Pessah, Judy Van de Water, and Robert F. Berman. 2012. “Maternal Autism-Associated IgG Antibodies Delay Development and Produce Anxiety in a Mouse Gestational Transfer Model.” *Journal of Neuroimmunology* 252 (1-2): 56–65.

Bronson, Stefanie L., and Tracy L. Bale. 2014. “Prenatal Stress-Induced Increases in Placental Inflammation and Offspring Hyperactivity Are Male-Specific and Ameliorated by Maternal Antiinflammatory Treatment.” *Endocrinology* 155 (7): 2635–46.

Brown, Alan S. 2012. “Epidemiologic Studies of Exposure to Prenatal Infection and Risk of Schizophrenia and Autism.” *Developmental Neurobiology* 72 (10): 1272–76.

Brown, Alan S. 2015. “The Kraepelinian Dichotomy From the Perspective of Prenatal Infectious and Immunologic Insults.” *Schizophrenia Bulletin* 41 (4): 786–91.

Brown, Alan S., Melissa D. Begg, Stefan Gravenstein, Catherine A. Schaefer, Richard J. Wyatt, Michaeline Bresnahan, Vicki P. Babulas, and Ezra S. Susser. 2004. “Serologic Evidence of Prenatal Influenza in the Etiology of Schizophrenia.” *Archives of General Psychiatry* 61 (8): 774–80.

Brown, Alan S., Patricia Cohen, Jill Harkavy-Friedman, Vicki Babulas, Dolores Malaspina, Jack M. Gorman, and Ezra S. Susser. 2001. “Prenatal Rubella, Premorbid Abnormalities, and Adult Schizophrenia.” *Biological Psychiatry* 49 (6): 473–86.

Brown, Alan S., and Elena J. Derkits. 2010. “Prenatal Infection and Schizophrenia: A Review of Epidemiologic and Translational Studies.” *The American Journal of Psychiatry* 167 (3): 261–80.

Brown, Alan S., Jonathan Hooton, Catherine A. Schaefer, Haiying Zhang, Eva Petkova, Vicki Babulas, Megan Perrin, Jack M. Gorman, and Ezra S. Susser. 2004. “Elevated Maternal Interleukin-8 Levels and Risk of Schizophrenia in Adult Offspring.” *American Journal of*

Psychiatry. https://doi.org/10.1176/appi.ajp.161.5.889.
Brown, Alan S., and Urs Meyer. 2018. "Maternal Immune Activation and Neuropsychiatric Illness: A Translational Research Perspective." *The American Journal of Psychiatry*, September, appiajp201817121311.
Brown, Alan S., Catherine A. Schaefer, Charles P. Quesenberry Jr, Liyan Liu, Vicki P. Babulas, and Ezra S. Susser. 2005. "Maternal Exposure to Toxoplasmosis and Risk of Schizophrenia in Adult Offspring." *The American Journal of Psychiatry* 162 (4): 767–73.
Brown, Alan S., Catherine A. Schaefer, Charles P. Quesenberry Jr, Ling Shen, and Ezra S. Susser. 2006. "No Evidence of Relation between Maternal Exposure to Herpes Simplex Virus Type 2 and Risk of Schizophrenia?" *The American Journal of Psychiatry* 163 (12): 2178–80.
Brown, Alan S., Heljä-Marja Surcel, Susanna Hinkka-Yli-Salomäki, Keely Cheslack-Postava, Yuanyuan Bao, and Andre Sourander. 2015. "Maternal Thyroid Autoantibody and Elevated Risk of Autism in a National Birth Cohort." *Progress in Neuro-Psychopharmacology & Biological Psychiatry* 57 (March): 86–92.
Brown, Alan S., and Ezra S. Susser. 2002. "In Utero Infection and Adult Schizophrenia." *Mental Retardation and Developmental Disabilities Research Reviews*. https://doi.org/10.1002/mrdd.10004.
Brown, A. S., P. Cohen, S. Greenwald, and E. Susser. 2000. "Nonaffective Psychosis after Prenatal Exposure to Rubella." *The American Journal of Psychiatry* 157 (3): 438–43.
Brucato, Martha, Christine Ladd-Acosta, Mengying Li, Deanna Caruso, Xiumei Hong, Jamie Kaczaniuk, Elizabeth A. Stuart, M. Daniele Fallin, and Xiaobin Wang. 2017. "Prenatal Exposure to Fever Is Associated with Autism Spectrum Disorder in the Boston Birth Cohort." *Autism Research: Official Journal of the International Society for Autism Research* 10 (11): 1878–90.
Buka, S. L., M. T. Tsuang, E. F. Torrey, M. A. Klebanoff, R. L. Wagner, and R. H. Yolken. 2001. "Maternal Cytokine Levels during Pregnancy and Adult Psychosis." *Brain, Behavior, and Immunity* 15 (4): 411–20.
Buka, Stephen L., Tyrone D. Cannon, E. Fuller Torrey, Robert H. Yolken, and Collaborative Study Group on the Perinatal Origins of Severe Psychiatric Disorders. 2008. "Maternal Exposure to Herpes Simplex Virus and Risk of Psychosis among Adult Offspring." *Biological Psychiatry* 63 (8): 809–15.
Buka, Stephen L., Ming T. Tsuang, E. Fuller Torrey, Mark A. Klebanoff, David Bernstein, and Robert H. Yolken. 2002. "Maternal Infections and Subsequent Psychosis Among Offspring." *Obstetrical and Gynecological Survey*. https://doi.org/10.1097/00006254-200204000-00005.
Burg, Jelske W. van der, Sarbattama Sen, Virginia R. Chomitz, Jaap C. Seidell, Alan Leviton, and Olaf Dammann. 2016. "The Role of Systemic Inflammation Linking Maternal BMI to Neurodevelopment in Children." *Pediatric Research* 79 (1-1): 3–12.
Busemeyer, Jerome R., and Adele Diederich. 2014. "Chapter 4 - Estimation and Testing of Computational Psychological Models." In *Neuroeconomics (Second Edition)*, edited by Paul W. Glimcher and Ernst Fehr, 49–61. San Diego: Academic Press.
Buss, Claudia, Elysia Poggi Davis, Babak Shahbaba, Jens C. Pruessner, Kevin Head, and Curt A. Sandman. 2012. "Maternal Cortisol over the Course of Pregnancy and Subsequent Child Amygdala and Hippocampus Volumes and Affective Problems." *Proceedings of the National Academy of Sciences of the United States of America* 109 (20): E1312–19.

Butt, Simon Jb, Jacqueline A. Stacey, Yayoi Teramoto, and Cristiana Vagnoni. 2017. “A Role for GABAergic Interneuron Diversity in Circuit Development and Plasticity of the Neonatal Cerebral Cortex.” *Current Opinion in Neurobiology* 43 (April): 149–55.

Cabral, G. A., E. S. Raborn, L. Griffin, J. Dennis, and F. Marciano-Cabral. 2008. “CB2 Receptors in the Brain: Role in Central Immune Function.” *British Journal of Pharmacology* 153 (2): 240–51.

Cahill, L. S., C. L. Laliberté, J. Ellegood, and S. Spring. 2012. “Preparation of Fixed Mouse Brains for MRI.” *NeuroImage*. https://www.sciencedirect.com/science/article/pii/S1053811912001188.

Calakos, Katina C., Shivani Bhatt, Dawn W. Foster, and Kelly P. Cosgrove. 2017. “Mechanisms Underlying Sex Differences in Cannabis Use.” *Current Addiction Reports* 4 (4): 439–53.

Canales, Cesar P., Myka L. Estes, Karol Cichewicz, Kartik Angara, John Paul Aboubechara, Scott Cameron, Kathryn Prendergast, et al. 2021. “Sequential Perturbations to Mouse Corticogenesis Following in Utero Maternal Immune Activation.” *eLife* 10 (March). https://doi.org/10.7554/eLife.60100.

Canetta, Sarah E., Yuanyuan Bao, Mary Dawn T. Co, Francis A. Ennis, John Cruz, Masanori Terajima, Ling Shen, Christoph Kellendonk, Catherine A. Schaefer, and Alan S. Brown. 2014. “Serological Documentation of Maternal Influenza Exposure and Bipolar Disorder in Adult Offspring.” *The American Journal of Psychiatry* 171 (5): 557–63.

Canetta, S., S. Bolkan, N. Padilla-Coreano, L. J. Song, R. Sahn, N. L. Harrison, J. A. Gordon, A. Brown, and C. Kellendonk. 2016. “Maternal Immune Activation Leads to Selective Functional Deficits in Offspring Parvalbumin Interneurons.” *Molecular Psychiatry* 21 (7): 956–68.

Canuso, Carla M., and Gahan Pandina. 2007. “Gender and Schizophrenia.” *Psychopharmacology Bulletin* 40 (4): 178–90.

Cao, Mingju, Marina Cortes, Craig S. Moore, Soo Yuen Leong, Lucien D. Durosier, Patrick Burns, Gilles Fecteau, et al. 2015. “Fetal Microglial Phenotype in Vitro Carries Memory of Prior in Vivo Exposure to Inflammation.” *Frontiers in Cellular Neuroscience* 9 (August): 294.

Careaga, Milo, Takeshi Murai, and Melissa D. Bauman. 2017. “Maternal Immune Activation and Autism Spectrum Disorder: From Rodents to Nonhuman and Human Primates.” *Biological Psychiatry* 81 (5): 391–401.

Carey, Luke C., Paulien L. Berbée, Peter Coyle, Jeffrey C. Philcox, and Allan M. Rofe. 2003. “Zinc Treatment Prevents Lipopolysaccharide-Induced Teratogenicity in Mice.” *Birth Defects Research Part A: Clinical and Molecular Teratology*. https://doi.org/10.1002/bdra.10035.

Casa, Luis Gonzalo De la, Luis Gonzalo De la Casa, Auxiliadora Mena, and Juan Carlos Ruiz-Salas. 2016. “Effect of Stress and Attention on Startle Response and Prepulse Inhibition.” *Physiology & Behavior*. https://doi.org/10.1016/j.physbeh.2016.07.022.

Caspi, Avshalom, Terrie E. Moffitt, Mary Cannon, Joseph McClay, Robin Murray, Honalee Harrington, Alan Taylor, Louise Arseneault, Ben Williams, Antony Braithwaite, and Others. 2005. “Moderation of the Effect of Adolescent-Onset Cannabis Use on Adult Psychosis by a Functional Polymorphism in the Catechol-O-Methyltransferase Gene: Longitudinal Evidence of a Gene X Environment Interaction.” *Biological Psychiatry* 57 (10): 1117–27.

Caspi, Avshalom, Terrie E. Moffitt, Mary Cannon, Joseph McClay, Robin Murray, Honalee

Harrington, Alan Taylor, Louise Arseneault, Ben Williams, Antony Braithwaite, Richie Poulton, et al. 2005. “Moderation of the Effect of Adolescent-Onset Cannabis Use on Adult Psychosis by a Functional Polymorphism in the Catechol-O-Methyltransferase Gene: Longitudinal Evidence of a Gene X Environment Interaction.” *Biological Psychiatry*. https://doi.org/10.1016/j.biopsych.2005.01.026.

Cassella, Sarah N., Ann M. Hemmerle, Kerstin H. Lundgren, Tara L. Kyser, Rebecca Ahlbrand, Stefanie L. Bronson, Neil M. Richtand, and Kim B. Seroogy. 2016. “Maternal Immune Activation Alters Glutamic Acid Decarboxylase-67 Expression in the Brains of Adult Rat Offspring.” *Schizophrenia Research* 171 (1-3): 195–99.

Chakravarty, M. Mallar, Patrick Steadman, Matthijs C. van Eede, Rebecca D. Calcott, Victoria Gu, Philip Shaw, Armin Raznahan, D. Louis Collins, and Jason P. Lerch. 2013. “Performing Label-Fusion-Based Segmentation Using Multiple Automatically Generated Templates.” *Human Brain Mapping* 34 (10): 2635–54.

Chandorkar, G. A., C. Ampasavate, J. F. Stobaugh, and K. L. Audus. 1999. “Peptide Transport and Metabolism across the Placenta.” *Advanced Drug Delivery Reviews* 38 (1): 59–67.

Chatterjee, Shampa. 2016. “Oxidative Stress, Inflammation, and Disease.” In *Oxidative Stress and Biomaterials*, 35–58. Elsevier.

Cheng, Chia-Hsiung, Pei-Ying S. Chan, Shih-Chieh Hsu, and Chia-Yih Liu. 2018. “Meta-Analysis of Sensorimotor Gating in Patients with Autism Spectrum Disorders.” *Psychiatry Research* 262 (April): 413–19.

Chen, Jingshan, Barbara K. Lipska, Nader Halim, Quang D. Ma, Mitsuyuki Matsumoto, Samer Melhem, Bhaskar S. Kolachana, et al. 2004. “Functional Analysis of Genetic Variation in Catechol-O-Methyltransferase (COMT): Effects on mRNA, Protein, and Enzyme Activity in Postmortem Human Brain.” *The American Journal of Human Genetics*. https://doi.org/10.1086/425589.

Choi, G. B., Y. S. Yim, H. Wong, S. Kim, H. Kim, S. V. Kim, C. A. Hoeffer, D. R. Littman, and J. R. Huh. 2016. “The Maternal Interleukin-17a Pathway in Mice Promotes Autism-like Phenotypes in Offspring.” *Science*. https://doi.org/10.1126/science.aad0314.

Choi, Gloria B., Yeong S. Yim, Helen Wong, Sangdoo Kim, Hyunju Kim, Sangwon V. Kim, Charles A. Hoeffer, Dan R. Littman, and Jun R. Huh. 2016. “The Maternal Interleukin-17a Pathway in Mice Promotes Autism-like Phenotypes in Offspring.” *Science* 351 (6276): 933–39.

Chua, Joanne S. C., Carina J. Cowley, Jim Manavis, Allan M. Rofe, and Peter Coyle. 2012. “Prenatal Exposure to Lipopolysaccharide Results in Neurodevelopmental Damage That Is Ameliorated by Zinc in Mice.” *Brain, Behavior, and Immunity* 26 (2): 326–36.

Chung, M. K., K. J. Worsley, T. Paus, C. Cherif, D. L. Collins, J. N. Giedd, J. L. Rapoport, and A. C. Evans. 2001. “A Unified Statistical Approach to Deformation-Based Morphometry.” *NeuroImage* 14 (3): 595–606.

Chung, Moo K., Keith J. Worsley, Steve Robbins, Tomáš Paus, Jonathan Taylor, Jay N. Giedd, Judith L. Rapoport, and Alan C. Evans. 2003. “Deformation-Based Surface Morphometry Applied to Gray Matter Deformation.” *NeuroImage*. https://doi.org/10.1016/s1053-8119(02)00017-4.

Clancy, Barbara, Barbara L. Finlay, Richard B. Darlington, and K. J. S. Anand. 2007. “Extrapolating Brain Development from Experimental Species to Humans.” *Neurotoxicology* 28 (5): 931–37.

Clarke, Mary C., Antti Tanskanen, Matti Huttunen, John C. Whittaker, and Mary Cannon. 2009.

"Evidence for an Interaction between Familial Liability and Prenatal Exposure to Infection in the Causation of Schizophrenia." *The American Journal of Psychiatry* 166 (9): 1025–30.
Cloyd, Ryan A., Shon A. Koren, and Jose F. Abisambra. 2018. "Manganese-Enhanced Magnetic Resonance Imaging: Overview and Central Nervous System Applications With a Focus on Neurodegeneration." *Frontiers in Aging Neuroscience* 10 (December): 403.
Cohen, Sheldon, Denise Janicki-Deverts, William J. Doyle, Gregory E. Miller, Ellen Frank, Bruce S. Rabin, and Ronald B. Turner. 2012. "Chronic Stress, Glucocorticoid Receptor Resistance, Inflammation, and Disease Risk." *Proceedings of the National Academy of Sciences of the United States of America* 109 (16): 5995–99.
Coiro, Pierluca, Ragunathan Padmashri, Anand Suresh, Elizabeth Spartz, Gurudutt Pendyala, Shinnyi Chou, Yoosun Jung, et al. 2015. "Impaired Synaptic Development in a Maternal Immune Activation Mouse Model of Neurodevelopmental Disorders." *Brain, Behavior, and Immunity* 50 (November): 249–58.
Colizzi, Marco, Philip McGuire, Vincent Giampietro, Steve Williams, Mick Brammer, and Sagnik Bhattacharyya. 2018. "Previous Cannabis Exposure Modulates the Acute Effects of Delta-9-Tetrahydrocannabinol on Attentional Salience and Fear Processing." *Experimental and Clinical Psychopharmacology*. https://doi.org/10.1037/pha0000221.
Connor, Caroline M., Aslihan Dincer, Juerg Straubhaar, Janina R. Galler, Isaac B. Houston, and Schahram Akbarian. 2012. "Maternal Immune Activation Alters Behavior in Adult Offspring, with Subtle Changes in the Cortical Transcriptome and Epigenome." *Schizophrenia Research* 140 (1-3): 175–84.
Cooper, Ziva D., and Rebecca M. Craft. 2018. "Sex-Dependent Effects of Cannabis and Cannabinoids: A Translational Perspective." *Neuropsychopharmacology: Official Publication of the American College of Neuropsychopharmacology* 43 (1): 34–51.
Corradini, Irene, Elisa Focchi, Marco Rasile, Raffaella Morini, Genni Desiato, Romana Tomasoni, Michela Lizier, et al. 2018. "Maternal Immune Activation Delays Excitatory-to-Inhibitory Gamma-Aminobutyric Acid Switch in Offspring." *Biological Psychiatry* 83 (8): 680–91.
Cossío, Lourdes Fernández de, Lourdes Fernández de Cossío, Andrea Guzmán, Suzanne van der Veldt, and Giamal N. Luheshi. 2017. "Prenatal Infection Leads to ASD-like Behavior and Altered Synaptic Pruning in the Mouse Offspring." *Brain, Behavior, and Immunity*. https://doi.org/10.1016/j.bbi.2016.09.028.
Cousijn, Janna, Reinout W. Wiers, K. Richard Ridderinkhof, Wim van den Brink, Dick J. Veltman, and Anna E. Goudriaan. 2012. "Grey Matter Alterations Associated with Cannabis Use: Results of a VBM Study in Heavy Cannabis Users and Healthy Controls." *NeuroImage* 59 (4): 3845–51.
Coussons-Read, Mary E., Marci Lobel, J. Chris Carey, Marianne O. Kreither, Kimberly D'Anna, Laura Argys, Randall G. Ross, Chandra Brandt, and Stephanie Cole. 2012. "The Occurrence of Preterm Delivery Is Linked to Pregnancy-Specific Distress and Elevated Inflammatory Markers across Gestation." *Brain, Behavior, and Immunity* 26 (4): 650–59.
Coussons-Read, Mary E., Michele L. Okun, Mischel P. Schmitt, and Scott Giese. 2005. "Prenatal Stress Alters Cytokine Levels in a Manner That May Endanger Human Pregnancy." *Psychosomatic Medicine* 67 (4): 625–31.
Coyle, Peter, Nancy Tran, Jenny N. T. Fung, Brooke L. Summers, and Allan M. Rofe. 2009. "Maternal Dietary Zinc Supplementation Prevents Aberrant Behaviour in an Object Recognition Task in Mice Offspring Exposed to LPS in Early Pregnancy." *Behavioural*

