The interaction between circadian disruption and prenatal immune activation in relation to neurodevelopmental disorders

Tara Christine Delorme

Doctor of Philosophy

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

August 8th, 2023

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

ABSTRACT

Neurodevelopmental disorders (NDDs), such as schizophrenia and autism spectrum disorders, are multifactorial in nature, caused by genetic risk variants, and environmental risk factors, such as prenatal exposure to maternal infection. Studying how multiple risk factors for NDDs interact with each other, rather than studying the effects of single risk factors, is necessary and becoming a recurrent theme in research. In the current thesis, we aimed to understand the interaction between a well-documented risk factor for NDDs, prenatal infection, and a less commonly discussed risk factor, circadian rhythm disruption. We used a combination of behavioral (including running wheel), cellular (immunohistochemistry, fluorescence microscopy), and molecular (RNA sequencing) approaches to uncover the mechanisms underlying the interaction between circadian disruption and NDDs, using a maternal immune activation (MIA) protocol, which models prenatal infection in mice.

Various sleep/circadian disturbances are highly prevalent in individuals with NDDs (discussed in **BACKGROUND)**. **Chapter 1** presents a published study showing that exposure to MIA altered circadian running patterns in mice, reminiscent of the disrupted rest/wake patterns in individuals with NDDs. Given that NDDs are thought to be caused by multiple interacting risk factors, we next sought to investigate whether environmental circadian disruption interacted with pre-existing risk factors for NDDs (prenatal infection) to affect disorder-related outcomes. Accordingly, we tested the hypothesis that MIA combined with circadian disruption synergistically induces sex–specific deficits in offspring. The protocol used to induce environmental circadian disruption was LL exposure, which was meant to mimic light at night. **Chapter 2** also presents a published study showing behavioral and microglial effects in MIA offspring before and after exposure to LL in adulthood. Poly IC exposure led to significant

behavioral differences, including reduced sociability specifically when males were tested after LL exposure. Poly IC exposure also led to increased microglial morphology index and density in dentate gyrus, an effect attenuated by LL exposure. **Chapter 3** builds upon these findings, by investigating the behavioral, microglial, and transcriptomic effects in MIA offspring after LL exposure in adolescence. Adolescence is a critical period of brain development and is when psychiatric disorders tend to emerge. When we combined prenatal poly IC exposure with adolescent LL exposure, we observed significant differences in cognitive, anxiety and sociability-related behaviours. For example, while MIA in males led to social deficits under LD, these differences were not observed between groups under LL. Additionally, MIA in males, but not females, caused a more activated microglial profile, while LL exposure induced a less reactive profile and less dense distribution of microglia. We also found that MIA led to many differentially expressed genes in males, which was diminished by LL. The rank-rank hypergeometric overlap algorithm identified transcriptional synchrony between groups within sexes, and discordant patterns between sexes. The weighted correlation network analysis identified significant clusters of co-expressed genes that were associated with either MIA or LL.

Finally, the **DISCUSSION** provides a summary and interpretation of the findings in this thesis. Altogether, this thesis integrates behavioral, cellular, and molecular modalities to understand the mechanisms by which circadian disruption interacts with prenatal poly IC exposure. It is critical to understand this interaction due to circadian disruption being ubiquitously experienced in our 24-hour society, and with the widespread transmission of viral infections (e.g., influenza, COVID-19) that affect many pregnant individuals. Our findings inform the development of circadian-based therapies that aim to prevent or mitigate symptoms in NDDs.

RÉSUMÉ

Les troubles neurodéveloppementaux (NDDs), tels que la schizophrénie et les troubles du spectre de l'autisme, sont considérés comme de nature multifactorielle, causés par des variants de risque génétiques et des facteurs de risque environnementaux, tels que l'exposition prénatale à l'infection maternelle. Dans cette thèse, nous avons cherché à comprendre l'interaction entre un facteur de risque bien documenté pour les NDDs, l'infection prénatale, et un facteur de risque moins couramment discuté, la perturbation des rythmes circadiens. Nous avons utilisé une combinaison d'approches comportementales (y compris analyses de roues), cellulaires (immunohistochimie, microscopie à fluorescence) et moléculaires (séquençage d'ARN) pour découvrir les mécanismes sous-jacents à l'interaction entre la perturbation circadienne et les NDDs, en utilisant une activation immunitaire maternelle (MIA), qui modélise l'infection prénatale chez la souris.