Brain Research 197 (1): 210–18.
Craft, R. M., A. E. Haas, J. L. Wiley, Z. Yu, and B. H. Clowers. 2017. "Gonadal Hormone Modulation of Δ9-Tetrahydrocannabinol-Induced Antinociception and Metabolism in Female versus Male Rats." *Pharmacology Biochemistry and Behavior*. https://doi.org/10.1016/j.pbb.2016.09.006.
Craig, Andrew, Ning Ling Luo, Douglas J. Beardsley, Nasiema Wingate-Pearse, David W. Walker, A. Roger Hohimer, and Stephen A. Back. 2003. "Quantitative Analysis of Perinatal Rodent Oligodendrocyte Lineage Progression and Its Correlation with Human." *Experimental Neurology* 181 (2): 231–40.
Crampton, Sean J., Louise M. Collins, Andre Toulouse, Yvonne M. Nolan, and Gerard W. O'Keeffe. 2012. "Exposure of Foetal Neural Progenitor Cells to IL-1β Impairs Their Proliferation and Alters Their Differentiation--a Role for Maternal Inflammation?" *Journal of Neurochemistry* 120 (6): 964–73.
Croen, Lisa A., Yinge Qian, Paul Ashwood, Ousseny Zerbo, Diana Schendel, Jennifer Pinto-Martin, M. Daniele Fallin, et al. 2019. "Infection and Fever in Pregnancy and Autism Spectrum Disorders: Findings from the Study to Explore Early Development." *Autism Research: Official Journal of the International Society for Autism Research* 12 (10): 1551–61.
Crum, William R., Stephen J. Sawiak, Winfred Chege, Jonathan D. Cooper, Steven C. R. Williams, and Anthony C. Vernon. 2017. "Evolution of Structural Abnormalities in the Rat Brain Following in Utero Exposure to Maternal Immune Activation: A Longitudinal in Vivo MRI Study." *Brain, Behavior, and Immunity* 63 (July): 50–59.
Cui, Ke, Helen Ashdown, Giamal N. Luheshi, and Patricia Boksa. 2009. "Effects of Prenatal Immune Activation on Hippocampal Neurogenesis in the Rat." *Schizophrenia Research* 113 (2-3): 288–97.
Cunningham, Christopher L., Verónica Martínez-Cerdeño, and Stephen C. Noctor. 2013. "Microglia Regulate the Number of Neural Precursor Cells in the Developing Cerebral Cortex." *The Journal of Neuroscience: The Official Journal of the Society for Neuroscience* 33 (10): 4216–33.
Cunningham, C. L., V. Martinez-Cerdeno, and S. C. Noctor. 2013. "Microglia Regulate the Number of Neural Precursor Cells in the Developing Cerebral Cortex." *Journal of Neuroscience*. https://doi.org/10.1523/jneurosci.3441-12.2013.
Cupo, Lani, Eric Plitman, Elisa Guma, and M. Mallar Chakravarty. 2021. "A Systematic Review of Neuroimaging and Acute Cannabis Exposure in Age-of-Risk for Psychosis." *Translational Psychiatry*. https://doi.org/10.1038/s41398-021-01295-w.
Curfs, J. H., J. F. Meis, and J. A. Hoogkamp-Korstanje. 1997. "A Primer on Cytokines: Sources, Receptors, Effects, and Inducers." *Clinical Microbiology Reviews* 10 (4): 742–80.
Currie, Stuart, Nigel Hoggard, Ian J. Craven, Marios Hadjivassiliou, and Iain D. Wilkinson. 2013. "Understanding MRI: Basic MR Physics for Physicians." *Postgraduate Medical Journal* 89 (1050): 209–23.
Cuthbert, Bruce N., and Thomas R. Insel. 2013. "Toward the Future of Psychiatric Diagnosis: The Seven Pillars of RDoC." *BMC Medicine* 11 (May): 126.
Dahlgren, Jovanna, Anne-Maj Samuelsson, Thomas Jansson, and Agneta Holmäng. 2006. "Interleukin-6 in the Maternal Circulation Reaches the Rat Fetus in Mid-Gestation." *Pediatric Research* 60 (2): 147–51.
Dahoun, T., S. V. Trossbach, N. J. Brandon, C. Korth, and O. D. Howes. 2017. "The Impact of

Disrupted-in-Schizophrenia 1 (DISC1) on the Dopaminergic System: A Systematic Review." *Translational Psychiatry* 7 (1): e1015.

Dalton, Victoria S., Mathieu Verdurand, Adam Walker, Deborah M. Hodgson, and Katerina Zavitsanou. 2012. "Synergistic Effect between Maternal Infection and Adolescent Cannabinoid Exposure on Serotonin 5HT1A Receptor Binding in the Hippocampus: Testing the 'Two Hit' Hypothesis for the Development of Schizophrenia." *ISRN Psychiatry* 2012 (June): 451865.

Dalwani, Manish S., Mary Agnes McMahon, Susan K. Mikulich-Gilbertson, Susan E. Young, Michael F. Regner, Kristen M. Raymond, Shannon K. McWilliams, et al. 2015. "Female Adolescents with Severe Substance and Conduct Problems Have Substantially Less Brain Gray Matter Volume." *PloS One* 10 (5): e0126368.

Dazai, Jun, Shoshana Spring, Lindsay S. Cahill, and R. Mark Henkelman. 2011. "Multiple-Mouse Neuroanatomical Magnetic Resonance Imaging." *Journal of Visualized Experiments: JoVE*, no. 48 (February). https://doi.org/10.3791/2497.

Dean, B., S. Sundram, R. Bradbury, E. Scarr, and D. Copolov. 2001. "Studies on [3H]CP-55940 Binding in the Human Central Nervous System: Regional Specific Changes in Density of Cannabinoid-1 Receptors Associated with Schizophrenia and Cannabis Use." *Neuroscience*. https://doi.org/10.1016/s0306-4522(00)00552-2.

Dean, Justin M., Yohan van de Looij, Stephane V. Sizonenko, Gregory A. Lodygensky, Francois Lazeyras, Hayde Bolouri, Ingemar Kjellmer, Petra S. Huppi, Henrik Hagberg, and Carina Mallard. 2011. "Delayed Cortical Impairment Following Lipopolysaccharide Exposure in Preterm Fetal Sheep." *Annals of Neurology* 70 (5): 846–56.

Degenhardt, Louisa, Carolyn Coffey, Helena Romaniuk, Wendy Swift, John B. Carlin, Wayne D. Hall, and George C. Patton. 2013. "The Persistence of the Association between Adolescent Cannabis Use and Common Mental Disorders into Young Adulthood." *Addiction*. https://doi.org/10.1111/j.1360-0443.2012.04015.x.

Degenhardt, Louisa, Wayne Hall, and Michael Lynskey. 2003. "Testing Hypotheses about the Relationship between Cannabis Use and Psychosis." *Drug and Alcohol Dependence* 71 (1): 37–48.

Dekel, Nava, Yulia Gnainsky, Irit Granot, and Gil Mor. 2010. "Inflammation and Implantation." *American Journal of Reproductive Immunology* 63 (1): 17–21.

DeLisi, Lynn E. 2008. "The Effect of Cannabis on the Brain: Can It Cause Brain Anomalies That Lead to Increased Risk for Schizophrenia?" *Current Opinion in Psychiatry* 21 (2): 140–50.

Demirakca, Traute, Alexander Sartorius, Gabriele Ende, Nadja Meyer, Helga Welzel, Gisela Skopp, Karl Mann, and Derik Hermann. 2011. "Diminished Gray Matter in the Hippocampus of Cannabis Users: Possible Protective Effects of Cannabidiol." *Drug and Alcohol Dependence* 114 (2-3): 242–45.

Denic, Aleksandar, Slobodan I. Macura, Prasanna Mishra, Jeffrey D. Gamez, Moses Rodriguez, and Istvan Pirko. 2011. "MRI in Rodent Models of Brain Disorders." *Neurotherapeutics: The Journal of the American Society for Experimental NeuroTherapeutics* 8 (1): 3–18.

Depino, A. M. 2015. "Early Prenatal Exposure to LPS Results in Anxiety- and Depression-Related Behaviors in Adulthood." *Neuroscience*. https://doi.org/10.1016/j.neuroscience.2015.04.065.

Despotović, Ivana, Bart Goossens, and Wilfried Philips. 2015. "MRI Segmentation of the Human Brain: Challenges, Methods, and Applications." *Computational and Mathematical Methods in Medicine* 2015 (March): 450341.

Devane, W. A., F. A. Dysarz 3rd, M. R. Johnson, L. S. Melvin, and A. C. Howlett. 1988. "Determination and Characterization of a Cannabinoid Receptor in Rat Brain." *Molecular Pharmacology* 34 (5): 605–13.
Dhenain, M., S. W. Ruffins, and R. E. Jacobs. 2001. "Three-Dimensional Digital Mouse Atlas Using High-Resolution MRI." *Developmental Biology* 232 (2): 458–70.
Dietert, Rodney R., Janice M. Dietert, and Jamie C. Dewitt. 2011. "Environmental Risk Factors for Autism." *Emerging Health Threats Journal* 4 (April): 7111.
Di Forti, Marta, Arianna Marconi, Elena Carra, Sara Fraietta, Antonella Trotta, Matteo Bonomo, Francesca Bianconi, et al. 2015. "Proportion of Patients in South London with First-Episode Psychosis Attributable to Use of High Potency Cannabis: A Case-Control Study." *The Lancet. Psychiatry* 2 (3): 233–38.
Di Forti, Marta, Hannah Sallis, Fabio Allegri, Antonella Trotta, Laura Ferraro, Simona A. Stilo, Arianna Marconi, et al. 2014. "Daily Use, Especially of High-Potency Cannabis, Drives the Earlier Onset of Psychosis in Cannabis Users." *Schizophrenia Bulletin* 40 (6): 1509–17.
Dinarello, C. A. 2000. "Pro-inflammatory Cytokines." *Chest* 118 (2): 503–8.
Dobbing, J., and J. Sands. 1979. "Comparative Aspects of the Brain Growth Spurt." *Early Human Development* 3 (1): 79–83.
Doudna, Jennifer A., and Emmanuelle Charpentier. 2014. "The New Frontier of Genome Engineering with CRISPR-Cas9." *Science* 346 (6213). https://doi.org/10.1126/science.1258096.
Dreier, Julie Werenberg, Anne-Marie Nybo Andersen, and Gabriele Berg-Beckhoff. 2014. "Systematic Review and Meta-Analyses: Fever in Pregnancy and Health Impacts in the Offspring." *Pediatrics* 133 (3): e674–88.
D'Souza, Deepak Cyril, Edward Perry, Lisa MacDougall, Yola Ammerman, Thomas Cooper, Yu-Te Wu, Gabriel Braley, Ralitza Gueorguieva, and John Harrison Krystal. 2004. "The Psychotomimetic Effects of Intravenous Delta-9-Tetrahydrocannabinol in Healthy Individuals: Implications for Psychosis." *Neuropsychopharmacology: Official Publication of the American College of Neuropsychopharmacology* 29 (8): 1558–72.
Durand, Christelle M., Catalina Betancur, Tobias M. Boeckers, Juergen Bockmann, Pauline Chaste, Fabien Fauchereau, Gudrun Nygren, et al. 2006. "Mutations in the Gene Encoding the Synaptic Scaffolding Protein SHANK3 Are Associated with Autism Spectrum Disorders." *Nature Genetics* 39 (1): 25–27.
Eckart, Carl, and Gale Young. 1936. "The Approximation of One Matrix by Another of Lower Rank." *Psychometrika* 1 (3): 211–18.
Edlow, Andrea G. 2017. "Maternal Obesity and Neurodevelopmental and Psychiatric Disorders in Offspring." *Prenatal Diagnosis* 37 (1): 95–110.
Edwards, Marshall J. 2006. "Review: Hyperthermia and Fever during Pregnancy." *Birth Defects Research Part A: Clinical and Molecular Teratology*. https://doi.org/10.1002/bdra.20277.
Edwards, M. J., R. D. Saunders, and K. Shiota. 2003. "Effects of Heat on Embryos and Foetuses." *International Journal of Hyperthermia: The Official Journal of European Society for Hyperthermic Oncology, North American Hyperthermia Group* 19 (3): 295–324.
Ellegood, J., E. Anagnostou, B. A. Babineau, J. N. Crawley, L. Lin, M. Genestine, E. DiCicco-Bloom, et al. 2015. "Clustering Autism: Using Neuroanatomical Differences in 26 Mouse Models to Gain Insight into the Heterogeneity." *Molecular Psychiatry* 20 (1): 118–25.
Ellis, Shaun, Abdeslam Mouihate, and Quentin J. Pittman. 2005. "Early Life Immune Challenge Alters Innate Immune Responses to Lipopolysaccharide: Implications for Host Defense as

Adults." *FASEB Journal: Official Publication of the Federation of American Societies for Experimental Biology* 19 (11): 1519–21.
Ellman, Lauren M., Raymond F. Deicken, Sophia Vinogradov, William S. Kremen, John H. Poole, David M. Kern, Wei Yann Tsai, Catherine A. Schaefer, and Alan S. Brown. 2010. "Structural Brain Alterations in Schizophrenia Following Fetal Exposure to the Inflammatory Cytokine Interleukin-8." *Schizophrenia Research* 121 (1-3): 46–54.
Ellulu, Mohammed S., Ismail Patimah, Huzwah Khaza'ai, Asmah Rahmat, and Yehia Abed. 2017. "Obesity and Inflammation: The Linking Mechanism and the Complications." *Archives of Medical Science: AMS* 13 (4): 851–63.
Elmaoğlu, Muhammed, and Azim Çelik. 2011. *MRI Handbook: MR Physics, Patient Positioning, and Protocols*. Springer Science & Business Media.
ElSohly, Mahmoud A., Zlatko Mehmedic, Susan Foster, Chandrani Gon, Suman Chandra, and James C. Church. 2016. "Changes in Cannabis Potency Over the Last 2 Decades (1995–2014): Analysis of Current Data in the United States." *Biological Psychiatry* 79 (7): 613–19.
Enevoldsen, Maj N., Artur Kochoyan, Monika Jurgenson, Külli Jaako, Oksana Dmytriyeva, Peter S. Walmod, Jesper D. Nielsen, et al. 2012. "Neuroprotective and Memory Enhancing Properties of a Dual Agonist of the FGF Receptor and NCAM." *Neurobiology of Disease* 48 (3): 533–45.
Erlenmeyer-Kimling, L., Z. Folnegović, V. Hrabak-Zerjavić, B. Borcić, V. Folnegović-Smalc, and E. Susser. 1994. "Schizophrenia and Prenatal Exposure to the 1957 A2 Influenza Epidemic in Croatia." *The American Journal of Psychiatry* 151 (10): 1496–98.
Estes, Myka L., and A. Kimberley McAllister. 2016. "Maternal Immune Activation: Implications for Neuropsychiatric Disorders." *Science* 353 (6301): 772–77.
Estrada, G., M. Fatjó-Vilas, M. J. Muñoz, G. Pulido, M. J. Miñano, E. Toledo, J. M. Illa, et al. 2011a. "Cannabis Use and Age at Onset of Psychosis: Further Evidence of Interaction with COMT Val158Met Polymorphism." *Acta Psychiatrica Scandinavica*. https://doi.org/10.1111/j.1600-0447.2010.01665.x.
Estrada, G., M. Fatjó-Vilas, M. J. Muñoz, G. Pulido, M. J. Miñano, E. Toledo, J. M. Illa, et al. 2011b. "Cannabis Use and Age at Onset of Psychosis: Further Evidence of Interaction with COMT Val158Met Polymorphism." *Acta Psychiatrica Scandinavica* 123 (6): 485–92.
Evans, S. J., P. V. Choudary, C. R. Neal, J. Z. Li, M. P. Vawter, H. Tomita, J. F. Lopez, et al. 2004. "Dysregulation of the Fibroblast Growth Factor System in Major Depression." *Proceedings of the National Academy of Sciences of the United States of America* 101 (43): 15506–11.
Fa, Francesca, Laetitia Laup, Laurent Mandelbrot, Jeanne Sibiude, and Olivier Picone. 2019. "Fetal and Neonatal Abnormalities due to Congenital Herpes Simplex Virus Infection: A Literature Review." *Prenatal Diagnosis*, October. https://doi.org/10.1002/pd.5587.
Fang, Shao-You, Sabrina Wang, Nicole Huang, Hsueh-Han Yeh, and Chuan-Yu Chen. 2015. "Prenatal Infection and Autism Spectrum Disorders in Childhood: A Population-Based Case-Control Study in Taiwan." *Paediatric and Perinatal Epidemiology* 29 (4): 307–16.
Farokhnia, Mehdi, Gray R. McDiarmid, Matthew N. Newmeyer, Vikas Munjal, Osama A. Abulseoud, Marilyn A. Huestis, and Lorenzo Leggio. 2020. "Effects of Oral, Smoked, and Vaporized Cannabis on Endocrine Pathways Related to Appetite and Metabolism: A Randomized, Double-Blind, Placebo-Controlled, Human Laboratory Study." *Translational Psychiatry* 10 (1): 71.

Fatemi, S. H., E. S. Emamian, D. Kist, R. W. Sidwell, K. Nakajima, P. Akhter, A. Shier, S. Sheikh, and K. Bailey. 1999. "Defective Corticogenesis and Reduction in Reelin Immunoreactivity in Cortex and Hippocampus of Prenatally Infected Neonatal Mice." *Molecular Psychiatry* 4 (2): 145–54.

Fattore, Liana, and Walter Fratta. 2010. "How Important Are Sex Differences in Cannabinoid Action?" *British Journal of Pharmacology* 160 (3): 544–48.

Feifel, David, and Paul D. Shilling. 2013. *Animal Models for the Study of Human Disease: Chapter 30. Modeling Schizophrenia in Animals*. Elsevier Inc. Chapters.

Fergusson, David M., L. John Horwood, and Elizabeth M. Ridder. 2005. "Tests of Causal Linkages between Cannabis Use and Psychotic Symptoms." *Addiction* 100 (3): 354–66.

Fitzmaurice, G. M., N. M. Laird, and J. H. Ware. n.d. "Applied Longitudinal Analysis. 2011, 2nd Edn. Hoboken." NJ: John Wiley & Sons.

Freeman, Tom P., Teodora Groshkova, Andrew Cunningham, Roumen Sedefov, Paul Griffiths, and Michael T. Lynskey. 2019. "Increasing Potency and Price of Cannabis in Europe, 2006-16: Cannabis in Europe." *Addiction* 114 (6): 1015–23.

French, Leon, Courtney Gray, Gabriel Leonard, Michel Perron, G. Bruce Pike, Louis Richer, Jean R. Séguin, et al. 2015. "Early Cannabis Use, Polygenic Risk Score for Schizophrenia and Brain Maturation in Adolescence." *JAMA Psychiatry* 72 (10): 1002–11.

Freund, Tamas F., Istvan Katona, and Daniele Piomelli. 2003. "Role of Endogenous Cannabinoids in Synaptic Signaling." *Physiological Reviews* 83 (3): 1017–66.

Friedel, Miriam, Matthijs C. van Eede, Jon Pipitone, M. Mallar Chakravarty, and Jason P. Lerch. 2014. "Pydpiper: A Flexible Toolkit for Constructing Novel Registration Pipelines." *Frontiers in Neuroinformatics* 8 (July): 67.

Fuller Torrey, E., R. Rawlings, and R. H. Yolken. 2000. "The Antecedents of Psychoses: A Case–control Study of Selected Risk Factors." *Schizophrenia Research* 46 (1): 17–23.

Fusar-Poli, P., R. Smieskova, M. J. Kempton, B. C. Ho, N. C. Andreasen, and S. Borgwardt. 2013. "Progressive Brain Changes in Schizophrenia Related to Antipsychotic Treatment? A Meta-Analysis of Longitudinal MRI Studies." *Neuroscience and Biobehavioral Reviews* 37 (8): 1680–91.

Galloway, Dylan A., Alexandra E. M. Phillips, David R. J. Owen, and Craig S. Moore. 2019. "Phagocytosis in the Brain: Homeostasis and Disease." *Frontiers in Immunology* 10 (April): 790.

Galvez-Buccollini, Juan A., Ashley C. Proal, Veronica Tomaselli, Melissa Trachtenberg, Cristinel Coconcea, Jinsoo Chun, Theo Manschreck, Jerry Fleming, and Lynn E. Delisi. 2012. "Association between Age at Onset of Psychosis and Age at Onset of Cannabis Use in Non-Affective Psychosis." *Schizophrenia Research* 139 (1-3): 157–60.

Garay, Paula A., Elaine Y. Hsiao, Paul H. Patterson, and A. K. McAllister. 2013. "Maternal Immune Activation Causes Age- and Region-Specific Changes in Brain Cytokines in Offspring throughout Development." *Brain, Behavior, and Immunity*. https://doi.org/10.1016/j.bbi.2012.07.008.

Gardener, Hannah, Donna Spiegelman, and Stephen L. Buka. 2009. "Prenatal Risk Factors for Autism: Comprehensive Meta-Analysis." *The British Journal of Psychiatry: The Journal of Mental Science* 195 (1): 7–14.

Gardner, R. M., C. Dalman, S. Wicks, B. K. Lee, and H. Karlsson. 2013. "Neonatal Levels of Acute Phase Proteins and Later Risk of Non-Affective Psychosis." *Translational Psychiatry* 3 (February): e228.

Gaston, Tyler E., and Daniel Friedman. 2017. “Pharmacology of Cannabinoids in the Treatment of Epilepsy.” *Epilepsy & Behavior: E&B* 70 (Pt B): 313–18.
Geschwind, Daniel H. 2011. “Genetics of Autism Spectrum Disorders.” *Trends in Cognitive Sciences* 15 (9): 409–16.
Geschwind, Daniel H., and Matthew W. State. 2015. “Gene Hunting in Autism Spectrum Disorder: On the Path to Precision Medicine.” *Lancet Neurology* 14 (11): 1109–20.
Geuns, Robert-Jan M. van, Piotr A. Wielopolski, Hein G. de Bruin, Benno J. Rensing, Peter M. A. van Ooijen, Marc Hulshoff, Matthijs Oudkerk, and Pim J. de Feyter. 1999. “Basic Principles of Magnetic Resonance Imaging☆.” *Progress in Cardiovascular Diseases*. https://doi.org/10.1016/s0033-0620(99)70014-9.
Giedd, J. N., J. Blumenthal, N. O. Jeffries, F. X. Castellanos, H. Liu, A. Zijdenbos, T. Paus, A. C. Evans, and J. L. Rapoport. 1999. “Brain Development during Childhood and Adolescence: A Longitudinal MRI Study.” *Nature Neuroscience* 2 (10): 861–63.
Gilman, Stephen E., Mady Hornig, Akhgar Ghassabian, Jill Hahn, Sara Cherkerzian, Paul S. Albert, Stephen L. Buka, and Jill M. Goldstein. 2017. “Socioeconomic Disadvantage, Gestational Immune Activity, and Neurodevelopment in Early Childhood.” *Proceedings of the National Academy of Sciences of the United States of America* 114 (26): 6728–33.
Gilmore, John H., Lars Fredrik Jarskog, Swarooparani Vadlamudi, and Jean M. Lauder. 2004. “Prenatal Infection and Risk for Schizophrenia: IL-1beta, IL-6, and TNFalpha Inhibit Cortical Neuron Dendrite Development.” *Neuropsychopharmacology: Official Publication of the American College of Neuropsychopharmacology* 29 (7): 1221–29.
Ginhoux, Florent, Melanie Greter, Marylene Leboeuf, Sayan Nandi, Peter See, Solen Gokhan, Mark F. Mehler, et al. 2010. “Fate Mapping Analysis Reveals That Adult Microglia Derive from Primitive Macrophages.” *Science* 330 (6005): 841–45.
Ginovart, Nathalie, Benjamin B. Tournier, Marcelle Moulin-Sallanon, Thierry Steimer, Vicente Ibanez, and Philippe Millet. 2012. “Chronic Δ9-Tetrahydrocannabinol Exposure Induces a Sensitization of Dopamine D2/3 Receptors in the Mesoaccumbens and Nigrostriatal Systems.” *Neuropsychopharmacology*. https://doi.org/10.1038/npp.2012.91.
Ginsberg, Yuval, Nizar Khatib, Boaz Weiss, Shay Arison, Michael G. Ross, Zeev Weiner, and Ron Beloosesky. 2017. “Magnesium Sulfate (MG) Prevents Maternal Inflammation Induced Offspring Cerebral Injury Evident on MRI but Not via IL-1β.” *Neuroscience* 353 (June): 98–105.
Giovanoli, Sandra, Harald Engler, Andrea Engler, Juliet Richetto, Mareike Voget, Roman Willi, Christine Winter, et al. 2013. “Stress in Puberty Unmasks Latent Neuropathological Consequences of Prenatal Immune Activation in Mice.” *Science* 339 (6123): 1095–99.
Giovanoli, Sandra, Tina Notter, Juliet Richetto, Marie A. Labouesse, Stéphanie Vuillermot, Marco A. Riva, and Urs Meyer. 2015. “Late Prenatal Immune Activation Causes Hippocampal Deficits in the Absence of Persistent Inflammation across Aging.” *Journal of Neuroinflammation* 12 (November): 221.
Giovanoli, Sandra, Ulrike Weber-Stadlbauer, Manfred Schedlowski, Urs Meyer, and Harald Engler. 2016. “Prenatal Immune Activation Causes Hippocampal Synaptic Deficits in the Absence of Overt Microglia Anomalies.” *Brain, Behavior, and Immunity* 55 (July): 25–38.
Girard, Sylvie, Luc Tremblay, Martin Lepage, and Guillaume Sébire. 2010. “IL-1 Receptor Antagonist Protects against Placental and Neurodevelopmental Defects Induced by Maternal Inflammation.” *Journal of Immunology* 184 (7): 3997–4005.
Girchenko, Polina, Marius Lahti-Pulkkinen, Kati Heinonen, Rebecca M. Reynolds, Hannele

Laivuori, Jari Lipsanen, Pia M. Villa, et al. 2020. “Persistently High Levels of Maternal Antenatal Inflammation Are Associated With and Mediate the Effect of Prenatal Environmental Adversities on Neurodevelopmental Delay in the Offspring.” *Biological Psychiatry* 87 (10): 898–907.

Gleason, K. A., S. G. Birnbaum, A. Shukla, and S. Ghose. 2012. “Susceptibility of the Adolescent Brain to Cannabinoids: Long-Term Hippocampal Effects and Relevance to Schizophrenia.” *Translational Psychiatry* 2 (November): e199.

Głodek, Joanna, Zbigniew Adamiak, and Adam Przeworski. 2016. “Magnetic Resonance Imaging of Reptiles, Rodents, and Lagomorphs for Clinical Diagnosis and Animal Research.” *Comparative Medicine* 66 (3): 216–19.

Gluckman, Peter D., Mark A. Hanson, Cyrus Cooper, and Kent L. Thornburg. 2008. “Effect of in Utero and Early-Life Conditions on Adult Health and Disease.” *The New England Journal of Medicine* 359 (1): 61–73.

Goerzen, Dana, Caitlin Fowler, Gabriel A. Devenyi, Jurgen Germann, Dan Madularu, M. Mallar Chakravarty, and Jamie Near. 2020. “An MRI-Derived Neuroanatomical Atlas of the Fischer 344 Rat Brain.” *Scientific Reports* 10 (1): 6952.

Gogos, Andrea, Alyssa Sbisa, Diede Witkamp, and Maarten van den Buuse. 2020. “Sex Differences in the Effect of Maternal Immune Activation on Cognitive and Psychosis-like Behaviour in Long Evans Rats.” *The European Journal of Neuroscience* 52 (1): 2614–26.

Gogtay, Nitin, Jay N. Giedd, Leslie Lusk, Kiralee M. Hayashi, Deanna Greenstein, A. Catherine Vaituzis, Tom F. Nugent 3rd, et al. 2004. “Dynamic Mapping of Human Cortical Development during Childhood through Early Adulthood.” *Proceedings of the National Academy of Sciences of the United States of America* 101 (21): 8174–79.

Goines, Paula E., Lisa A. Croen, Daniel Braunschweig, Cathleen K. Yoshida, Judith Grether, Robin Hansen, Martin Kharrazi, Paul Ashwood, and Judy Van de Water. 2011. “Increased Midgestational IFN-γ, IL-4 and IL-5 in Women Bearing a Child with Autism: A Case-Control Study.” *Molecular Autism* 2 (August): 13.

Gomes, Felipe V., Francisco S. Guimarães, and Anthony A. Grace. 2014. “Effects of Pubertal Cannabinoid Administration on Attentional Set-Shifting and Dopaminergic Hyper-Responsivity in a Developmental Disruption Model of Schizophrenia.” *The International Journal of Neuropsychopharmacology / Official Scientific Journal of the Collegium Internationale Neuropsychopharmacologicum* 18 (2). https://doi.org/10.1093/ijnp/pyu018.

Gomes, Felipe V., Millie Rincón-Cortés, and Anthony A. Grace. 2016. “Adolescence as a Period of Vulnerability and Intervention in Schizophrenia: Insights from the MAM Model.” *Neuroscience and Biobehavioral Reviews* 70 (November): 260–70.

Goronzy, Jörg J., and Cornelia M. Weyand. 2012. “The Innate and Adaptive Immune Systems.” *Goldman’s Cecil Medicine*. https://doi.org/10.1016/b978-1-4377-1604-7.00044-0.

Gow, Rachel V., and Joseph R. Hibbeln. 2014. “Omega-3 Fatty Acid and Nutrient Deficits in Adverse Neurodevelopment and Childhood Behaviors.” *Child and Adolescent Psychiatric Clinics of North America* 23 (3): 555–90.

Graciarena, Mariana, Valeria Roca, Patricia Mathieu, Amaicha M. Depino, and Fernando J. Pitossi. 2013. “Differential Vulnerability of Adult Neurogenesis by Adult and Prenatal Inflammation: Role of TGF-β1.” *Brain, Behavior, and Immunity* 34 (November): 17–28.

Graham, Alice M., Jerod M. Rasmussen, Marc D. Rudolph, Christine M. Heim, John H. Gilmore, Martin Styner, Steven G. Potkin, et al. 2018. “Maternal Systemic Interleukin-6 During Pregnancy Is Associated With Newborn Amygdala Phenotypes and Subsequent

Behavior at 2 Years of Age." *Biological Psychiatry* 83 (2): 109–19.
Grether, Judith K., Lisa A. Croen, Meredith C. Anderson, Karin B. Nelson, and Robert H. Yolken. 2010. "Neonatally Measured Immunoglobulins and Risk of Autism." *Autism Research: Official Journal of the International Society for Autism Research* 3 (6): 323–32.
Guma, Elisa, Eric Plitman, and M. Mallar Chakravarty. 2019. "The Role of Maternal Immune Activation in Altering the Neurodevelopmental Trajectories of Offspring: A Translational Review of Neuroimaging Studies with Implications for Autism Spectrum Disorder and Schizophrenia." *Neuroscience and Biobehavioral Reviews*. https://www.sciencedirect.com/science/article/pii/S0149763419302088.
Gumusoglu, Serena B., Rebecca S. Fine, Samuel J. Murray, Jada L. Bittle, and Hanna E. Stevens. 2017. "The Role of IL-6 in Neurodevelopment after Prenatal Stress." *Brain, Behavior, and Immunity* 65 (October): 274–83.
Gumusoglu, Serena B., and Hanna E. Stevens. 2019. "Maternal Inflammation and Neurodevelopmental Programming: A Review of Preclinical Outcomes and Implications for Translational Psychiatry." *Biological Psychiatry* 85 (2): 107–21.
Haida, Obelia, Tareq Al Sagheer, Anais Balbous, Maureen Francheteau, Emmanuel Matas, Federico Soria, Pierre Olivier Fernagut, and Mohamed Jaber. 2019. "Sex-Dependent Behavioral Deficits and Neuropathology in a Maternal Immune Activation Model of Autism." *Translational Psychiatry* 9 (1): 124.
Hall, Wayne, Louisa Degenhardt, and Maree Teesson. 2004. "Cannabis Use and Psychotic Disorders: An Update." *Drug and Alcohol Review* 23 (4): 433–43.
Hanamsagar, Richa, and Staci D. Bilbo. 2016. "Sex Differences in Neurodevelopmental and Neurodegenerative Disorders: Focus on Microglial Function and Neuroinflammation during Development." *The Journal of Steroid Biochemistry and Molecular Biology* 160 (June): 127–33.
Hantsoo, Liisa, Sara Kornfield, Montserrat C. Anguera, and C. Neill Epperson. 2019. "Inflammation: A Proposed Intermediary Between Maternal Stress and Offspring Neuropsychiatric Risk." *Biological Psychiatry* 85 (2): 97–106.
Han, Velda X., Shrujna Patel, Hannah F. Jones, Timothy C. Nielsen, Shekeeb S. Mohammad, Markus J. Hofer, Wendy Gold, et al. 2021. "Maternal Acute and Chronic Inflammation in Pregnancy Is Associated with Common Neurodevelopmental Disorders: A Systematic Review." *Translational Psychiatry* 11 (1): 71.
Hao, L. Y., X. Q. Hao, S. H. Li, and X. H. Li. 2010. "Prenatal Exposure to Lipopolysaccharide Results in Cognitive Deficits in Age-Increasing Offspring Rats." *Neuroscience* 166 (3): 763–70.
Harkany, Tibor, Manuel Guzmán, Ismael Galve-Roperh, Paul Berghuis, Lakshmi A. Devi, and Ken Mackie. 2007. "The Emerging Functions of Endocannabinoid Signaling during CNS Development." *Trends in Pharmacological Sciences* 28 (2): 83–92.
Harkany, Tibor, Erik Keimpema, Klaudia Barabás, and Jan Mulder. 2008. "Endocannabinoid Functions Controlling Neuronal Specification during Brain Development." *Molecular and Cellular Endocrinology* 286 (1-2 Suppl 1): S84–90.
Harrison, Xavier A., Lynda Donaldson, Maria Eugenia Correa-Cano, Julian Evans, David N. Fisher, Cecily Goodwin, Beth Robinson, David J. Hodgson, and Richard Inger. n.d. "A Brief Introduction to Mixed Effects Modelling and Multi-Model Inference in Ecology." https://doi.org/10.7287/peerj.preprints.3113.
Harvey, Louise, and Patricia Boksa. 2014a. "Do Prenatal Immune Activation and Maternal Iron

Deficiency Interact to Affect Neurodevelopment and Early Behavior in Rat Offspring?" *Brain, Behavior, and Immunity* 35 (January): 144–54.

Harvey, Louise, and Patricia Boksa. 2014b. "Additive Effects of Maternal Iron Deficiency and Prenatal Immune Activation on Adult Behaviors in Rat Offspring." *Brain, Behavior, and Immunity* 40 (August): 27–37.

Hava, Golan, Lev Vered, Mazar Yael, Hallak Mordechai, and Huleihel Mahoud. 2006. "Alterations in Behavior in Adult Offspring Mice Following Maternal Inflammation during Pregnancy." *Developmental Psychobiology* 48 (2): 162–68.

Heerema-McKenney, Amy. 2018. "Defense and Infection of the Human Placenta." *APMIS: Acta Pathologica, Microbiologica, et Immunologica Scandinavica* 126 (7): 570–88.

Henquet, Cécile, Lydia Krabbendam, Janneke Spauwen, Charles Kaplan, Roselind Lieb, Hans-Ulrich Wittchen, and Jim van Os. 2004. "Prospective Cohort Study of Cannabis Use, Predisposition for Psychosis, and Psychotic Symptoms in Young People." *BMJ* 330 (7481): 11.

Herbert, C., G. Lassalle, and C. Alcouffe. 2014. Approaches targeting the FGF–FGFR system: A review of the recent patent literature and associated advanced therapeutic agents. *Pharmaceutical Patent*, issued 2014. https://www.future-science.com/doi/abs/10.4155/ppa.14.45.

Herkenham, M., A. B. Lynn, M. R. Johnson, L. S. Melvin, B. R. de Costa, and K. C. Rice. 1991. "Characterization and Localization of Cannabinoid Receptors in Rat Brain: A Quantitative in Vitro Autoradiographic Study." *The Journal of Neuroscience: The Official Journal of the Society for Neuroscience* 11 (2): 563–83.

Hickman, Matthew, Peter Vickerman, John Macleod, James Kirkbride, and Peter B. Jones. 2007. "Cannabis and Schizophrenia: Model Projections of the Impact of the Rise in Cannabis Use on Historical and Future Trends in Schizophrenia in England and Wales." *Addiction* 102 (4): 597–606.

Hicks, Steven D., and Frank A. Middleton. 2016. "A Comparative Review of microRNA Expression Patterns in Autism Spectrum Disorder." *Frontiers in Psychiatry / Frontiers Research Foundation* 7 (November): 176.

Hindley, Guy, Katherine Beck, Faith Borgan, Cedric E. Ginestet, Robert McCutcheon, Daniel Kleinloog, Suhas Ganesh, Rajiv Radhakrishnan, Deepak Cyril D'Souza, and Oliver D. Howes. 2020. "Psychiatric Symptoms Caused by Cannabis Constituents: A Systematic Review and Meta-Analysis." *The Lancet. Psychiatry* 7 (4): 344–53.

Hollins, Sharon L., Katerina Zavitsanou, Frederick Rohan Walker, and Murray J. Cairns. 2016. "Alteration of Transcriptional Networks in the Entorhinal Cortex after Maternal Immune Activation and Adolescent Cannabinoid Exposure." *Brain, Behavior, and Immunity* 56 (August): 187–96.

Hollins, S. L., K. Zavitsanou, F. R. Walker, and M. J. Cairns. 2014. "Alteration of Imprinted Dlk1-Dio3 miRNA Cluster Expression in the Entorhinal Cortex Induced by Maternal Immune Activation and Adolescent Cannabinoid Exposure." *Translational Psychiatry* 4 (September): e452.

Hornig, M., M. A. Bresnahan, X. Che, A. F. Schultz, J. E. Ukaigwe, M. L. Eddy, D. Hirtz, et al. 2018. "Prenatal Fever and Autism Risk." *Molecular Psychiatry* 23 (3): 759–66.