Divers troubles du sommeil et des rythmes circadiens sont très répandus chez les personnes atteintes de NDD (abordé au **BACKGROUND)**. Le **chapitre 1** présente une étude publiée montrant que l'exposition au MIA altère les rythmes d'activité chez la souris, rappelant les cycles de repos/éveil perturbés chez les personnes atteintes de NDD. Étant donné que l'on pense que les NDDs sont causés par plusieurs facteurs de risque en interaction, nous avons ensuite cherché à déterminer si la perturbation circadienne environnementale interagissait avec des facteurs de risque préexistants de NDD (infection prénatale) pour affecter les résultats liés à ces maladies. Le protocole utilisé pour induire une perturbation circadienne environnementale était l'exposition LL. Le **chapitre 2** présente les effets comportementaux et microgliaux chez les descendants MIA avant et après que les souris aient été exposées à LL à l'âge adulte. Nous avons constaté que l'exposition au poly IC entraînait des différences de comportement significatives, notamment une sociabilité

réduite, lorsque les mâles étaient testés après une exposition à la LL. L'exposition au poly IC a entraîné une augmentation de l'indice de morphologie microgliale et de la densité dans le gyrus denté, un effet atténué par l'exposition à la LL. Le **chapitre 3** s'appuie sur ces résultats en étudiant les effets comportementaux, microgliaux et transcriptomiques chez les descendants MIA après une exposition à la LL à l'adolescence. Nous avons observé des différences significatives dans les comportements cognitifs, anxieux et liés à la sociabilité. De plus, le MIA chez les mâles, mais pas chez les femmelles, a provoqué un profil microglial plus activé, tandis que l'exposition à la LL a induit un profil moins réactif et une distribution moins dense des microglies. Nous avons également constaté que MIA conduisait à de nombreux gènes exprimés de manière différentielle chez les mâles, ce qui était diminué par LL. L'algorithme de chevauchement hypergéométrique rang-rang a identifié une synchronie transcriptionnelle entre les groupes au sein des sexes et les modèles discordants entre les sexes. L'analyse du réseaux de corrélation pondérée a identifié des modules importantes de gènes co-exprimés qui étaient associés à MIA ou LL.

La **DISCUSSION** fournit une discussion des résultats de cette thèse. Cette thèse intègre les modalités comportementales, cellulaires et moléculaires pour comprendre les mécanismes par lequel la perturbation circadienne interagit avec l'exposition prénatale au poly IC. Il est essentiel de comprendre cette interaction en raison de la perturbation circadienne omniprésente dans notre société active 24 heures sur 24 et de la transmission généralisée des infections virales qui affectent de nombreuses personnes enceintes. Nos découvertes éclairent le développement de thérapies basées sur les rythmes circadiens qui visent à prévenir ou à atténuer les symptômes des NDDs.

TABLE OF CONTENTS

LIST OF FIGURES

Background

Chapter 1: Altered Circadian Rhythms in a Mouse Model of Neurodevelopmental Disorders Based on Prenatal Maternal Immune Activation

LIST OF TABLES

Chapter 3: Effects of prenatal immune activation and exposure to circadian disruption during adolescence: exploring the two-hit model of neurodevelopmental disorders

LIST OF ABBREVIATIONS

NDD = neurodevelopmental disorders

ASD = autism spectrum disorders

MIA = maternal immune activation

Poly IC = polyinosinic:polycytidylic acid

CNS = central nervous system

LL = constant light

EPM = elevated plus maze

PPI = prepulse inhibition of acoustic startle

DG = dentate gyrus

CA1 = cornu ammonis 1

PFC = prefrontal cortex

SCN = suprachiasmatic nucleus

ipRGCs = intrinsically photosensitive retinal ganglion cells

ACKNOWLEDGEMENTS

I extend my greatest gratitude to my PhD supervisors Dr. Nicolas Cermakian and Dr. Lalit Srivastava. This thesis would not have been possible without your expertise, valuable insight, and commitment to my personal and professional development. Thank you both for being pillars of support, and excellent mentors throughout my PhD. Nico, your quick and constructive feedback, unwavering support, and encouragement have been instrumental these past years. Thank you for always providing me with opportunities to showcase my work and helping me navigate difficult decisions. Lalit, thank you for your invaluable feedback, for being supportive and a constant source of motivation. Thank you for having an open-door policy and always being willing to discuss. I am extremely grateful for the dedication of both my supervisors in my growth as a scientist and for the development of this thesis. I could not have done it without you both, and I thank you both greatly.