Horwood, L. John, David M. Fergusson, Carolyn Coffey, George C. Patton, Robert Tait, Diana Smart, Primrose Letcher, Edmund Silins, and Delyse M. Hutchinson. 2012. "Cannabis and Depression: An Integrative Data Analysis of Four Australasian Cohorts." *Drug and Alcohol*

Dependence 126 (3): 369–78.
Howerton, Christopher L., and Tracy L. Bale. 2012. "Prenatal Programing: At the Intersection of Maternal Stress and Immune Activation." *Hormones and Behavior* 62 (3): 237–42.
Hsiao, Elaine Y., Sara W. McBride, Sophia Hsien, Gil Sharon, Embriette R. Hyde, Tyler McCue, Julian A. Codelli, et al. 2013. "Microbiota Modulate Behavioral and Physiological Abnormalities Associated with Neurodevelopmental Disorders." *Cell* 155 (7): 1451–63.
Hsiao, Elaine Y., and Paul H. Patterson. 2012. "Placental Regulation of Maternal-Fetal Interactions and Brain Development." *Developmental Neurobiology* 72 (10): 1317–26.
Huestis, M. A., J. E. Henningfield, and E. J. Cone. 1992. "Blood Cannabinoids. I. Absorption of THC and Formation of 11-OH-THC and THCCOOH during and after Smoking Marijuana." *Journal of Analytical Toxicology* 16 (5): 276–82.
Huestis, Marilyn A. 2007. "Human Cannabinoid Pharmacokinetics." *Chemistry & Biodiversity* 4 (8): 1770–1804.
Huestis, M. A., A. H. Sampson, B. J. Holicky, J. E. Henningfield, and E. J. Cone. 1992. "Characterization of the Absorption Phase of Marijuana Smoking." *Clinical Pharmacology and Therapeutics* 52 (1): 31–41.
Hui, Chin Wai, Haley A. Vecchiarelli, Étienne Gervais, Xiao Luo, Félix Michaud, Lisa Scheefhals, Kanchan Bisht, Kaushik Sharma, Lisa Topolnik, and Marie-Ève Tremblay. 2020. "Sex Differences of Microglia and Synapses in the Hippocampal Dentate Gyrus of Adult Mouse Offspring Exposed to Maternal Immune Activation." *Frontiers in Cellular Neuroscience* 14 (October): 558181.
Hui, Chin W., Abygaël St-Pierre, Hassan El Hajj, Yvan Remy, Sébastien S. Hébert, Giamal N. Luheshi, Lalit K. Srivastava, and Marie-Ève Tremblay. 2018. "Prenatal Immune Challenge in Mice Leads to Partly Sex-Dependent Behavioral, Microglial, and Molecular Abnormalities Associated with Schizophrenia." *Frontiers in Molecular Neuroscience* 11 (February): 13.
Hutchins, Kenneth D., Dennis W. Dickson, William K. Rashbaum, and William D. Lyman. 1990. "Localization of Morphologically Distinct Microglial Populations in the Developing Human Fetal Brain: Implications for Ontogeny." *Developmental Brain Research*. https://doi.org/10.1016/0165-3806(90)90109-c.
Hutton, Lisa C., Margie Castillo-Melendez, George A. Smythe, and David W. Walker. 2008. "Microglial Activation, Macrophage Infiltration, and Evidence of Cell Death in the Fetal Brain after Uteroplacental Administration of Lipopolysaccharide in Sheep in Late Gestation." *American Journal of Obstetrics and Gynecology*. https://doi.org/10.1016/j.ajog.2007.06.035.
Iede, Montaha Al, Kenneth Nunn, Bronwyn Milne, and Dominic A. Fitzgerald. 2017. "The Consequences of Chronic Cannabis Smoking in Vulnerable Adolescents." *Paediatric Respiratory Reviews* 24 (September): 44–53.
Inui, Toshio, Shinichiro Kumagaya, and Masako Myowa-Yamakoshi. 2017. "Neurodevelopmental Hypothesis about the Etiology of Autism Spectrum Disorders." *Frontiers in Human Neuroscience* 11 (July): 354.
Jacobs, R. E., E. T. Ahrens, M. E. Dickinson, and D. Laidlaw. 1999. "Towards a microMRI Atlas of Mouse Development." *Computerized Medical Imaging and Graphics: The Official Journal of the Computerized Medical Imaging Society* 23 (1): 15–24.
Jamain, Stéphane, Paris Autism Research International Sibpair Study, Hélène Quach, Catalina Betancur, Maria Råstam, Catherine Colineaux, I. Carina Gillberg, et al. 2003. "Mutations of

the X-Linked Genes Encoding Neuroligins NLGN3 and NLGN4 Are Associated with Autism." *Nature Genetics*. https://doi.org/10.1038/ng1136.
Jamieson, Denise J., Regan N. Theiler, and Sonja A. Rasmussen. 2006. "Emerging Infections and Pregnancy." *Emerging Infectious Diseases* 12 (11): 1638–43.
Jarskog, L. Fredrik, L. Fredrik Jarskog, Hong Xiao, Mary Beth Wilkie, Jean M. Lauder, and John H. Gilmore. 1997. "Cytokine Regulation of Embryonic Rat Dopamine and Serotonin Neuronal Survival in Vitro." *International Journal of Developmental Neuroscience*. https://doi.org/10.1016/s0736-5748(97)00029-4.
Jiang, Hai-Yin, Lian-Lian Xu, Li Shao, Rong-Man Xia, Zheng-He Yu, Zong-Xin Ling, Fan Yang, Min Deng, and Bing Ruan. 2016. "Maternal Infection during Pregnancy and Risk of Autism Spectrum Disorders: A Systematic Review and Meta-Analysis." *Brain, Behavior, and Immunity* 58 (November): 165–72.
Jockers-Scherübl, Maria C., Johannes Rentzsch, Heidi Danker-Hopfe, Nicole Radzei, Falk Schürer, Sharif Bahri, and Rainer Hellweg. 2006. "Adequate Antipsychotic Treatment Normalizes Serum Nerve Growth Factor Concentrations in Schizophrenia with and without Cannabis or Additional Substance Abuse." *Neuroscience Letters* 400 (3): 262–66.
Jonakait, G. Miller. 2007. "The Effects of Maternal Inflammation on Neuronal Development: Possible Mechanisms." *International Journal of Developmental Neuroscience: The Official Journal of the International Society for Developmental Neuroscience* 25 (7): 415–25.
Joseph, Robert M., Helen Tager-Flusberg, and Catherine Lord. 2002. "Cognitive Profiles and Social-Communicative Functioning in Children with Autism Spectrum Disorder." *Journal of Child Psychology and Psychiatry, and Allied Disciplines* 43 (6): 807–21.
Joshi, Gagan, Stephen V. Faraone, Janet Wozniak, Laura Tarko, Ronna Fried, Maribel Galdo, Stephannie L. Furtak, and Joseph Biederman. 2017. "Symptom Profile of ADHD in Youth With High-Functioning Autism Spectrum Disorder: A Comparative Study in Psychiatrically Referred Populations." *Journal of Attention Disorders* 21 (10): 846–55.
Juckel, Georg, Marie Pierre Manitz, Martin Brüne, Astrid Friebe, Michael T. Heneka, and Rainer J. Wolf. 2011. "Microglial Activation in a Neuroinflammational Animal Model of Schizophrenia — a Pilot Study." *Schizophrenia Research*. https://doi.org/10.1016/j.schres.2011.06.018.
Kahn, René S., Iris E. Sommer, Robin M. Murray, Andreas Meyer-Lindenberg, Daniel R. Weinberger, Tyrone D. Cannon, Michael O'Donovan, et al. 2015. "Schizophrenia." *Nature Reviews Disease Primers*. https://doi.org/10.1038/nrdp.2015.67.
Kalish, Brian T., Eunha Kim, Benjamin Finander, Erin E. Duffy, Hyunju Kim, Casey K. Gilman, Yeong Shin Yim, et al. 2020. "Maternal Immune Activation in Mice Disrupts Proteostasis in the Fetal Brain." *Nature Neuroscience*, December. https://doi.org/10.1038/s41593-020-00762-9.
Kang, Silvia S., Aishe Kurti, Damien A. Fair, and John D. Fryer. 2014. "Dietary Intervention Rescues Maternal Obesity Induced Behavior Deficits and Neuroinflammation in Offspring." *Journal of Neuroinflammation* 11 (September): 156.
Karalis, K., L. Crofford, R. L. Wilder, and G. P. Chrousos. 1995. "Glucocorticoid And/or Glucocorticoid Antagonist Effects in Inflammatory Disease-Susceptible Lewis Rats and Inflammatory Disease-Resistant Fischer Rats." *Endocrinology* 136 (7): 3107–12.
Kendell, R. E., and I. W. Kemp. 1989. "Maternal Influenza in the Etiology of Schizophrenia." *Archives of General Psychiatry* 46 (10): 878–82.
Kent, A. S., M. H. Sullivan, and M. G. Elder. 1994. "Transfer of Cytokines through Human Fetal

Membranes." *Journal of Reproduction and Fertility* 100 (1): 81–84.
Kentner, Amanda C., Staci D. Bilbo, Alan S. Brown, Elaine Y. Hsiao, A. Kimberley McAllister, Urs Meyer, Brad D. Pearce, Mikhail V. Pletnikov, Robert H. Yolken, and Melissa D. Bauman. 2019. "Maternal Immune Activation: Reporting Guidelines to Improve the Rigor, Reproducibility, and Transparency of the Model." *Neuropsychopharmacology: Official Publication of the American College of Neuropsychopharmacology* 44 (2): 245–58.
Keshavan, Matcheri S., Jay Giedd, Jennifer Y. F. Lau, David A. Lewis, and Tomáš Paus. 2014. "Changes in the Adolescent Brain and the Pathophysiology of Psychotic Disorders." *The Lancet. Psychiatry* 1 (7): 549–58.
Keshavan, Matcheri S., and Tomas Paus. 2015. "Neurodevelopmental Trajectories, Disconnection, and Schizophrenia Risk." *JAMA Psychiatry* .
Khan, D., P. Fernando, A. Cicvaric, A. Berger, A. Pollak, F. J. Monje, and D. D. Pollak. 2014. "Long-Term Effects of Maternal Immune Activation on Depression-like Behavior in the Mouse." *Translational Psychiatry* 4 (February): e363.
Kilbourne, Edwin D. 2006. "Influenza Pandemics of the 20th Century." *Emerging Infectious Diseases* 12 (1): 9.
Kim, S., Y. Hwang, M. J. Webster, and D. Lee. 2016. "Differential Activation of Immune/inflammatory Response-Related Co-Expression Modules in the Hippocampus across the Major Psychiatric Disorders." *Molecular Psychiatry* 21 (3): 376–85.
Kimura, Akihiro, and Tadamitsu Kishimoto. 2010. "IL-6: Regulator of Treg/Th17 Balance." *European Journal of Immunology* 40 (7): 1830–35.
Kirschner, Matthias, Golia Shafiei, Ross D. Markello, Carolina Makowski, Alexandra Talpalaru, Benazir Hodzic-Santor, Gabriel A. Devenyi, et al. 2020. "Latent Clinical-Anatomical Dimensions of Schizophrenia." *Schizophrenia Bulletin* 46 (6): 1426–38.
Klebanoff, Mark A. 2009. "The Collaborative Perinatal Project: A 50-Year Retrospective." *Paediatric and Perinatal Epidemiology* 23 (1): 2–8.
Kleinmans, Maren, and David K. Bilkey. 2018. "Reversal Learning Impairments in the Maternal Immune Activation Rat Model of Schizophrenia." *Behavioral Neuroscience* 132 (6): 520–25.
Kluger, M. J., W. Kozak, C. A. Conn, L. R. Leon, and D. Soszynski. 1998. "Role of Fever in Disease." *Annals of the New York Academy of Sciences* 856 (September): 224–33.
Knuesel, Irene, Laurie Chicha, Markus Britschgi, Scott A. Schobel, Michael Bodmer, Jessica A. Hellings, Stephen Toovey, and Eric P. Prinssen. 2014. "Maternal Immune Activation and Abnormal Brain Development across CNS Disorders." *Nature Reviews. Neurology* 10 (11): 643–60.
Koenders, Laura, Janna Cousijn, Wilhelmina A. M. Vingerhoets, Wim van den Brink, Reinout W. Wiers, Carin J. Meijer, Marise W. J. Machielsen, Dick J. Veltman, Anneke E. Goudriaan, and Lieuwe de Haan. 2016. "Grey Matter Changes Associated with Heavy Cannabis Use: A Longitudinal sMRI Study." *PloS One* 11 (5): e0152482.
Kohn, Laurence, France Kittel, and Danielle Piette. 2004. "Peer, Family Integration and Other Determinants of Cannabis Use among Teenagers." *International Journal of Adolescent Medicine and Health* 16 (4): 359–70.
Kowash, H. M., H. G. Potter, M. E. Edye, E. P. Prinssen, S. Bandinelli, J. C. Neill, R. Hager, and J. D. Glazier. 2019. "Poly(I:C) Source, Molecular Weight and Endotoxin Contamination Affect Dam and Prenatal Outcomes, Implications for Models of Maternal Immune Activation." *Brain, Behavior, and Immunity* 82 (November): 160–66.

Koyama, Ryuta, and Yuji Ikegaya. 2015. “Microglia in the Pathogenesis of Autism Spectrum Disorders.” *Neuroscience Research* 100 (November): 1–5.
Kukurba, Kimberly R., and Stephen B. Montgomery. 2015. “RNA Sequencing and Analysis.” *Cold Spring Harbor Protocols* 2015 (11): 951–69.
Labrousse, V. F., Q. Leyrolle, C. Amadieu, A. Aubert, A. Sere, E. Coutureau, S. Grégoire, et al. 2018. “Dietary Omega-3 Deficiency Exacerbates Inflammation and Reveals Spatial Memory Deficits in Mice Exposed to Lipopolysaccharide during Gestation.” *Brain, Behavior, and Immunity* 73 (October): 427–40.
Lanté, Fabien, Johann Meunier, Janique Guiramand, Marie-Céleste De Jesus Ferreira, Gilles Cambonie, Rose Aimar, Catherine Cohen-Solal, Tangui Maurice, Michel Vignes, and Gérard Barbanel. 2008. “LateN-Acetylcysteine Treatment Prevents the Deficits Induced in the Offspring of Dams Exposed to an Immune Stress during Gestation.” *Hippocampus* 18 (6): 602–9.
Lanté, Fabien, Johann Meunier, Janique Guiramand, Tangui Maurice, Mélanie Cavalier, Marie-Céleste de Jesus Ferreira, Rose Aimar, Catherine Cohen-Solal, Michel Vignes, and Gérard Barbanel. 2007. “Neurodevelopmental Damage after Prenatal Infection: Role of Oxidative Stress in the Fetal Brain.” *Free Radical Biology & Medicine* 42 (8): 1231–45.
LaRossa, R. A. 2013. “Transcriptome.”
Lavdas, A. A., M. Grigoriou, V. Pachnis, and J. G. Parnavelas. 1999. “The Medial Ganglionic Eminence Gives Rise to a Population of Early Neurons in the Developing Cerebral Cortex.” *The Journal of Neuroscience: The Official Journal of the Society for Neuroscience* 19 (18): 7881–88.
Law, B., P. A. Mason, A. C. Moffat, R. I. Gleadle, and L. J. King. 1984. “Forensic Aspects of the Metabolism and Excretion of Cannabinoids Following Oral Ingestion of Cannabis Resin.” *The Journal of Pharmacy and Pharmacology* 36 (5): 289–94.
Le Belle, Janel E., Jantzen Sperry, Amy Ngo, Yasmin Ghochani, Dan R. Laks, Manuel López-Aranda, Alcino J. Silva, and Harley I. Kornblum. 2014. “Maternal Inflammation Contributes to Brain Overgrowth and Autism-Associated Behaviors through Altered Redox Signaling in Stem and Progenitor Cells.” *Stem Cell Reports* 3 (5): 725–34.
Lecca, Salvatore, Antonio Luchicchi, Maria Scherma, Paola Fadda, Anna Lisa Muntoni, and Marco Pistis. 2019. “Δ9-Tetrahydrocannabinol During Adolescence Attenuates Disruption of Dopamine Function Induced in Rats by Maternal Immune Activation.” *Frontiers in Behavioral Neuroscience* 13 (September): 202.
Lee, Hansongyi, In Seok Lee, and Ryowon Choue. 2013. “Obesity, Inflammation and Diet.” *Pediatric Gastroenterology, Hepatology & Nutrition*. https://doi.org/10.5223/pghn.2013.16.3.143.
Lee, Younga H., Sara Cherkerzian, Larry J. Seidman, George D. Papandonatos, David A. Savitz, Ming T. Tsuang, Jill M. Goldstein, and Stephen L. Buka. 2020. “Maternal Bacterial Infection During Pregnancy and Offspring Risk of Psychotic Disorders: Variation by Severity of Infection and Offspring Sex.” *The American Journal of Psychiatry* 177 (1): 66–75.
Lemberger, L., S. D. Silberstein, J. Axelrod, and I. J. Kopin. 1970. “Marihuana: Studies on the Disposition and Metabolism of Delta-9-Tetrahydrocannabinol in Man.” *Science* 170 (3964): 1320–22.
Lenroot, Rhoshel K., and Jay N. Giedd. 2006. “Brain Development in Children and Adolescents: Insights from Anatomical Magnetic Resonance Imaging.” *Neuroscience & Biobehavioral*

Reviews. https://doi.org/10.1016/j.neubiorev.2006.06.001.
Lenz, Kathryn M., Bridget M. Nugent, Rachana Haliyur, and Margaret M. McCarthy. 2013. "Microglia Are Essential to Masculinization of Brain and Behavior." *The Journal of Neuroscience: The Official Journal of the Society for Neuroscience* 33 (7): 2761–72.
Lepore, Natasha, Caroline A. Brun, Ming-Chang Chiang, Yi-Yu Chou, Rebecca A. Dutton, Kiralee M. Hayashi, Oscar L. Lopez, et al. 2006. "Multivariate Statistics of the Jacobian Matrices in Tensor Based Morphometry and Their Application to HIV/AIDS." *Medical Image Computing and Computer-Assisted Intervention: MICCAI ... International Conference on Medical Image Computing and Computer-Assisted Intervention* 9 (Pt 1): 191–98.
Lerch, Jason P., Lisa Gazdzinski, Jürgen Germann, John G. Sled, R. Mark Henkelman, and Brian J. Nieman. 2012. "Wanted Dead or Alive? The Tradeoff between in-Vivo versus Ex-Vivo MR Brain Imaging in the Mouse." *Frontiers in Neuroinformatics* 6 (March): 6.
Lerch, Jason P., André J. W. van der Kouwe, Armin Raznahan, Tomáš Paus, Heidi Johansen-Berg, Karla L. Miller, Stephen M. Smith, Bruce Fischl, and Stamatios N. Sotiropoulos. 2017. "Studying Neuroanatomy Using MRI." *Nature Neuroscience* 20 (3): 314–26.
Letterio, J. J., A. G. Geiser, A. B. Kulkarni, N. S. Roche, M. B. Sporn, and A. B. Roberts. 1994. "Maternal Rescue of Transforming Growth Factor-Beta 1 Null Mice." *Science* 264 (5167): 1936–38.
Levin, S. G., and O. V. Godukhin. 2017. "Modulating Effect of Cytokines on Mechanisms of Synaptic Plasticity in the Brain." *Biochemistry. Biokhimiia* 82 (3): 264–74.
Lewis, Fraser, Adam Butler, and Lucy Gilbert. 2011. "A Unified Approach to Model Selection Using the Likelihood Ratio Test." *Methods in Ecology and Evolution / British Ecological Society* 2 (2): 155–62.
Lim, K-L, P. Jacobs, A. Ohinmaa, D. Schopflocher, and C. S. Dewa. 2008. "A New Population-Based Measure of the Economic Burden of Mental Illness in Canada." *Chronic Diseases in Canada* 28 (3): 92–98.
Ling, Zaodung, Yuangui Zhu, Chong Wai Tong, Joshua A. Snyder, Jack W. Lipton, and Paul M. Carvey. 2006. "Progressive Dopamine Neuron Loss Following Supra-Nigral Lipopolysaccharide (LPS) Infusion into Rats Exposed to LPS Prenatally." *Experimental Neurology* 199 (2): 499–512.
Ling, Z. D., E. D. Potter, J. W. Lipton, and P. M. Carvey. 1998. "Differentiation of Mesencephalic Progenitor Cells into Dopaminergic Neurons by Cytokines." *Experimental Neurology* 149 (2): 411–23.
Lin, Yi-Jen, and Alan P. Koretsky. 1997. "Manganese Ion Enhances T1-Weighted MRI during Brain Activation: An Approach to Direct Imaging of Brain Function." *Magnetic Resonance in Medicine*. https://doi.org/10.1002/mrm.1910380305.
Lin, Yu-Lung, and Sabrina Wang. 2014. "Prenatal Lipopolysaccharide Exposure Increases Depression-like Behaviors and Reduces Hippocampal Neurogenesis in Adult Rats." *Behavioural Brain Research* 259 (February): 24–34.
Lipina, Tatiana V., Clement Zai, Daniela Hlousek, John C. Roder, and Albert H. C. Wong. 2013. "Maternal Immune Activation during Gestation Interacts with Disc1 Point Mutation to Exacerbate Schizophrenia-Related Behaviors in Mice." *The Journal of Neuroscience: The Official Journal of the Society for Neuroscience* 33 (18): 7654–66.
Li, Q., Y. O. Leung, I. Zhou, L. C. Ho, W. Kong, P. Basil, R. Wei, et al. 2015. "Dietary Supplementation with N-3 Fatty Acids from Weaning Limits Brain Biochemistry and

Behavioural Changes Elicited by Prenatal Exposure to Maternal Inflammation in the Mouse Model." *Translational Psychiatry* 5 (September): e641.
Liu, L., D. Zhang, J. K. Rodzinka-Pasko, and Y-M Li. 2016. "Environmental Risk Factors for Autism Spectrum Disorders." *Der Nervenarzt* 87 (Suppl 2): 55–61.
Liu, Yuan-Hsuan, Wen-Sung Lai, Huey-Jen Tsay, Tsu-Wei Wang, and Jenn-Yah Yu. 2013. "Effects of Maternal Immune Activation on Adult Neurogenesis in the Subventricular Zone–olfactory Bulb Pathway and Olfactory Discrimination." *Schizophrenia Research* 151 (1): 1–11.
Liuzzi, Juan P., Louis A. Lichten, Seth Rivera, Raymond K. Blanchard, Tolunay Beker Aydemir, Mitchell D. Knutson, Tomas Ganz, and Robert J. Cousins. 2005. "Interleukin-6 Regulates the Zinc Transporter Zip14 in Liver and Contributes to the Hypozincemia of the Acute-Phase Response." *Proceedings of the National Academy of Sciences of the United States of America* 102 (19): 6843–48.
Li, Wai-Yu, Yi-Chun Chang, L. Jyuhn-Hsiarn Lee, and Li-Jen Lee. 2014. "Prenatal Infection Affects the Neuronal Architecture and Cognitive Function in Adult Mice." *Developmental Neuroscience* 36 (5): 359–70.
Li, Yong-Gang, Uamporn Siripanyaphinyo, Uranan Tumkosit, Nitchakarn Noranate, Atchareeya A-nuegoonpipat, Yang Pan, Masanori Kameoka, et al. 2012. "Poly (I:C), an Agonist of Toll-like Receptor-3, Inhibits Replication of the Chikungunya Virus in BEAS-2B Cells." *Virology Journal*. https://doi.org/10.1186/1743-422x-9-114.
Llorente-Berzal, Alvaro, Emma Puighermanal, Aurelijus Burokas, Andrés Ozaita, Rafael Maldonado, Eva M. Marco, and Maria-Paz Viveros. 2013. "Correction: Sex-Dependent Psychoneuroendocrine Effects of THC and MDMA in an Animal Model of Adolescent Drug Consumption." *PloS One* 8 (12): 10.1371/annotation/89e38eb6–e8e3–402d – a6fb – 84ebf9fcbb27.
Lombardo, M. V., H. M. Moon, J. Su, T. D. Palmer, E. Courchesne, and T. Pramparo. 2018. "Maternal Immune Activation Dysregulation of the Fetal Brain Transcriptome and Relevance to the Pathophysiology of Autism Spectrum Disorder." *Molecular Psychiatry* 23 (4): 1001–13.
Loubser, Michael. 2012. "The Immune System: Development and the Immune Response." *Textbook of Clinical Pediatrics*. https://doi.org/10.1007/978-3-642-02202-9_122.
Luan, Wei, Luke Alexander Hammond, Stephanie Vuillermot, Urs Meyer, and Darryl Walter Eyles. 2018. "Maternal Vitamin D Prevents Abnormal Dopaminergic Development and Function in a Mouse Model of Prenatal Immune Activation." *Scientific Reports* 8 (1): 9741.
Lucas, Catherine J., Peter Galettis, and Jennifer Schneider. 2018. "The Pharmacokinetics and the Pharmacodynamics of Cannabinoids." *British Journal of Clinical Pharmacology* 84 (11): 2477–82.
Lyall, Kristen, Rebecca J. Schmidt, and Irva Hertz-Picciotto. 2014. "Maternal Lifestyle and Environmental Risk Factors for Autism Spectrum Disorders." *International Journal of Epidemiology* 43 (2): 443–64.
MacDowell, Karina S., Eva Munarriz-Cuezva, Javier R. Caso, José L. M. Madrigal, Arantzazu Zabala, J. Javier Meana, Borja García-Bueno, and Juan C. Leza. 2017. "Paliperidone Reverts Toll-like Receptor 3 Signaling Pathway Activation and Cognitive Deficits in a Maternal Immune Activation Mouse Model of Schizophrenia." *Neuropharmacology* 116 (April): 196–207.
Machado, Christopher J., Alexander M. Whitaker, Stephen E. P. Smith, Paul H. Patterson, and

Melissa D. Bauman. 2015. “Maternal Immune Activation in Nonhuman Primates Alters Social Attention in Juvenile Offspring.” *Biological Psychiatry* 77 (9): 823–32.
Machon, Ricardo A., Sarnoff A. Mednick, and Matti O. Huttunen. 1997. “Adult Major Affective Disorder after Prenatal Exposure to an Influenza Epidemic.” *Archives of General Psychiatry* 54 (4): 322–28.
Mackenzie-Graham, Allan. 2012. “In Vivo vs. Ex Vivo Magnetic Resonance Imaging In Mice.” *Frontiers in Neuroinformatics* 6 (May): 19.
MacKinnon, Nathalie, Mila Kingsbury, Liam Mahedy, Jonathan Evans, and Ian Colman. 2018. “The Association Between Prenatal Stress and Externalizing Symptoms in Childhood: Evidence From the Avon Longitudinal Study of Parents and Children.” *Biological Psychiatry* 83 (2): 100–108.
Macleod, John, Rachel Oakes, Alex Copello, Ilana Crome, Matthias Egger, Mathew Hickman, Thomas Oppenkowski, Helen Stokes-Lampard, and George Davey Smith. 2004. “Psychological and Social Sequelae of Cannabis and Other Illicit Drug Use by Young People: A Systematic Review of Longitudinal, General Population Studies.” *The Lancet* 363 (9421): 1579–88.
Malkova, Natalia V., Collin Z. Yu, Elaine Y. Hsiao, Marlyn J. Moore, and Paul H. Patterson. 2012. “Maternal Immune Activation Yields Offspring Displaying Mouse Versions of the Three Core Symptoms of Autism.” *Brain, Behavior, and Immunity* 26 (4): 607–16.
Malone, Daniel T., Matthew N. Hill, and Tiziana Rubino. 2010. “Adolescent Cannabis Use and Psychosis: Epidemiology and Neurodevelopmental Models.” *British Journal of Pharmacology* 160 (3): 511–22.
Manitz, Marie Pierre, Jennifer Plümper, Seray Demir, Maike Ahrens, Manuela Eßlinger, Simone Wachholz, Martin Eisenacher, Georg Juckel, and Astrid Friebe. 2016. “Flow Cytometric Characterization of Microglia in the Offspring of PolyI:C Treated Mice.” *Brain Research*. https://doi.org/10.1016/j.brainres.2016.02.004.
Maric, Nadja, Lydia Krabbendam, Wilma Vollebergh, Ron de Graaf, and Jim van Os. 2003. “Sex Differences in Symptoms of Psychosis in a Non-Selected, General Population Sample.” *Schizophrenia Research* 63 (1-2): 89–95.
Marseglia, Lucia, Gabriella D’Angelo, Sara Manti, Teresa Arrigo, Ignazio Barberi, Russel J. Reiter, and Eloisa Gitto. 2014. “Oxidative Stress-Mediated Aging during the Fetal and Perinatal Periods.” *Oxidative Medicine and Cellular Longevity* 2014 (August): 358375.
Martin, Bill, Stig Agurell, Marianne Nordqvist, and And Jan-Erik Lindgren. 1976. “Dioxygenated Metabolites of Cannabidiol Formed by Rat Liver.” *Journal of Pharmacy and Pharmacology*. https://doi.org/10.1111/j.2042-7158.1976.tb02809.x.
Martínez-Cerdeño, Verónica, Jasmin Camacho, Elizabeth Fox, Elaine Miller, Jeanelle Ariza, Devon Kienzle, Kaela Plank, Stephen C. Noctor, and Judy Van de Water. 2016. “Prenatal Exposure to Autism-Specific Maternal Autoantibodies Alters Proliferation of Cortical Neural Precursor Cells, Enlarges Brain, and Increases Neuronal Size in Adult Animals.” *Cerebral Cortex*. https://doi.org/10.1093/cercor/bhu291.
Martin, Joanna, Mark J. Taylor, and Paul Lichtenstein. 2018. “Assessing the Evidence for Shared Genetic Risks across Psychiatric Disorders and Traits.” *Psychological Medicine* 48 (11): 1759–74.
Martin, Loren A., Paul Ashwood, Daniel Braunschweig, Maricel Cabanlit, Judy Van de Water, and David G. Amaral. 2008. “Stereotypies and Hyperactivity in Rhesus Monkeys Exposed to IgG from Mothers of Children with Autism.” *Brain, Behavior, and Immunity* 22 (6):

806–16.
Mateos, B., E. Borcel, R. Loriga, W. Luesu, V. Bini, R. Llorente, M. P. Castelli, and M-P Viveros. 2011. “Adolescent Exposure to Nicotine And/or the Cannabinoid Agonist CP 55,940 Induces Gender-Dependent Long-Lasting Memory Impairments and Changes in Brain Nicotinic and CB1 Cannabinoid Receptors.” *Journal of Psychopharmacology* 25 (12): 1676–90.
Mathew, R. J., W. H. Wilson, D. F. Humphreys, J. V. Lowe, and K. E. Wiethe. 1992. “Regional Cerebral Blood Flow after Marijuana Smoking.” *Journal of Cerebral Blood Flow and Metabolism: Official Journal of the International Society of Cerebral Blood Flow and Metabolism* 12 (5): 750–58.
Matochik, John A., Dana A. Eldreth, Jean-Lud Cadet, and Karen I. Bolla. 2005. “Altered Brain Tissue Composition in Heavy Marijuana Users.” *Drug and Alcohol Dependence* 77 (1): 23–30.
Mattei, Daniele, Anaïs Djodari-Irani, Ravit Hadar, Andreas Pelz, Lourdes Fernandez de Cossío, Thomas Goetz, Marina Matyash, Helmut Kettenmann, Christine Winter, and Susanne A. Wolf. 2014. “Minocycline Rescues Decrease in Neurogenesis, Increase in Microglia Cytokines and Deficits in Sensorimotor Gating in an Animal Model of Schizophrenia.” *Brain, Behavior, and Immunity* 38 (May): 175–84.
Mattei, D., A. Ivanov, C. Ferrai, P. Jordan, D. Guneykaya, A. Buonfiglioli, W. Schaafsma, et al. 2017. “Maternal Immune Activation Results in Complex Microglial Transcriptome Signature in the Adult Offspring That Is Reversed by Minocycline Treatment.” *Translational Psychiatry* 7 (5): e1120.
McAllister, A. Kimberley. 2007. “Dynamic Aspects of CNS Synapse Formation.” *Annual Review of Neuroscience* 30: 425–50.
McGettigan, Paul A. 2013. “Transcriptomics in the RNA-Seq Era.” *Current Opinion in Chemical Biology* 17 (1): 4–11.
McGilveray, Iain J. 2005. “Pharmacokinetics of Cannabinoids.” *Pain Research & Management: The Journal of the Canadian Pain Society = Journal de La Societe Canadienne Pour Le Traitement de La Douleur* 10 Suppl A (Autumn): 15A – 22A.
McIntosh, Anthony Randal, and Nancy J. Lobaugh. 2004. “Partial Least Squares Analysis of Neuroimaging Data: Applications and Advances.” *NeuroImage* 23 Suppl 1: S250–63.
McIntosh, Anthony R., and Bratislav Mišić. 2013. “Multivariate Statistical Analyses for Neuroimaging Data.” *Annual Review of Psychology* 64: 499–525.
McMillan, Katherine A., Murray W. Enns, Brian James Cox, and Jitender Sareen. 2009. “Comorbidity of Axis I and II Mental Disorders with Schizophrenia and Psychotic Disorders: Findings from the National Epidemiologic Survey on Alcohol and Related Conditions.” *The Canadian Journal of Psychiatry* 54 (7): 477–86.
McQueeny, Tim, Claudia B. Padula, Jenessa Price, Krista Lisdahl Medina, Patrick Logan, and Susan F. Tapert. 2011. “Gender Effects on Amygdala Morphometry in Adolescent Marijuana Users.” *Behavioural Brain Research* 224 (1): 128–34.
Mednick, S. A., M. O. Huttunen, and R. A. Machón. 1994. “Prenatal Influenza Infections and Adult Schizophrenia.” *Schizophrenia Bulletin* 20 (2): 263–67.
Mednick, S. A., R. A. Machon, M. O. Huttunen, and D. Bonett. 1988. “Adult Schizophrenia Following Prenatal Exposure to an Influenza Epidemic.” *Archives of General Psychiatry* 45 (2): 189–92.
Mehler, M. F., and J. A. Kessler. 1997. “Hematolymphopoietic and Inflammatory Cytokines in

Neural Development." *Trends in Neurosciences* 20 (8): 357–65.
Menassa, David A., and Diego Gomez-Nicola. 2018. "Microglial Dynamics During Human Brain Development." *Frontiers in Immunology* 9 (May): 1014.
"Mental Health Commission of Canada Commends Federal Investment in Child and Youth Mental Health: (505522013-001)." 2011. *PsycEXTRA Dataset*. American Psychological Association (APA). https://doi.org/10.1037/e505522013-001.
Meyer, U., P. J. Murray, A. Urwyler, B. K. Yee, M. Schedlowski, and J. Feldon. 2008. "Adult Behavioral and Pharmacological Dysfunctions Following Disruption of the Fetal Brain Balance between pro-Inflammatory and IL-10-Mediated Anti-Inflammatory Signaling." *Molecular Psychiatry*. https://doi.org/10.1038/sj.mp.4002042.
Meyer, Urs. 2013. "Developmental Neuroinflammation and Schizophrenia." *Progress in Neuro-Psychopharmacology & Biological Psychiatry* 42 (April): 20–34.
———. 2014. "Prenatal poly(i:C) Exposure and Other Developmental Immune Activation Models in Rodent Systems." *Biological Psychiatry* 75 (4): 307–15.
Meyer, Urs, and Joram Feldon. 2012. "To poly(I:C) or Not to poly(I:C): Advancing Preclinical Schizophrenia Research through the Use of Prenatal Immune Activation Models." *Neuropharmacology* 62 (3): 1308–21.
Meyer, Urs, Joram Feldon, and Olaf Dammann. 2011. "Schizophrenia and Autism: Both Shared and Disorder-Specific Pathogenesis via Perinatal Inflammation?" *Pediatric Research* 69 (5 Pt 2): 26R – 33R.
Meyer, Urs, Joram Feldon, and Benjamin K. Yee. 2009. "A Review of the Fetal Brain Cytokine Imbalance Hypothesis of Schizophrenia." *Schizophrenia Bulletin* 35 (5): 959–72.
Meyer, Urs, Myriel Nyffeler, Andrea Engler, Adrian Urwyler, Manfred Schedlowski, Irene Knuesel, Benjamin K. Yee, and Joram Feldon. 2006. "The Time of Prenatal Immune Challenge Determines the Specificity of Inflammation-Mediated Brain and Behavioral Pathology." *The Journal of Neuroscience: The Official Journal of the Society for Neuroscience* 26 (18): 4752–62.
Meyer, Urs, Myriel Nyffeler, Severin Schwendener, Irene Knuesel, Benjamin K. Yee, and Joram Feldon. 2008. "Relative Prenatal and Postnatal Maternal Contributions to Schizophrenia-Related Neurochemical Dysfunction after in Utero Immune Challenge." *Neuropsychopharmacology: Official Publication of the American College of Neuropsychopharmacology* 33 (2): 441–56.
Meyer, Urs, Myriel Nyffeler, Benjamin K. Yee, Irene Knuesel, and Joram Feldon. 2008. "Adult Brain and Behavioral Pathological Markers of Prenatal Immune Challenge during Early/middle and Late Fetal Development in Mice." *Brain, Behavior, and Immunity* 22 (4): 469–86.
Mier, Daniela, and Peter Kirsch. 2015. "Social-Cognitive Deficits in Schizophrenia." In *Social Behavior from Rodents to Humans*, 397–409. Springer.
Mietchen, Daniel, and Christian Gaser. 2009. "Computational Morphometry for Detecting Changes in Brain Structure due to Development, Aging, Learning, Disease and Evolution." *Frontiers in Neuroinformatics* 3 (August): 25.
Miller, Gregory E., Jennifer Culhane, William Grobman, Hyagriv Simhan, Douglas E. Williamson, Emma K. Adam, Claudia Buss, et al. 2017. "Mothers' Childhood Hardship Forecasts Adverse Pregnancy Outcomes: Role of Inflammatory, Lifestyle, and Psychosocial Pathways." *Brain, Behavior, and Immunity* 65 (October): 11–19.
Miller, Michael L., Benjamin Chadwick, Dara L. Dickstein, Immanuel Purushothaman, Gabor

Egervari, Tanni Rahman, Chloe Tessereau, et al. 2019. “Adolescent Exposure to Δ9-Tetrahydrocannabinol Alters the Transcriptional Trajectory and Dendritic Architecture of Prefrontal Pyramidal Neurons.” *Molecular Psychiatry*. https://doi.org/10.1038/s41380-018-0243-x.

Minakova, Elena, and Barbara B. Warner. 2018. “Maternal Immune Activation, Central Nervous System Development and Behavioral Phenotypes.” *Birth Defects Research* 110 (20): 1539–50.

Monji, Akira, Takahiro A. Kato, Yoshito Mizoguchi, Hideki Horikawa, Yoshihiro Seki, Mina Kasai, Yusuke Yamauchi, Shigeto Yamada, and Shigenobu Kanba. 2013. “Neuroinflammation in Schizophrenia Especially Focused on the Role of Microglia.” *Progress in Neuro-Psychopharmacology & Biological Psychiatry* 42 (April): 115–21.

Montag, Christian, Ulrike Basten, Christine Stelzel, Christian J. Fiebach, and Martin Reuter. 2010. “The BDNF Val66Met Polymorphism and Anxiety: Support for Animal Knock-in Studies from a Genetic Association Study in Humans.” *Psychiatry Research* 179 (1): 86–90.

Montag, Christian, Magdalena Jurkiewicz, and Martin Reuter. 2012. “The Role of the Catechol-O-Methyltransferase (COMT) Gene in Personality and Related Psychopathological Disorders.” *CNS & Neurological Disorders Drug Targets* 11 (3): 236–50.

Moore, Theresa H. M., Stanley Zammit, Anne Lingford-Hughes, Thomas R. E. Barnes, Peter B. Jones, Margaret Burke, and Glyn Lewis. 2007. “Cannabis Use and Risk of Psychotic or Affective Mental Health Outcomes: A Systematic Review.” *The Lancet* 370 (9584): 319–28.

Morelli, Sara S., Mili Mandal, Laura T. Goldsmith, Banafsheh N. Kashani, and Nicholas M. Ponzio. 2015. “The Maternal Immune System during Pregnancy and Its Influence on Fetal Development.” *Research and Reports in Biology* 6: 171–89.

Morgan, V., D. Castle, A. Page, S. Fazio, L. Gurrin, P. Burton, P. Montgomery, and A. Jablensky. 1997. “Influenza Epidemics and Incidence of Schizophrenia, Affective Disorders and Mental Retardation in Western Australia: No Evidence of a Major Effect.” *Schizophrenia Research* 26 (1): 25–39.

Mor, Gil, and Ingrid Cardenas. 2010. “The Immune System in Pregnancy: A Unique Complexity.” *American Journal of Reproductive Immunology* 63 (6): 425–33.

Mori, S., R. Itoh, J. Zhang, W. E. Kaufmann, P. C. van Zijl, M. Solaiyappan, and P. Yarowsky. 2001. “Diffusion Tensor Imaging of the Developing Mouse Brain.” *Magnetic Resonance in Medicine: Official Journal of the Society of Magnetic Resonance in Medicine / Society of Magnetic Resonance in Medicine* 46 (1): 18–23.

Mortensen, Preben Bo, Bent Nørgaard-Pedersen, Berit Lindum Waltoft, Tina L. Sørensen, David Hougaard, E. Fuller Torrey, and Robert H. Yolken. 2007. “Toxoplasma Gondii as a Risk Factor for Early-Onset Schizophrenia: Analysis of Filter Paper Blood Samples Obtained at Birth.” *Biological Psychiatry* 61 (5): 688–93.

Mortensen, Preben Bo, Carsten Bøcker Pedersen, John Joseph McGrath, David Michael Hougaard, Bent Nørgaard-Petersen, Ole Mors, Anders D. Børglum, and Robert H. Yolken. 2011. “Neonatal Antibodies to Infectious Agents and Risk of Bipolar Disorder: A Population-Based Case--Control Study.” *Bipolar Disorders* 13 (7-8): 624–29.

Mott, Natasha N., Wilson C. J. Chung, Pei-San Tsai, and Toni R. Pak. 2010. “Differential Fibroblast Growth Factor 8 (FGF8)-Mediated Autoregulation of Its Cognate Receptors, Fgfr1 and Fgfr3, in Neuronal Cell Lines.” *PloS One* 5 (4): e10143.