I could not have undertaken this thesis without the members of my committee. Thank you Dr. Mallar Chakravarty and Dr. Laura Fonken for serving as members of my defense committee and Dr. Tak Pan Wong for serving as chair. Thank you for your time, expertise, and interest in this thesis. I also thank Dr. Florian Storch, the late Dr. Giamal Luheshi and Dr. Dominique Walker for serving as members of my committee throughout my degree. Your constructive and thoughtful feedback and insightful suggestions have helped me improve the quality of this thesis and have pushed me to think more deeply about the implications of my findings.

I also thank our collaborators Dr. Patricia Silveira, Dr. Danusa Arcego, and Dr. Nicholas O'Toole for all your help on the transcriptomics experiment. Your expertise has been invaluable, and it was, and continues to be, a pleasure to work with you all. A special thank you to Dr. Elisa Guma for teaching me about maternal immune activation, always making the time to discuss protocols and data with me and helping me navigate postdoctoral opportunities.

A major thank you to my colleagues. Shashank Srikanta, i.e. Shanky with the stones, thank you for being my gym buddy, for choosing the best restaurants in town, for all the great chats and for your friendship. Pri (Priscilla Cabral), thank you for always being so supportive, always sending such kind messages and for bringing Max in to lift our spirits! Mec (Marie-Ève Cloutier), thank you for all your hard work, knowledge, and willingness to help, and for always bringing the sass. Nam (Namasthée Harris-Gauthier), thank you for the laughs that literally took our breath away, for the chats about making soap and all the fun times. Andy (Dr. Andrea Harée Pantoja Urbán), my brain reach partner, thank you for your friendship and always sticking up for me. Dr. Clément Bourguignon, run club enthusiast, thank you for all your help with the PIR sensors and for your extravagant desserts. Rafa (Rafael Pérez Medina) thank you for all the fun times, Spanish lessons, and bike rides to work. Amanda Larosa thank you for the supportive and fun office chats and for your kindness. Christine Kirady, thank you for hosting the best board game nights, and being the best Zumba partner. Thank you to Dr. Laura Kervezee for introducing me to werewolf and encouraging me on my biking journey, and Anna Koshy for being very welcoming, funny and kind. Sai (Saishree Badrinarayanan), Mary Loka, Jose Maria Restropo, Pratap Singh Markam, Geneviève Dubeau Laramée, and Dr. Nicolas Ludin thank you all for your support!

Big thank you to the Cermakian and Srivastava labs. Thank you to Sophia Stegeman for all the dog and plant chats, Maryam Malki for being the best office mate, Ahmed Abderaouf Bouteldja for the good laughs at the gym, Danae Penichet for being so kind and asking great questions, Sebastian Boy Waxman for your genuine interest in my project, and Genavieve Maloney for being so accommodating, and for being the coolest singer in the coolest band, and Sanjeev Bhardwaj for the helpful discussions.

Thank you to everyone at the Douglas Research Center. Thank you to the animal facility staff, including Eve-Marie Charbonneau, Tara Thomson, Jodie Middleton, Jessica Balbin, Vanessa Léal-Lapointe, Julien and especially Guylaine Gadoury, who I thank for always being willing to chat, for sharing dog stories and plant cuttings. I thank Melina Jaramillo Garcia and Dr. Bita Khadivjam for your expert microscopy knowledge. As well, thank you to Andrew for always being so kind and positive, I will miss our corridor chats! Lastly, thank you to the security guards who always stopped by to play ping pong with Shashank and I.

Thank you to all the undergraduate students I've had over the years. I really appreciate all the help with experiments, and data analysis. I'm proud to say that many of you received summer scholarships, presentation awards and got your name on publications! Big thank you to Katherine Lord, William Ozell, Victoria Light, Nikki Huebener, and Margaret Sayeh.

Finally, I thank my family and friends. My mother Lucia Di Liello for modeling ingenuity for me from a young age, and for always supporting and being there for me. My late father, Jacques Delorme, who I miss every day, for always believing in me and encouraging me to chase my dreams. My brother Eric Delorme, who is as clever as he is kind, for always being a calm presence and always willing to lend an ear. To our sweet dog Toby who was with us for 16 years and always offered a warm cuddle. Thank you deeply to the Di Liello family. Thank you to my uncle and aunt, Joe and Anna Di Liello, I'm lucky to have your support and I know that you're always a call away. My cousins Tessa and Jesse Di Liello, who are more like siblings to me than cousins, thank you for all the good laughs and harsh jokes. I miei nonni, Giuseppina and Angelo Di Liello (i.e., MA! and PA!), for teaching me the value of hard work and perseverance. Big thank you to my childhood best friends Alyssa Brown for always forcing me into nature, Megan Drupals for always making the time to meet up, and Nikole Gregory and Nova for walks by the water. Thank you to the Valyears (Luisa, Tim, Ris, Kiara, Rose, and Fortuna) for always being supportive and making the best pasta dishes. Last, but certainly not least, thank you to my partner Dr. Milan Valyear for the unconditional love and support, for picking me up whenever I was down, for reminding me to enjoy the simple things and for supporting me every step of my career.

This work would not have been possible without the support from various funding agencies. Thank you to the Canadian Institutes of Health Research (PJT-153299), Velux Stiftung (Project 927) and Ludmer – MI4 Collaborative Seed Fund Grant. Lastly, thank you to the Canadian College of Neuropsychopharmacology, The Schizophrenia Society of Canada Foundation and the Fonds de la recherche en santé du Quebec for funding me throughout my degree.

CONTRIBUTION OF AUTHORS

The original work in this thesis spans Chapters **1, 2, and 3**. For each manuscript, I led the experimental design, data acquisition, data processing, statistical analyses, and interpretation of results. I wrote the first draft of each manuscripts and incorporated revisions from co-authors. Each study features co-authors whose contributions were integral and invaluable to the completion of this work, especially my supervisors Dr. Nicolas Cermakian and Dr. Lalit Srivastava.

Chapter 1: Tara C. Delorme, Lalit K. Srivastava and Nicolas Cermakian. Altered Circadian Rhythms in a Mouse Model of Neurodevelopmental Disorders Based on Prenatal Maternal Immune Activation. *Brain Behavior, and Immunity*. 2021;93:119–31.
Published: https://doi.org/10.1016/j.bbi.2020.12.030

- LKS and NC: supervised the study, were involved in the conception and design of the experiments, interpretation of results, and revising manuscript for publication.

Chapter 2: Tara C. Delorme, William Ozell-Landry, Nicolas Cermakian and Lalit K. Srivastava. Behavioral and cellular responses to circadian disruption and prenatal immune activation in mice.
Published: https://doi.org/10.1038/s41598-023-34363-w

- WOL: was involved in data acquisition, data analysis and interpretation of results.
- NC and LKS: supervised the study, were involved in the conception and design of the experiments, interpretation of results, and revising manuscript for publication.

Chapter 3: Tara C. Delorme, Nikki Huebener, Danusa M. Arcego, Nick O'Toole, Patrícia P. Silveira, Lalit K. Srivastava and Nicolas Cermakian. Effects of prenatal maternal immune activation and exposure to circadian disruption during adolescence: exploring the two-hit model. *In preparation*.

- NH: was involved in data acquisition, data analysis and interpretation of results.
- DMA and NO: were involved in data analysis and interpretation of results.
- PPS: was involved in choosing ways to analyze data and interpretation of results.
- LKS and NC: supervised the study, were involved in the conception and design of the experiments, interpretation of results, and revising manuscript for publication.