Mousa, A., SeigerA, A. Kjaeldgaard, and M. Bakhiet. 1999. “Human First Trimester Forebrain

Cells Express Genes for Inflammatory and Anti-Inflammatory Cytokines." *Cytokine* 11 (1): 55–60.
Mueller, Flavia S., Juliet Richetto, Lindsay N. Hayes, Alice Zambon, Daniela D. Pollak, Akira Sawa, Urs Meyer, and Ulrike Weber-Stadlbauer. 2019. "Influence of poly(I:C) Variability on Thermoregulation, Immune Responses and Pregnancy Outcomes in Mouse Models of Maternal Immune Activation." *Brain, Behavior, and Immunity* 80 (August): 406–18.
Mueller, Flavia S., Joseph Scarborough, Sina M. Schalbetter, Juliet Richetto, Eugene Kim, Amalie Couch, Yohan Yee, et al. 2021. "Behavioral, Neuroanatomical, and Molecular Correlates of Resilience and Susceptibility to Maternal Immune Activation." *Molecular Psychiatry* 26 (2): 396–410.
Mulder, J., T. Aguado, E. Keimpema, K. Barabas, C. J. Ballester Rosado, L. Nguyen, K. Monory, et al. 2008. "Endocannabinoid Signaling Controls Pyramidal Cell Specification and Long-Range Axon Patterning." *Proceedings of the National Academy of Sciences*. https://doi.org/10.1073/pnas.0803545105.
Nashed, Mina G., Daniel B. Hardy, and Steven R. Laviolette. 2020. "Prenatal Cannabinoid Exposure: Emerging Evidence of Physiological and Neuropsychiatric Abnormalities." *Frontiers in Psychiatry / Frontiers Research Foundation* 11: 624275.
Nelson, Lars H., Angela I. Saulsbery, and Kathryn M. Lenz. 2019. "Small Cells with Big Implications: Microglia and Sex Differences in Brain Development, Plasticity and Behavioral Health." *Progress in Neurobiology* 176 (May): 103–19.
Nestler, Eric J., and Steven E. Hyman. 2010. "Animal Models of Neuropsychiatric Disorders." *Nature Neuroscience* 13 (10): 1161–69.
Nguyen, Vu H., Mathieu Verdurand, Stefanie Dedeurwaerdere, Hongqin Wang, David Zahra, Marie-Claude Gregoire, and Katerina Zavitsanou. 2012. "Increased Brain Metabolism after Acute Administration of the Synthetic Cannabinoid HU210: A Small Animal PET Imaging Study with 18F-FDG." *Brain Research Bulletin* 87 (2-3): 172–79.
Nielsen, Philip Rising, Esben Agerbo, Kristin Skogstrand, David Michael Hougaard, Urs Meyer, and Preben Bo Mortensen. 2015. "Neonatal Levels of Inflammatory Markers and Later Risk of Schizophrenia." *Biological Psychiatry* 77 (6): 548–55.
Nishimura, Dwight George. 1996. *Principles of Magnetic Resonance Imaging*. Stanford University.
Nordentoft, M. 2010. "Review: Pharmacological and Psychological Interventions Decrease Cannabis Use in People with Depressive and Psychotic Disorders in the Short Term." *Evidence-Based Mental Health*. https://doi.org/10.1136/ebmh.13.4.124.
Noriega, Daniela Briceno, and Huub F. J. Savelkoul. 2014. "Immune Dysregulation in Autism Spectrum Disorder." *European Journal of Pediatrics* 173 (1): 33–43.
Norris, Francesca C., Bernard M. Siow, Jon O. Cleary, Jack A. Wells, Sandra C. P. De Castro, Roger J. Ordidge, Nicholas D. E. Greene, et al. 2015. "Diffusion Microscopic MRI of the Mouse Embryo: Protocol and Practical Implementation in the Splotch Mouse Model." *Magnetic Resonance in Medicine: Official Journal of the Society of Magnetic Resonance in Medicine / Society of Magnetic Resonance in Medicine* 73 (2): 731–39.
Nouel, Dominique, Melissa Burt, Ying Zhang, Louise Harvey, and Patricia Boksa. 2012. "Prenatal Exposure to Bacterial Endotoxin Reduces the Number of GAD67- and Reelin-Immunoreactive Neurons in the Hippocampus of Rat Offspring." *European Neuropsychopharmacology*. https://doi.org/10.1016/j.euroneuro.2011.08.001.
Nugent, Bridget M., and Tracy L. Bale. 2015. "The Omniscient Placenta: Metabolic and

Epigenetic Regulation of Fetal Programming." *Frontiers in Neuroendocrinology* 39 (October): 28–37.
O'Callaghan, E., D. Cotter, K. Colgan, C. Larkin, D. Walsh, and J. L. Waddington. 1995. "Confinement of Winter Birth Excess in Schizophrenia to the Urban-Born and Its Gender Specificity." *The British Journal of Psychiatry: The Journal of Mental Science* 166 (1): 51–54.
O'Callaghan, E., P. Sham, N. Takei, G. Glover, and R. M. Murray. 1991. "Schizophrenia after Prenatal Exposure to 1957 A2 Influenza Epidemic." *The Lancet* 337 (8752): 1248–50.
Ohmura, Yoshiyuki, and Yasuo Kuniyoshi. 2017. "A Translational Model to Determine Rodent's Age from Human Foetal Age." *Scientific Reports* 7 (1): 17248.
Oh-Nishi, Arata, Shigeru Obayashi, Izumi Sugihara, Takafumi Minamimoto, and Tetsuya Suhara. 2010. "Maternal Immune Activation by Polyriboinosinic-Polyribocytidilic Acid Injection Produces Synaptic Dysfunction but Not Neuronal Loss in the Hippocampus of Juvenile Rat Offspring." *Brain Research* 1363 (December): 170–79.
Olsen, J., M. Melbye, S. F. Olsen, T. I. Sørensen, P. Aaby, A. M. Andersen, D. Taxbøl, et al. 2001. "The Danish National Birth Cohort--Its Background, Structure and Aim." *Scandinavian Journal of Public Health* 29 (4): 300–307.
Opal, S. M., and V. A. DePalo. 2000. "Anti-Inflammatory Cytokines." *Chest* 117 (4): 1162–72.
Orr, Catherine, Philip Spechler, Zhipeng Cao, Matthew Albaugh, Bader Chaarani, Scott Mackey, Deepak D'Souza, et al. 2019. "Grey Matter Volume Differences Associated with Extremely Low Levels of Cannabis Use in Adolescence." *The Journal of Neuroscience: The Official Journal of the Society for Neuroscience* 39 (10): 1817–27.
O'Shea, Melanie, Iain S. McGregor, and Paul E. Mallet. 2006. "Repeated Cannabinoid Exposure during Perinatal, Adolescent or Early Adult Ages Produces Similar Longlasting Deficits in Object Recognition and Reduced Social Interaction in Rats." *Journal of Psychopharmacology* 20 (5): 611–21.
Os, Jim van, Gunter Kenis, and Bart P. F. Rutten. 2010. "The Environment and Schizophrenia." *Nature* 468 (7321): 203–12.
Oskvig, Devon B., Abdel G. Elkahloun, Kory R. Johnson, Terry M. Phillips, and Miles Herkenham. 2012. "Maternal Immune Activation by LPS Selectively Alters Specific Gene Expression Profiles of Interneuron Migration and Oxidative Stress in the Fetus without Triggering a Fetal Immune Response." *Brain, Behavior, and Immunity*. https://doi.org/10.1016/j.bbi.2012.01.015.
O'Tuathaigh, Colm M. P., Magdalena Hryniewiecka, Aine Behan, Orna Tighe, Catherine Coughlan, Lieve Desbonnet, Mary Cannon, et al. 2010. "Chronic Adolescent Exposure to Δ-9-Tetrahydrocannabinol in COMT Mutant Mice: Impact on Psychosis-Related and Other Phenotypes." *Neuropsychopharmacology: Official Publication of the American College of Neuropsychopharmacology* 35 (11): 2262–73.
Ozawa, Kimiyoshi, Kenji Hashimoto, Takashi Kishimoto, Eiji Shimizu, Hiroshi Ishikura, and Masaomi Iyo. 2006. "Immune Activation During Pregnancy in Mice Leads to Dopaminergic Hyperfunction and Cognitive Impairment in the Offspring: A Neurodevelopmental Animal Model of Schizophrenia." *Biological Psychiatry*. https://doi.org/10.1016/j.biopsych.2005.07.031.
Paintlia, Manjeet K., Ajaib S. Paintlia, Ernest Barbosa, Inderjit Singh, and Avtar K. Singh. 2004. "N-Acetylcysteine Prevents Endotoxin-Induced Degeneration of Oligodendrocyte Progenitors and Hypomyelination in Developing Rat Brain." *Journal of Neuroscience*

Research. https://doi.org/10.1002/jnr.20261.
Paintlia, Manjeet K., Ajaib S. Paintlia, Miguel A. Contreras, Inderjit Singh, and Avtar K. Singh. 2008. "Lipopolysaccharide-Induced Peroxisomal Dysfunction Exacerbates Cerebral White Matter Injury: Attenuation by N-Acetyl Cysteine." *Experimental Neurology* 210 (2): 560–76.
Pan, Dipanjan, Anne H. Schmieder, Samuel A. Wickline, and Gregory M. Lanza. 2011. "Manganese-Based MRI Contrast Agents: Past, Present and Future." *Tetrahedron* 67 (44): 8431–44.
Paolicelli, Rosa C., and Cornelius T. Gross. 2011. "Microglia in Development: Linking Brain Wiring to Brain Environment." *Neuron Glia Biology* 7 (1): 77–83.
Parboosing, Raveen, Yuanyuan Bao, Ling Shen, Catherine A. Schaefer, and Alan S. Brown. 2013. "Gestational Influenza and Bipolar Disorder in Adult Offspring." *JAMA Psychiatry* 70 (7): 677–85.
Parker-Athill, E. Carla, E. Carla Parker-Athill, and Jun Tan. 2010. "Maternal Immune Activation and Autism Spectrum Disorder: Interleukin-6 Signaling as a Key Mechanistic Pathway." *Neurosignals*. https://doi.org/10.1159/000319828.
Patel, Raihaan, Christopher J. Steele, Anthony G. X. Chen, Sejal Patel, Gabriel A. Devenyi, Jürgen Germann, Christine L. Tardif, and M. Mallar Chakravarty. 2020. "Investigating Microstructural Variation in the Human Hippocampus Using Non-Negative Matrix Factorization." *NeuroImage* 207 (February): 116348.
Patterson, Paul H. 2009. "Immune Involvement in Schizophrenia and Autism: Etiology, Pathology and Animal Models." *Behavioural Brain Research* 204 (2): 313–21.
Paus, Tomás, Matcheri Keshavan, and Jay N. Giedd. 2008. "Why Do Many Psychiatric Disorders Emerge during Adolescence?" *Nature Reviews. Neuroscience* 9 (12): 947–57.
Pelayo-Terán, José María, Rocío Pérez-Iglesias, Ignacio Mata, Eugenio Carrasco-Marín, José Luis Vázquez-Barquero, and Benedicto Crespo-Facorro. 2010. "Catechol-O-Methyltransferase (COMT) Val158Met Variations and Cannabis Use in First-Episode Non-Affective Psychosis: Clinical-Onset Implications." *Psychiatry Research* 179 (3): 291–96.
Pendyala, Gurudutt, Shinnyi Chou, Yoosun Jung, Pierluca Coiro, Elizabeth Spartz, Ragunathan Padmashri, Ming Li, and Anna Dunaevsky. 2017. "Maternal Immune Activation Causes Behavioral Impairments and Altered Cerebellar Cytokine and Synaptic Protein Expression." *Neuropsychopharmacology: Official Publication of the American College of Neuropsychopharmacology* 42 (7): 1435–46.
Penzes, Peter, Michael E. Cahill, Kelly A. Jones, Jon-Eric VanLeeuwen, and Kevin M. Woolfrey. 2011. "Dendritic Spine Pathology in Neuropsychiatric Disorders." *Nature Neuroscience* 14 (3): 285–93.
Pierre, Joseph M. 2010. "Psychosis Associated with Medical Marijuana: Risk vs. Benefits of Medicinal Cannabis Use." *The American Journal of Psychiatry* 167 (5): 598–99.
Piontkewitz, Yael, Michal Arad, and Ina Weiner. 2011. "Abnormal Trajectories of Neurodevelopment and Behavior Following in Utero Insult in the Rat." *Biological Psychiatry* 70 (9): 842–51.
Piontkewitz, Yael, Yaniv Assaf, and Ina Weiner. 2009. "Clozapine Administration in Adolescence Prevents Postpubertal Emergence of Brain Structural Pathology in an Animal Model of Schizophrenia." *Biological Psychiatry* 66 (11): 1038–46.
Piontkewitz, Yael, Hans-Gert Bernstein, Henrik Dobrowolny, Bernhard Bogerts, Ina Weiner, and Gerburg Keilhoff. 2012. "Effects of Risperidone Treatment in Adolescence on

Hippocampal Neurogenesis, Parvalbumin Expression, and Vascularization Following Prenatal Immune Activation in Rats." *Brain, Behavior, and Immunity*. https://doi.org/10.1016/j.bbi.2011.11.004.
Pipitone, Jon, Min Tae M. Park, Julie Winterburn, Tristram A. Lett, Jason P. Lerch, Jens C. Pruessner, Martin Lepage, Aristotle N. Voineskos, M. Mallar Chakravarty, and Alzheimer's Disease Neuroimaging Initiative. 2014. "Multi-Atlas Segmentation of the Whole Hippocampus and Subfields Using Multiple Automatically Generated Templates." *NeuroImage* 101 (November): 494–512.
Pleasure, S. J., S. Anderson, R. Hevner, A. Bagri, O. Marin, D. H. Lowenstein, and J. L. Rubenstein. 2000. "Cell Migration from the Ganglionic Eminences Is Required for the Development of Hippocampal GABAergic Interneurons." *Neuron* 28 (3): 727–40.
Plewes, Donald B., and Walter Kucharczyk. 2012. "Physics of MRI: A Primer." *Journal of Magnetic Resonance Imaging: JMRI* 35 (5): 1038–54.
Potter, E. D., Z. D. Ling, and P. M. Carvey. 1999. "Cytokine-Induced Conversion of Mesencephalic-Derived Progenitor Cells into Dopamine Neurons." *Cell and Tissue Research* 296 (2): 235–46.
Prata, Joana, Susana G. Santos, Maria Inês Almeida, Rui Coelho, and Mário A. Barbosa. 2017. "Bridging Autism Spectrum Disorders and Schizophrenia through Inflammation and Biomarkers - Pre-Clinical and Clinical Investigations." *Journal of Neuroinflammation* 14 (1): 179.
Pratt, Lorelei, Li Ni, Nicholas M. Ponzio, and G. Miller Jonakait. 2013. "Maternal Inflammation Promotes Fetal Microglial Activation and Increased Cholinergic Expression in the Fetal Basal Forebrain: Role of Interleukin-6." *Pediatric Research* 74 (4): 393–401.
Prini, Pamela, Franceso Rusconi, Erica Zamberletti, Marina Gabaglio, Federica Penna, Mauro Fasano, Elena Battaglioli, Daniela Parolaro, and Tiziana Rubino. 2018. "Adolescent THC Exposure in Female Rats Leads to Cognitive Deficits through a Mechanism Involving Chromatin Modifications in the Prefrontal Cortex." *Journal of Psychiatry & Neuroscience: JPN* 43 (2): 87–101.
Pykett, Ian L. 1984. "Instrumentation for Nuclear Magnetic Resonance Imaging." *Magnetic Resonance Imaging*. https://doi.org/10.1016/0730-725x(84)90110-3.
Qiu, Lily R., Darren J. Fernandes, Kamila U. Szulc-Lerch, Jun Dazai, Brian J. Nieman, Daniel H. Turnbull, Jane A. Foster, Mark R. Palmert, and Jason P. Lerch. 2018. "Mouse MRI Shows Brain Areas Relatively Larger in Males Emerge before Those Larger in Females." *Nature Communications* 9 (1): 2615.
Rabinak, Christine A., Craig Peters, Hilary A. Marusak, Samiran Ghosh, and K. Luan Phan. 2018. "Effects of Acute Δ9-Tetrahydrocannabinol on next-Day Extinction Recall Is Mediated by Post-Extinction Resting-State Brain Dynamics." *Neuropharmacology* 143 (December): 289–98.
Rakic, P., and R. S. Nowakowski. 1981. "The Time of Origin of Neurons in the Hippocampal Region of the Rhesus Monkey." *The Journal of Comparative Neurology* 196 (1): 99–128.
Rapoport, J. L., A. M. Addington, S. Frangou, and M. R. C. Psych. 2005. "The Neurodevelopmental Model of Schizophrenia: Update 2005." *Molecular Psychiatry* 10 (5): 434–49.
Rapoport, J. L., J. N. Giedd, and N. Gogtay. 2012. "Neurodevelopmental Model of Schizophrenia: Update 2012." *Molecular Psychiatry* 17 (12): 1228–38.
Rapp, Charlotte, Hilal Bugra, Anita Riecher-Rössler, Corinne Tamagni, and Stefan Borgwardt.