Other related lead-author publications:

Tara C. Delorme, Lalit K. Srivastava and Nicolas Cermakian. Are Circadian Disturbances a Core Pathophysiological Component of Schizophrenia? *Journal of Biological Rhythms*. 2020;35:325–39.
Published: https://doi.org/10.1177/0748730420929448

Delorme TC, Srikanta SB, Fisk AS, Cloutier M-È, Sato M, Pothecary CA, Merz C, Foster RG, Brown SA, Peirson SN, Cermakian N, Banks GT. Chronic Exposure to Dim Light at Night or Irregular Lighting Conditions Impact Circadian Behavior, Motor Coordination, and Neuronal Morphology. *Frontiers in Neuroscience*. 2022;16:855154.
Published: https://doi.org/10.3389/fnins.2022.855154

ORIGINAL CONTRIBUTIONS OF THESIS

Background:

- Comprehensive literature review on the evidence supporting maternal immune activation as a risk factor for neurodevelopmental disorders, such as schizophrenia and autism spectrum disorders, in humans and in animals.
- Additionally, a review of the literature exploring sleep and circadian disturbances in schizophrenia and autism spectrum disorders (and related animal models), with an emphasis on sex differences and age of exposure to circadian disruption.
- We discuss the hypothesis that circadian rhythm disruption interacts with existing risk factors for neurodevelopmental disorders to promote relevant behavioral and neurobiological abnormalities.
- We also identify areas for future investigation, such as:
  - The need to understand the role of antipsychotics and sleep or circadian-promoting therapies or drugs on symptom severity in individuals with neurodevelopmental disorders and related animal models.
  - The need to uncover mechanistic links between neurodevelopmental disorders and the sleep and circadian systems.
  - The need for inclusion of both males and females.

Chapter 1: Altered Circadian Rhythms in a Mouse Model of Neurodevelopmental Disorders Based on Prenatal Maternal Immune Activation.

- Maternal immune activation (MIA) led to altered circadian rhythms largely in males.
- Female offspring showed milder effects of MIA exposure (induced by poly IC).
- Adult MIA male offspring exhibited increased (subjective) day locomotor activity.
- MIA males appear to have more activity at the start of the (subjective) day.

Chapter 2: Behavioral and cellular responses to circadian disruption and prenatal immune activation in mice.

- Behavioral phenotyping of male and female poly IC exposed mice before and after constant light (LL) exposure revealed behavioral differences, including reduced sociability (males only) and deficits in prepulse inhibition.
- In line with our hypothesis, adulthood LL exposure interacted with MIA to exacerbate behaviors related to neurodevelopmental disorders. Namely, MIA led to reduced sociability specifically when males were tested after LL exposure.
- Microglial phenotyping of male poly IC exposed mice after either standard lighting or LL revealed that poly IC exposure led to increased microglial morphology index and density in dentate gyrus; an effect attenuated by LL exposure.

Chapter 3: Effects of prenatal maternal immune activation and exposure to circadian disruption during adolescence: exploring the two-hit model.

- Behavioral phenotyping of male and female poly IC exposed mice after either standard lighting or LL revealed differences in cognitive, anxiety and sociability-related behaviours.

In particular, MIA in males led to social deficits under LD, whereas this difference were not observed under LL.

- Microglial phenotyping of male and female poly IC-exposed mice after either standard lighting or LL revealed that poly IC exposure in males only led to increased microglial morphology index and density in dentate gyrus while LL exposure induced a less reactive profile and less dense distribution of microglia.
- Transcriptional phenotyping of male and female poly IC-exposed mice after either standard lighting or LL revealed many differentially expressed genes in males, which was diminished by LL.
- The rank-rank hypergeometric overlap algorithm identified transcriptional synchrony between groups within sexes, and discordant patterns between sexes.
- A weighted correlation network analysis identified significant clusters of co-expressed genes that were associated with either MIA or LL.

INTRODUCTION

Sleep and circadian disturbances are prevalent in individuals with neurodevelopmental disorders (NDDs), with up to 80% of individuals with schizophrenia and autism spectrum disorders (ASD) being affected (Cohrs 2008, Wulff, Dijk et al. 2012, Carmassi, Palagini et al. 2019). Studies report that individuals with chronic schizophrenia exhibit alterations in circadian gene expression in various tissues (Johansson, Owe-Larsson et al. 2016, Seney, Cahill et al. 2019), and poorer sleep quality is correlated with greater symptom severity (Korenic, Klingaman et al. 2019). Sleep disturbances, including increased sleep latency and increased nighttime waking, are prevalent in children with ASD (Krakowiak, Goodlin-Jones et al. 2008, Souders, Mason et al. 2009). Although problems in sleep and circadian rhythms have been documents in these individuals for decades, few studies have investigated the underlying mechanisms driving these disturbances.