2012. “Effects of Cannabis Use on Human Brain Structure in Psychosis: A Systematic Review Combining in Vivo Structural Neuroimaging and Post Mortem Studies.” *Current Pharmaceutical Design* 18 (32): 5070–80.
Ratajczak, C. K., J. C. Fay, and L. J. Muglia. 2010. “Preventing Preterm Birth: The Past Limitations and New Potential of Animal Models.” *Disease Models & Mechanisms*. https://doi.org/10.1242/dmm.001701.
Ratering, David, Christof Baltes, Jurek Nordmeyer-Massner, Daniel Marek, and Markus Rudin. 2008. “Performance of a 200-MHz Cryogenic RF Probe Designed for MRI and MRS of the Murine Brain.” *Magnetic Resonance in Medicine: An Official Journal of the International Society for Magnetic Resonance in Medicine* 59 (6): 1440–47.
Ratnayake, Udani, Tracey Quinn, David W. Walker, and Hayley Dickinson. 2013. “Cytokines and the Neurodevelopmental Basis of Mental Illness.” *Frontiers in Neuroscience* 7 (October): 180.
Rees, Elliott, and Michael J. Owen. 2020. “Translating Insights from Neuropsychiatric Genetics and Genomics for Precision Psychiatry.” *Genome Medicine* 12 (1): 43.
Rees, Sandra, and Terrie Inder. 2005. “Fetal and Neonatal Origins of Altered Brain Development.” *Early Human Development* 81 (9): 753–61.
Reid, Andrew T., John Lewis, Gleb Bezgin, Budhachandra Khundrakpam, Simon B. Eickhoff, Anthony R. McIntosh, Pierre Bellec, and Alan C. Evans. 2016. “A Cross-Modal, Cross-Species Comparison of Connectivity Measures in the Primate Brain.” *NeuroImage*. https://doi.org/10.1016/j.neuroimage.2015.10.057.
Reisenberger, K., C. Egarter, S. Vogl, B. Sternberger, H. Kiss, and P. Husslein. 1996. “The Transfer of Interleukin-8 across the Human Placenta Perfused in Vitro.” *Obstetrics and Gynecology* 87 (4): 613–16.
Reisinger, Sonali, Deeba Khan, Eryan Kong, Angelika Berger, Arnold Pollak, and Daniela D. Pollak. 2015. “The Poly(I:C)-Induced Maternal Immune Activation Model in Preclinical Neuropsychiatric Drug Discovery.” *Pharmacology & Therapeutics* 149 (May): 213–26.
Réus, G. Z., G. R. Fries, L. Stertz, M. Badawy, I. C. Passos, T. Barichello, F. Kapczinski, and J. Quevedo. 2015. “The Role of Inflammation and Microglial Activation in the Pathophysiology of Psychiatric Disorders.” *Neuroscience* 300 (August): 141–54.
Ricci, S., R. Businaro, F. Ippoliti, V. R. Lo Vasco, F. Massoni, E. Onofri, G. M. Troili, et al. 2013. “Altered Cytokine and BDNF Levels in Autism Spectrum Disorder.” *Neurotoxicity Research* 24 (4): 491–501.
Rilling, James K. 2014. “Comparative Primate Neuroimaging: Insights into Human Brain Evolution.” *Trends in Cognitive Sciences* 18 (1): 46–55.
Robertson, Sarah A., Alison S. Care, and Lachlan M. Moldenhauer. 2018. “Regulatory T Cells in Embryo Implantation and the Immune Response to Pregnancy.” *The Journal of Clinical Investigation* 128 (10): 4224–35.
Rollins, Colleen P. E., Daniel Gallino, Vincent Kong, Gülebru Ayranci, Gabriel A. Devenyi, Jürgen Germann, and M. Mallar Chakravarty. 2019. “Contributions of a High-Fat Diet to Alzheimer’s Disease-Related Decline: A Longitudinal Behavioural and Structural Neuroimaging Study in Mouse Models.” *NeuroImage: Clinical* 21 (January): 101606.
Romero, Roberto, Jimmy Espinoza, Luís F. Gonçalves, Juan Pedro Kusanovic, Lara A. Friel, and Jyh Kae Nien. 2006. “Inflammation in Preterm and Term Labour and Delivery.” *Seminars in Fetal & Neonatal Medicine* 11 (5): 317–26.
Romijn, H. J., M. A. Hofman, and A. Gramsbergen. 1991. “At What Age Is the Developing

Cerebral Cortex of the Rat Comparable to that of the Full-Term Newborn Human Baby?" *Early Human Development* 26 (1): 61–67.
Ronovsky, Marianne, Stefanie Berger, Barbara Molz, Angelika Berger, and Daniela D. Pollak. 2016. "Animal Models of Maternal Immune Activation in Depression Research." *Current Neuropharmacology* 14 (7): 688–704.
Ronovsky, Marianne, Stefanie Berger, Alice Zambon, Sonali N. Reisinger, Orsolya Horvath, Arnold Pollak, Claudia Lindtner, Angelika Berger, and Daniela D. Pollak. 2017. "Maternal Immune Activation Transgenerationally Modulates Maternal Care and Offspring Depression-like Behavior." *Brain, Behavior, and Immunity* 63 (July): 127–36.
Rostène, William, Patrick Kitabgi, and Stéphane Mélik Parsadaniantz. 2007. "Chemokines: A New Class of Neuromodulator?" *Nature Reviews. Neuroscience* 8 (11): 895–903.
Rubenstein, John L. R. 2011. "Annual Research Review: Development of the Cerebral Cortex: Implications for Neurodevelopmental Disorders: Development of the Cerebral Cortex." *Journal of Child Psychology and Psychiatry, and Allied Disciplines* 52 (4): 339–55.
Rubino, Tiziana, and Daniela Parolaro. 2016. "The Impact of Exposure to Cannabinoids in Adolescence: Insights From Animal Models." *Biological Psychiatry* 79 (7): 578–85.
Rubino, Tiziana, Natalia Realini, Daniela Braida, Sandra Guidi, Valeria Capurro, Daniela Viganò, Cinzia Guidali, et al. 2009. "Changes in Hippocampal Morphology and Neuroplasticity Induced by Adolescent THC Treatment Are Associated with Cognitive Impairment in Adulthood." *Hippocampus* 19 (8): 763–72.
Rubino, Tiziana, Daniela Vigano', Natalia Realini, Cinzia Guidali, Daniela Braida, Valeria Capurro, Chiara Castiglioni, et al. 2008. "Chronic Delta 9-Tetrahydrocannabinol during Adolescence Provokes Sex-Dependent Changes in the Emotional Profile in Adult Rats: Behavioral and Biochemical Correlates." *Neuropsychopharmacology: Official Publication of the American College of Neuropsychopharmacology* 33 (11): 2760–71.
Rudolph, Marc D., Alice M. Graham, Eric Feczko, Oscar Miranda-Dominguez, Jerod M. Rasmussen, Rahel Nardos, Sonja Entringer, Pathik D. Wadhwa, Claudia Buss, and Damien A. Fair. 2018. "Maternal IL-6 during Pregnancy Can Be Estimated from Newborn Brain Connectivity and Predicts Future Working Memory in Offspring." *Nature Neuroscience* 21 (5): 765–72.
Sankar, Tejas, M. Mallar Chakravarty, Agustin Bescos, Monica Lara, Toshiki Obuchi, Adrian W. Laxton, Mary Pat McAndrews, et al. 2015. "Deep Brain Stimulation Influences Brain Structure in Alzheimer's Disease." *Brain Stimulation* 8 (3): 645–54.
Sapolsky, R. M., L. M. Romero, and A. U. Munck. 2000. "How Do Glucocorticoids Influence Stress Responses? Integrating Permissive, Suppressive, Stimulatory, and Preparative Actions." *Endocrine Reviews* 21 (1): 55–89.
Sass, L., S. K. Urhoj, J. Kjærgaard, J. W. Dreier, K. Strandberg-Larsen, and A-M Nybo Andersen. 2017. "Fever in Pregnancy and the Risk of Congenital Malformations: A Cohort Study." *BMC Pregnancy and Childbirth* 17 (1): 413.
Scattoni, Maria Luisa, Jacqueline Crawley, and Laura Ricceri. 2009. "Ultrasonic Vocalizations: A Tool for Behavioural Phenotyping of Mouse Models of Neurodevelopmental Disorders." *Neuroscience and Biobehavioral Reviews* 33 (4): 508–15.
Schaafsma, Wandert, Laura Bozal Basterra, Sabrina Jacobs, Nieske Brouwer, Peter Meerlo, Anne Schaafsma, Erik W G, and Bart J. L. Eggen. 2017. "Maternal Inflammation Induces Immune Activation of Fetal Microglia and Leads to Disrupted Microglia Immune Responses, Behavior, and Learning Performance in Adulthood." *Neurobiology of Disease*.

https://doi.org/10.1016/j.nbd.2017.07.017.
Schmidt, André, Diana M. Morales-Prieto, Jana Pastuschek, Karolin Fröhlich, and Udo R. Markert. 2015. “Only Humans Have Human Placentas: Molecular Differences between Mice and Humans.” *Journal of Reproductive Immunology* 108 (April): 65–71.
Schneider, Miriam, and Michael Koch. 2003. “Chronic Pubertal, but Not Adult Chronic Cannabinoid Treatment Impairs Sensorimotor Gating, Recognition Memory, and the Performance in a Progressive Ratio Task in Adult Rats.” *Neuropsychopharmacology: Official Publication of the American College of Neuropsychopharmacology* 28 (10): 1760–69.
Schumann, Cynthia M., Cinnamon S. Bloss, Cynthia Carter Barnes, Graham M. Wideman, Ruth A. Carper, Natacha Akshoomoff, Karen Pierce, et al. 2010. “Longitudinal Magnetic Resonance Imaging Study of Cortical Development through Early Childhood in Autism.” *The Journal of Neuroscience: The Official Journal of the Society for Neuroscience* 30 (12): 4419–27.
Schwartzer, Jared J., Milo Careaga, Morgan A. Coburn, Destanie R. Rose, Heather K. Hughes, and Paul Ashwood. 2017. “Behavioral Impact of Maternal Allergic-Asthma in Two Genetically Distinct Mouse Strains.” *Brain, Behavior, and Immunity* 63 (July): 99–107.
Schwartzer, J. J., M. Careaga, C. Chang, C. E. Onore, and P. Ashwood. 2015. “Allergic Fetal Priming Leads to Developmental, Behavioral and Neurobiological Changes in Mice.” *Translational Psychiatry* 5 (April): e543.
Schwartzer, J. J., M. Careaga, C. E. Onore, J. A. Rushakoff, R. F. Berman, and P. Ashwood. 2013. “Maternal Immune Activation and Strain Specific Interactions in the Development of Autism-like Behaviors in Mice.” *Translational Psychiatry* 3 (March): e240.
Schwarz, Jaclyn M. 2016. “Sex and the Developing Brain.” *Sex Differences in the Central Nervous System*. https://doi.org/10.1016/b978-0-12-802114-9.00010-x.
Schwarz, Jaclyn M., Paige W. Sholar, and Staci D. Bilbo. 2012. “Sex Differences in Microglial Colonization of the Developing Rat Brain.” *Journal of Neurochemistry* 120 (6): 948–63.
Seeman, Philip. 1990. “Elevation of Dopamine D2 Receptors in Schizophrenia Is Underestimated by Radioactive Raclopride.” *Archives of General Psychiatry*. https://doi.org/10.1001/archpsyc.1990.01810240090014.
Selemon, L. D., and N. Zecevic. 2015. “Schizophrenia: A Tale of Two Critical Periods for Prefrontal Cortical Development.” *Translational Psychiatry* 5 (August): e623.
Sellgren, Carl M., Jessica Gracias, Bradley Watmuff, Jonathan D. Biag, Jessica M. Thanos, Paul B. Whittredge, Ting Fu, et al. 2019. “Increased Synapse Elimination by Microglia in Schizophrenia Patient-Derived Models of Synaptic Pruning.” *Nature Neuroscience* 22 (3): 374–85.
Semple, Bridgette D., Klas Blomgren, Kayleen Gimlin, Donna M. Ferriero, and Linda J. Noble-Haeusslein. 2013. “Brain Development in Rodents and Humans: Identifying Benchmarks of Maturation and Vulnerability to Injury across Species.” *Progress in Neurobiology*. https://doi.org/10.1016/j.pneurobio.2013.04.001.
Sharabi, Hila, Nizar Khatib, Yuval Ginsberg, Zeev Weiner, Michael G. Ross, Blumenfeld-Katzir Tamar, Sasson Efrat, Hallak Mordechai, and Ron Beloosesky. 2018. “Therapeutic N-Acetyl-Cysteine (Nac) Following Initiation of Maternal Inflammation Attenuates Long-Term Offspring Cerebral Injury, as Evident in Magnetic Resonance Imaging (MRI).” *Neuroscience*, February. https://doi.org/10.1016/j.neuroscience.2018.01.013.
Shaw, Philip, Noor J. Kabani, Jason P. Lerch, Kristen Eckstrand, Rhoshel Lenroot, Nitin Gogtay,

Deanna Greenstein, et al. 2008. “Neurodevelopmental Trajectories of the Human Cerebral Cortex.” *The Journal of Neuroscience: The Official Journal of the Society for Neuroscience* 28 (14): 3586–94.
Shi, Limin, S. Hossein Fatemi, Robert W. Sidwell, and Paul H. Patterson. 2003. “Maternal Influenza Infection Causes Marked Behavioral and Pharmacological Changes in the Offspring.” *The Journal of Neuroscience: The Official Journal of the Society for Neuroscience* 23 (1): 297–302.
Shimamura, K., and J. L. Rubenstein. 1997. “Inductive Interactions Direct Early Regionalization of the Mouse Forebrain.” *Development* 124 (14): 2709–18.
Shimoya, K., N. Matsuzaki, T. Taniguchi, T. Okada, F. Saji, and Y. Murata. 1997. “Interleukin-8 Level in Maternal Serum as a Marker for Screening of Histological Chorioamnionitis at Term.” *International Journal of Gynecology & Obstetrics*. https://doi.org/10.1016/s0020-7292(97)02891-9.
Shin Yim, Yeong, Ashley Park, Janet Berrios, Mathieu Lafourcade, Leila M. Pascual, Natalie Soares, Joo Yeon Kim, et al. 2017. “Reversing Behavioural Abnormalities in Mice Exposed to Maternal Inflammation.” *Nature* 549 (7673): 482–87.
Shrivastava, Amresh, Megan Johnston, Kristen Terpstra, and Yves Bureau. 2014. “Cannabis and Psychosis: Neurobiology.” *Indian Journal of Psychiatry* 56 (1): 8–16.
Silva, Lindsay, Lauren Harte-Hargrove, Sari Izenwasser, Ashley Frank, Dean Wade, and Diana Dow-Edwards. 2015. “Sex-Specific Alterations in Hippocampal Cannabinoid 1 Receptor Expression Following Adolescent Delta-9-Tetrahydrocannabinol Treatment in the Rat.” *Neuroscience Letters* 602 (August): 89–94.
Sloot, W. N., and J. B. Gramsbergen. 1994. “Axonal Transport of Manganese and Its Relevance to Selective Neurotoxicity in the Rat Basal Ganglia.” *Brain Research* 657 (1-2): 124–32.
Ślusarczyk, Joanna, Ewa Trojan, Katarzyna Głombik, Bogusława Budziszewska, Marta Kubera, Władysław Lasoń, Katarzyna Popiołek-Barczyk, Joanna Mika, Krzysztof Wędzony, and Agnieszka Basta-Kaim. 2015. “Prenatal Stress Is a Vulnerability Factor for Altered Morphology and Biological Activity of Microglia Cells.” *Frontiers in Cellular Neuroscience* 9 (March): 82.
Smith, Stephen E. P., Jennifer Li, Krassimira Garbett, Karoly Mirnics, and Paul H. Patterson. 2007. “Maternal Immune Activation Alters Fetal Brain Development through Interleukin-6.” *The Journal of Neuroscience: The Official Journal of the Society for Neuroscience* 27 (40): 10695–702.
Smolders, Silke, Sophie M. T. Smolders, Nina Swinnen, Annette Gärtner, Jean-Michel Rigo, Pascal Legendre, and Bert Brône. 2015. “Maternal Immune Activation Evoked by Polyinosinic:polycytidylic Acid Does Not Evoke Microglial Cell Activation in the Embryo.” *Frontiers in Cellular Neuroscience* 9 (August): 301.
Solek, Cynthia M., Nasr Farooqi, Myriam Verly, Tony K. Lim, and Edward S. Ruthazer. 2018. “Maternal Immune Activation in Neurodevelopmental Disorders.” *Developmental Dynamics*. https://doi.org/10.1002/dvdy.24612.
Solowij, Nadia, and Patricia T. Michie. 2007. “Cannabis and Cognitive Dysfunction: Parallels with Endophenotypes of Schizophrenia?” *Journal of Psychiatry & Neuroscience: JPN* 32 (1): 30–52.
Sorensen, H. J., E. L. Mortensen, J. M. Reinisch, and S. A. Mednick. 2009. “Association Between Prenatal Exposure to Bacterial Infection and Risk of Schizophrenia.” *Schizophrenia Bulletin*. https://doi.org/10.1093/schbul/sbn121.

Spann, Marisa N., Catherine Monk, Dustin Scheinost, and Bradley S. Peterson. 2018. “Maternal Immune Activation During the Third Trimester Is Associated with Neonatal Functional Connectivity of the Salience Network and Fetal to Toddler Behavior.” *The Journal of Neuroscience: The Official Journal of the Society for Neuroscience* 38 (11): 2877–86.
Stachowiak, M. K., A. Kucinski, R. Curl, C. Syposs, Y. Yang, S. Narla, C. Terranova, et al. 2013. “Schizophrenia: A Neurodevelopmental Disorder — Integrative Genomic Hypothesis and Therapeutic Implications from a Transgenic Mouse Model.” *Schizophrenia Research*. https://doi.org/10.1016/j.schres.2012.11.004.
Stefanis, N. C., P. Delespaul, C. Henquet, C. Bakoula, C. N. Stefanis, and J. Van Os. 2004. “Early Adolescent Cannabis Exposure and Positive and Negative Dimensions of Psychosis.” *Addiction* 99 (10): 1333–41.
Stewart, Adam Michael, and Allan V. Kalueff. 2015. “Developing Better and More Valid Animal Models of Brain Disorders.” *Behavioural Brain Research* 276 (January): 28–31.
Sugranyes, Gisela, Itziar Flamarique, Eduard Parellada, Immaculada Baeza, Javier Goti, Emilio Fernandez-Egea, and Miquel Bernardo. 2009. “Cannabis Use and Age of Diagnosis of Schizophrenia.” *European Psychiatry: The Journal of the Association of European Psychiatrists* 24 (5): 282–86.
Sunwoo, Jun-Sang, Daejong Jeon, Soon-Tae Lee, Jangsup Moon, Jung-Suk Yu, Dong-Kyu Park, Ji-Yeon Bae, et al. 2018. “Maternal Immune Activation Alters Brain microRNA Expression in Mouse Offspring.” *Annals of Clinical and Translational Neurology* 5 (10): 1264–76.
Susser, E., S. P. Lin, A. S. Brown, L. H. Lumey, and L. Erlenmeyer-Kimling. 1994. “No Relation between Risk of Schizophrenia and Prenatal Exposure to Influenza in Holland.” *The American Journal of Psychiatry* 151 (6): 922–24.
Susser, E. S., C. A. Schaefer, A. S. Brown, M. D. Begg, and R. J. Wyatt. 2000. “The Design of the Prenatal Determinants of Schizophrenia Study.” *Schizophrenia Bulletin* 26 (2): 257–73.
Suzuki, Katsuaki, Genichi Sugihara, Yasuomi Ouchi, Kazuhiko Nakamura, Masami Futatsubashi, Kiyokazu Takebayashi, Yujiro Yoshihara, et al. 2013. “Microglial Activation in Young Adults with Autism Spectrum Disorder.” *JAMA Psychiatry* 70 (1): 49–58.
Szulc, Kamila U., Jason P. Lerch, Brian J. Nieman, Benjamin B. Bartelle, Miriam Friedel, Giselle A. Suero-Abreu, Charles Watson, Alexandra L. Joyner, and Daniel H. Turnbull. 2015. “4D MEMRI Atlas of Neonatal FVB/N Mouse Brain Development.” *NeuroImage* 118 (September): 49–62.
Takeda, A., Y. Kodama, S. Ishiwatari, and S. Okada. 1998. “Manganese Transport in the Neural Circuit of Rat CNS.” *Brain Research Bulletin* 45 (2): 149–52.
Takei, N., P. Sham, E. O’Callaghan, G. K. Murray, G. Glover, and R. M. Murray. 1994. “Prenatal Exposure to Influenza and the Development of Schizophrenia: Is the Effect Confined to Females?” *The American Journal of Psychiatry* 151 (1): 117–19.
Terwisscha van Scheltinga, Afke F., Steven C. Bakker, and René S. Kahn. 2010. “Fibroblast Growth Factors in Schizophrenia.” *Schizophrenia Bulletin* 36 (6): 1157–66.
Thion, Morgane S., Florent Ginhoux, and Sonia Garel. 2018. “Microglia and Early Brain Development: An Intimate Journey.” *Science* 362 (6411): 185–89.
Thion, Morgane Sonia, and Sonia Garel. 2017. “On Place and Time: Microglia in Embryonic and Perinatal Brain Development.” *Current Opinion in Neurobiology* 47 (December): 121–30.
Thion, Morgane Sonia, Coralie-Anne Mosser, Isabelle Férézou, Pauline Grisel, Sofia Baptista, Donovan Low, Florent Ginhoux, Sonia Garel, and Etienne Audinat. 2019. “Biphasic Impact of Prenatal Inflammation and Macrophage Depletion on the Wiring of Neocortical

Inhibitory Circuits." *Cell Reports* 28 (5): 1119–26.e4.
Toennes, Stefan W., Johannes G. Ramaekers, Eef L. Theunissen, Manfred R. Moeller, and Gerold F. Kauert. 2008. "Comparison of Cannabinoid Pharmacokinetic Properties in Occasional and Heavy Users Smoking a Marijuana or Placebo Joint." *Journal of Analytical Toxicology* 32 (7): 470–77.
Tordjman, Sylvie, Dominique Drapier, Olivier Bonnot, Rozenn Graignic, Sylvia Fortes, David Cohen, Bruno Millet, Claudine Laurent, and Pierre L. Roubertoux. 2007. "Animal Models Relevant to Schizophrenia and Autism: Validity and Limitations." *Behavior Genetics* 37 (1): 61–78.
Torrey, E. F., R. Rawlings, and I. N. Waldman. 1988. "Schizophrenic Births and Viral Diseases in Two States." *Schizophrenia Research* 1 (1): 73–77.
Tracy, J. I., J. de Leon, G. Qureshi, E. M. McCann, A. McGrory, and R. C. Josiassen. 1996. "Repetitive Behaviors in Schizophrenia: A Single Disturbance or Discrete Symptoms?" *Schizophrenia Research* 20 (1-2): 221–29.
Tremblay, Marie-Ève, and Ania K. Majewska. 2011. "A Role for Microglia in Synaptic Plasticity?" *Communicative & Integrative Biology* 4 (2): 220–22.
Tronnes, Ashlie A., Jenna Koschnitzky, Ray Daza, Jane Hitti, Jan Marino Ramirez, and Robert Hevner. 2016. "Effects of Lipopolysaccharide and Progesterone Exposures on Embryonic Cerebral Cortex Development in Mice." *Reproductive Sciences* 23 (6): 771–78.
Tseng, Alan H., and Rebecca M. Craft. 2004. "CB(1) Receptor Mediation of Cannabinoid Behavioral Effects in Male and Female Rats." *Psychopharmacology* 172 (1): 25–30.
Tsukada, Tsuyoshi, Eriko Simamura, Hiroki Shimada, Takuma Arai, Nobuaki Higashi, Takuya Akai, Hideaki Iizuka, and Toshihisa Hatta. 2015. "The Suppression of Maternal-Fetal Leukemia Inhibitory Factor Signal Relay Pathway by Maternal Immune Activation Impairs Brain Development in Mice." *PloS One* 10 (6): e0129011.
Turner, Cortney A., Emine Eren-Koçak, Edny G. Inui, Stanley J. Watson, and Huda Akil. 2016. "Dysregulated Fibroblast Growth Factor (FGF) Signaling in Neurological and Psychiatric Disorders." *Seminars in Cell & Developmental Biology* 53 (May): 136–43.
Tzilos, Golfo K., Christina B. Cintron, Jonas B. R. Wood, Norah S. Simpson, Ashley D. Young, Harrison G. Pope Jr, and Deborah A. Yurgelun-Todd. 2005. "Lack of Hippocampal Volume Change in Long-Term Heavy Cannabis Users." *The American Journal on Addictions / American Academy of Psychiatrists in Alcoholism and Addictions* 14 (1): 64–72.
Vaccarino, Flora M., Elena L. Grigorenko, Karen Müller Smith, and Hanna E. Stevens. 2009. "Regulation of Cerebral Cortical Size and Neuron Number by Fibroblast Growth Factors: Implications for Autism." *Journal of Autism and Developmental Disorders* 39 (3): 511–20.
Vaessen, Thomas Stephanus Johannes, Lea de Jong, Annika Theresia Schäfer, Thomas Damen, Aniek Uittenboogaard, Pauline Krolinski, Chinyere Vicky Nwosu, et al. 2018. "The Interaction between Cannabis Use and the Val158Met Polymorphism of the COMT Gene in Psychosis: A Transdiagnostic Meta - Analysis." *PloS One* 13 (2): e0192658.
Van den Berge, Koen, Katharina M. Hembach, Charlotte Soneson, Simone Tiberi, Lieven Clement, Michael I. Love, Rob Patro, and Mark D. Robinson. 2019. "RNA Sequencing Data: Hitchhiker's Guide to Expression Analysis," July. https://doi.org/10.1146/annurev-biodatasci-072018-021255.
Van den Eynde, Karlien, Stephan Missault, Erik Fransen, Leen Raeymaekers, Roland Willems, Wilhelmus Drinkenburg, Jean-Pierre Timmermans, Samir Kumar-Singh, and Stefanie Dedeurwaerdere. 2014. "Hypolocomotive Behaviour Associated with Increased Microglia

in a Prenatal Immune Activation Model with Relevance to Schizophrenia." *Behavioural Brain Research* 258 (January): 179–86.
Van Os, J., Maarten Bak, M. Hanssen, R. V. Bijl, R. De Graaf, and H. Verdoux. 2002. "Cannabis Use and Psychosis: A Longitudinal Population-Based Study." *American Journal of Epidemiology* 156 (4): 319–27.
Varghese, Merina, Neha Keshav, Sarah Jacot-Descombes, Tahia Warda, Bridget Wicinski, Dara L. Dickstein, Hala Harony-Nicolas, et al. 2017. "Autism Spectrum Disorder: Neuropathology and Animal Models." *Acta Neuropathologica* 134 (4): 537–66.
Verdurand, Mathieu, Victoria S. Dalton, Vu Nguyen, Marie-Claude Grégoire, David Zahra, Naomi Wyatt, Leena Burgess, Ivan Greguric, and Katerina Zavitsanou. 2014. "Prenatal Poly I:C Age-Dependently Alters Cannabinoid Type 1 Receptors in Offspring: A Longitudinal Small Animal PET Study Using [(18)F]MK-9470." *Experimental Neurology* 257 (July): 162–69.
Verrico, Christopher D., Hong Gu, Melanie L. Peterson, Allan R. Sampson, and David A. Lewis. 2014. "Repeated Δ9-Tetrahydrocannabinol Exposure in Adolescent Monkeys: Persistent Effects Selective for Spatial Working Memory." *The American Journal of Psychiatry* 171 (4): 416–25.
Vinet, Évelyne, Christian A. Pineau, Ann E. Clarke, Susan Scott, Éric Fombonne, Lawrence Joseph, Robert W. Platt, and Sasha Bernatsky. 2015. "Increased Risk of Autism Spectrum Disorders in Children Born to Women with Systemic Lupus Erythematosus: Results from a Large Population-Based Cohort." *Arthritis & Rheumatology* 67 (12): 3201–8.
Vogel Ciernia, Annie, Milo Careaga, Janine M. LaSalle, and Paul Ashwood. 2018. "Microglia from Offspring of Dams with Allergic Asthma Exhibit Epigenomic Alterations in Genes Dysregulated in Autism." *Glia* 66 (3): 505–21.
Volkow, Nora D., George F. Koob, Robert T. Croyle, Diana W. Bianchi, Joshua A. Gordon, Walter J. Koroshetz, Eliseo J. Pérez-Stable, et al. 2018. "The Conception of the ABCD Study: From Substance Use to a Broad NIH Collaboration." *Developmental Cognitive Neuroscience* 32 (August): 4–7.
Volkow, Nora D., James M. Swanson, A. Eden Evins, Lynn E. DeLisi, Madeline H. Meier, Raul Gonzalez, Michael A. P. Bloomfield, H. Valerie Curran, and Ruben Baler. 2016. "Effects of Cannabis Use on Human Behavior, Including Cognition, Motivation, and Psychosis: A Review." *JAMA Psychiatry* 73 (3): 292–97.
Vousden, Dulcie A., Elizabeth Cox, Rylan Allemang-Grand, Christine Laliberté, Lily R. Qiu, Zsuzsa Lindenmaier, Brian J. Nieman, and Jason P. Lerch. 2018. "Continuous Manganese Delivery via Osmotic Pumps for Manganese-Enhanced Mouse MRI Does Not Impair Spatial Learning but Leads to Skin Ulceration." *NeuroImage* 173 (June): 411–20.
Vries, Petrus J. de. 2009. "Genetics and Neuropsychiatric Disorders: Genome-Wide, yet Narrow." *Nature Medicine* 15 (8): 850–51.
Vuillermot, Stephanie, Wei Luan, Urs Meyer, and Darryl Eyles. 2017. "Vitamin D Treatment during Pregnancy Prevents Autism-Related Phenotypes in a Mouse Model of Maternal Immune Activation." *Molecular Autism* 8 (March): 9.
Wallace, K., S. Veerisetty, I. Paul, W. May, J. J. Miguel-Hidalgo, and W. Bennett. 2010. "Prenatal Infection Decreases Calbindin, Decreases Purkinje Cell Volume and Density and Produces Long-Term Motor Deficits in Sprague-Dawley Rats." *Developmental Neuroscience* 32 (4): 302–12.
Walter, Lisa, Allyn Franklin, Anke Witting, Christian Wade, Yiheng Xie, George Kunos, Ken

Mackie, and Nephi Stella. 2003. “Nonpsychotropic Cannabinoid Receptors Regulate Microglial Cell Migration.” *The Journal of Neuroscience: The Official Journal of the Society for Neuroscience* 23 (4): 1398–1405.
Wang, Ti, Zhen Zeng, Zhiwei Hu, Linqing Zheng, Tao Li, You Li, Jie Liu, et al. 2012. “FGFR2 Is Associated with Bipolar Disorder: A Large-Scale Case–control Study of Three Psychiatric Disorders in the Chinese Han Population.” *The World Journal of Biological Psychiatry: The Official Journal of the World Federation of Societies of Biological Psychiatry* 13 (8): 599–604.
Wang, Zhong, Mark Gerstein, and Michael Snyder. 2009. “RNA-Seq: A Revolutionary Tool for Transcriptomics.” *Nature Reviews. Genetics* 10 (1): 57–63.
Weber-Stadlbauer, Ulrike, and Urs Meyer. 2019. “Challenges and Opportunities of a-Priori and a-Posteriori Variability in Maternal Immune Activation Models.” *Current Opinion in Behavioral Sciences* 28 (August): 119–28.
Wei, E. T., J. G. Kiang, P. Buchan, and T. W. Smith. 1986. “Corticotropin-Releasing Factor Inhibits Neurogenic Plasma Extravasation in the Rat Paw.” *The Journal of Pharmacology and Experimental Therapeutics* 238 (3): 783–87.
Weinhard, Laetitia, Giulia di Bartolomei, Giulia Bolasco, Pedro Machado, Nicole L. Schieber, Urte Neniskyte, Melanie Exiga, et al. 2018. “Microglia Remodel Synapses by Presynaptic Trogocytosis and Spine Head Filopodia Induction.” *Nature Communications* 9 (1): 1228.
Weinstein, Aviv, Orit Brickner, Hedva Lerman, Mazal Greemland, Miki Bloch, Hava Lester, Roland Chisin, et al. 2008. “Brain Imaging Study of the Acute Effects of Delta9-Tetrahydrocannabinol (THC) on Attention and Motor Coordination in Regular Users of Marijuana.” *Psychopharmacology* 196 (1): 119–31.
Welberg, Leonie. 2014. “Synaptic Plasticity: A Synaptic Role for Microglia.” *Nature Reviews. Neuroscience*.
Werling, Donna M., Sirisha Pochareddy, Jinmyung Choi, Joon-Yong An, Brooke Sheppard, Minshi Peng, Zhen Li, et al. 2020. “Whole-Genome and RNA Sequencing Reveal Variation and Transcriptomic Coordination in the Developing Human Prefrontal Cortex.” *Cell Reports* 31 (1): 107489.
Wetherby, Amy M., Nola Watt, Lindee Morgan, and Stacy Shumway. 2007. “Social Communication Profiles of Children with Autism Spectrum Disorders Late in the Second Year of Life.” *Journal of Autism and Developmental Disorders* 37 (5): 960–75.
Wiley, Jenny L., Mary M. O’Connell, Mary E. Tokarz, and M. Jerry Wright. 2007. “Pharmacological Effects of Acute and Repeated Administration of Δ9-Tetrahydrocannabinol in Adolescent and Adult Rats.” *The Journal of Pharmacology and Experimental Therapeutics* 320 (3): 1097–1105.
Willner, P. 1984. “The Validity of Animal Models of Depression.” *Psychopharmacology* 83 (1): 1–16.
Winterburn, Julie L., Jens C. Pruessner, Sofia Chavez, Mark M. Schira, Nancy J. Lobaugh, Aristotle N. Voineskos, and M. Mallar Chakravarty. 2013. “A Novel in Vivo Atlas of Human Hippocampal Subfields Using High-Resolution 3 T Magnetic Resonance Imaging.” *NeuroImage* 74 (July): 254–65.
Wischhof, Lena, Ellen Irrsack, Carmen Osorio, and Michael Koch. 2015. “Prenatal LPS-Exposure – a Neurodevelopmental Rat Model of Schizophrenia – Differentially Affects Cognitive Functions, Myelination and Parvalbumin Expression in Male and Female Offspring.” *Progress in Neuro-Psychopharmacology and Biological Psychiatry*.

https://doi.org/10.1016/j.pnpbp.2014.10.004.
Wojcik, Sophie, Sasha Bernatsky, Robert W. Platt, Christian A. Pineau, Ann E. Clarke, Éric Fombonne, Anick Bérard, and Évelyne Vinet. 2017. "Risk of Autism Spectrum Disorders in Children Born to Mothers With Rheumatoid Arthritis: A Systematic Literature Review." *Arthritis Care & Research* 69 (12): 1926–31.
Wolman, Benjamin B. 1988. "The Immune System." *Psychosomatic Disorders*. https://doi.org/10.1007/978-1-4684-5520-5_5.
Wong, Michael D., Adrienne E. Dorr, Johnathon R. Walls, Jason P. Lerch, and R. Mark Henkelman. 2012. "A Novel 3D Mouse Embryo Atlas Based on Micro-CT." *Development* 139 (17): 3248–56.
Wong, Michael D., Matthijs C. van Eede, Shoshana Spring, Stefan Jevtic, Julia C. Boughner, Jason P. Lerch, and R. Mark Henkelman. 2015. "4D Atlas of the Mouse Embryo for Precise Morphological Staging." *Development* 142 (20): 3583–91.
Wong, Michael D., Yoshiro Maezawa, Jason P. Lerch, and R. Mark Henkelman. 2014. "Automated Pipeline for Anatomical Phenotyping of Mouse Embryos Using Micro-CT." *Development* 141 (12): 2533–41.
Wong, Michael D., Shoshana Spring, and R. Mark Henkelman. 2013. "Structural Stabilization of Tissue for Embryo Phenotyping Using Micro-CT with Iodine Staining." *PloS One* 8 (12): e84321.
Wormser, G. P., and R. W. Tolan. 2006. "Infectious Diseases of the Fetus and Newborn Infant, 6th Edition Edited by Jack S. Remington, Jerome O. Klein, Christopher B. Wilson, and Carol J. Baker Philadelphia: Elsevier Saunders, 2006. 1328 Pp., Illustrated. $229.00 (cloth)." *Clinical Infectious Diseases*. https://doi.org/10.1086/501023.
Wu, Dan, Jiadi Xu, Michael T. McMahon, Peter C. M. van Zijl, Susumu Mori, Frances J. Northington, and Jiangyang Zhang. 2013. "In Vivo High-Resolution Diffusion Tensor Imaging of the Mouse Brain." *NeuroImage* 83 (December): 18–26.
Xia, Cedric Huchuan, Zongming Ma, Rastko Ciric, Shi Gu, Richard F. Betzel, Antonia N. Kaczkurkin, Monica E. Calkins, et al. 2018. "Linked Dimensions of Psychopathology and Connectivity in Functional Brain Networks." *Nature Communications* 9 (1): 3003.
Xiang, Anny H., Xinhui Wang, Mayra P. Martinez, Kathleen Page, Thomas A. Buchanan, and R. Klara Feldman. 2018. "Maternal Type 1 Diabetes and Risk of Autism in Offspring." *JAMA: The Journal of the American Medical Association* 320 (1): 89–91.
Xiao, Jianchun, Stephen L. Buka, Tyrone D. Cannon, Yasuhiro Suzuki, Raphael P. Viscidi, E. Fuller Torrey, and Robert H. Yolken. 2009. "Serological Pattern Consistent with Infection with Type I Toxoplasma Gondii in Mothers and Risk of Psychosis among Adult Offspring." *Microbes and Infection / Institut Pasteur* 11 (13): 1011–18.
Xuan, Ingrid C. Y., and David R. Hampson. 2014. "Gender-Dependent Effects of Maternal Immune Activation on the Behavior of Mouse Offspring." *PloS One* 9 (8): e104433.
Yanguas-Casás, Natalia. n.d. "Physiological Sex Differences in Microglia and Their Relevance in Neurological Disorders." https://nnjournal.net/article/view/3357.
Yücel, Murat, Nadia Solowij, Colleen Respondek, Sarah Whittle, Alex Fornito, Christos Pantelis, and Dan I. Lubman. 2008. "Regional Brain Abnormalities Associated with Long-Term Heavy Cannabis Use." *Archives of General Psychiatry* 65 (6): 694–701.
Yudofsky, Stuart C. 2009. "Contracting Schizophrenia." *JAMA: The Journal of the American Medical Association* 301 (3): 324–26.
Zalesky, Andrew, Nadia Solowij, Murat Yücel, Dan I. Lubman, Michael Takagi, Ian H. Harding,

Valentina Lorenzetti, et al. 2012. “Effect of Long-Term Cannabis Use on Axonal Fibre Connectivity.” *Brain: A Journal of Neurology* 135 (Pt 7): 2245–55.
Zammit, Stanley, Peter Allebeck, Sven Andreasson, Ingvar Lundberg, and Glyn Lewis. 2002. “Self Reported Cannabis Use as a Risk Factor for Schizophrenia in Swedish Conscripts of 1969: Historical Cohort Study.” *BMJ* 325 (7374): 1199.
Zaretsky, Michael V., James M. Alexander, William Byrd, and Roger E. Bawdon. 2004. “Transfer of Inflammatory Cytokines across the Placenta.” *Obstetrics and Gynecology* 103 (3): 546–50.
Zeighami, Yashar, Seyed-Mohammad Fereshtehnejad, Mahsa Dadar, D. Louis Collins, Ronald B. Postuma, Bratislav Mišić, and Alain Dagher. 2019. “A Clinical-Anatomical Signature of Parkinson’s Disease Identified with Partial Least Squares and Magnetic Resonance Imaging.” *NeuroImage* 190 (April): 69–78.
Zerbo, Ousseny, Ana-Maria Iosif, Cheryl Walker, Sally Ozonoff, Robin L. Hansen, and Irva Hertz-Picciotto. 2013. “Is Maternal Influenza or Fever during Pregnancy Associated with Autism or Developmental Delays? Results from the CHARGE (CHildhood Autism Risks from Genetics and Environment) Study.” *Journal of Autism and Developmental Disorders* 43 (1): 25–33.
Zerbo, Ousseny, Yinge Qian, Cathleen Yoshida, Judith K. Grether, Judy Van de Water, and Lisa A. Croen. 2015. “Maternal Infection During Pregnancy and Autism Spectrum Disorders.” *Journal of Autism and Developmental Disorders* 45 (12): 4015–25.
Zhang, C., X-Y Li, L. Zhao, H. Wang, and D-X Xu. 2007. “Lipopolysaccharide (LPS) up-Regulates the Expression of Haem Oxygenase-1 in Mouse Placenta.” *Placenta* 28 (8-9): 951–57.
Zhang, Jun-Ming, and Jianxiong An. 2007. “Cytokines, Inflammation, and Pain.” *International Anesthesiology Clinics* 45 (2): 27–37.
Zhang, Ying, Brittany N. Cazakoff, Chester A. Thai, and John G. Howland. 2012. “Prenatal Exposure to a Viral Mimetic Alters Behavioural Flexibility in Male, but Not Female, Rats.” *Neuropharmacology* 62 (3): 1299–1307.
Zhang, Zhi, and Henriette van Praag. 2015. “Maternal Immune Activation Differentially Impacts Mature and Adult-Born Hippocampal Neurons in Male Mice.” *Brain, Behavior, and Immunity* 45 (March): 60–70.
Zou, Jian, Taro Kawai, Tetsuo Tsuchida, Tatsuya Kozaki, Hiroki Tanaka, Kyung-Sue Shin, Himanshu Kumar, and Shizuo Akira. 2013. “Poly IC Triggers a Cathepsin D- and IPS-1-Dependent Pathway to Enhance Cytokine Production and Mediate Dendritic Cell Necroptosis.” *Immunity* 38 (4): 717–28.
Zuckerman, Lee, Moshe Rehavi, Rachel Nachman, and Ina Weiner. 2003. “Immune Activation During Pregnancy in Rats Leads to a PostPubertal Emergence of Disrupted Latent Inhibition, Dopaminergic Hyperfunction, and Altered Limbic Morphology in the Offspring: A Novel Neurodevelopmental Model of Schizophrenia.” *Neuropsychopharmacology*. https://doi.org/10.1038/sj.npp.1300248.