A related but distinct topic of study is if environmental circadian disruption is a risk factor for NDDs. Circadian rhythms are involved in the regulation of numerous activities in the body including sleep/wake cycles, hormones, metabolism, immune function, and various behaviors. Thus, it is no surprise that disruptions in the circadian system can greatly affect physical and mental health (Reppert and Weaver 2002, Puttonen, Harma et al. 2010, Karatsoreos 2012, Evans and Davidson 2013). Circadian disruption can result from a variety of factors, including exposure to artificial light at night or dim daytime light, jetlag, “social jetlag”, and shiftwork (Fishbein, Knutson et al. 2021). Evidently, exposure to circadian disruption is ubiquitously experienced in the 24-hour society, and this could impact many aspects of health. How environmental circadian disturbances interacts with NDD, where individuals are already known to experiences various problems with sleep and circadian rhythms, is largely unknown and is a focus of this thesis.

Another focus of this thesis was the age of exposure to environmental circadian disruption. Namely, exposure during adulthood versus adolescence, and how this specifically interacted with NDDs. Given that circadian disruption affects a large portion of the adult population, adulthood presents an opportune time to examine the effects of environmental circadian disruption. Alternatively, adolescence is a crucial period of brain development (Spear 2000) and heightened sensitivity to stressors (Slawecki, Thorsell et al. 2004, McCormick, Mongillo et al. 2013), with significant changes in sleep and circadian rhythms (Logan and McClung 2019), and is when psychiatric disorders tend to emerge (Kessler, Amminger et al. 2007). Thus, exposure to environmental circadian disruption at both ages is of particular interest to this thesis.

NDDs are likely caused by complex interactions between many genetic and environmental risk factors (Cheroni, Caporale et al. 2020, Schmitt, Falkai et al. 2022), one such risk factor being maternal immune activation (MIA). Specifically, MIA may alter neurodevelopment, potentially leading to schizophrenia (Brown, Begg et al. 2004), and ASD (Atladottir, Henriksen et al. 2012) later in life. Importantly, not all prenatal infections lead to the development of schizophrenia and ASD. MIA is thought to be a disease primer, which makes the brain more sensitive to exposure to other risk factors later in life. One way to model MIA in rodents is by injecting pregnant mouse with a viral mimic during gestation. Resulting offspring exhibit a variety of behavioral and neurobiological abnormalities related to NDDs (Meyer 2014, Fernandez de Cossio, Guzman et al. 2017, Haddad, Patel et al. 2020).

Specific Objectives of the Thesis:

The overarching goal of this thesis is to investigate the bidirectional relationship between a well-documented risk factor for NDDs, prenatal infection, and a less commonly discussed risk

factor, circadian rhythm disruption. The **BACKGROUND** is comprised of a comprehensive literature review on the evidence supporting MIA as a risk factor for schizophrenia and ASD in humans and related animal models. As well as sleep and circadian disturbances in schizophrenia and ASD, with an emphasis on sex differences and age of exposure to circadian disruption. In **Chapter 1**, we explored if we could recapitulate the disruptions in the circadian system observed in patients with NDDs in male and female mice exposed to MIA. We analyzed circadian wheel running parameters in adult male and female mice that were exposed to prenatal maternal immune activation under 3 different lighting conditions: standard lighting, constant dark and constant light. Next, given the prevalence of light at night and the growing number of shift workers worldwide, in **Chapter 2**, we decided to explore the effects of exposure to a second risk factor, environmental circadian disruption during adulthood, in mice exposed to prenatal MIA. Given that adolescence is a time of major neural development, when most psychiatric disorders begin to emerge and is when sleep patterns and circadian rhythms change compared to childhood, we next sought to explore the impact of circadian disruption during juvenility/adolescence, which is described in **Chapter 3**.

BACKGROUND

Neurodevelopmental disorders (NDDs): schizophrenia and autism spectrum disorders (ASD)

Characteristics, prevalence, and disease burden of schizophrenia and ASD

Schizophrenia and ASD are complex and chronic NDDs, with a multifactorial etiology. The development of these disorders is influenced by a combination of genetic and environmental risk factors, which affect healthy brain development (Brown 2011, Giusti-Rodriguez and Sullivan 2013). Both disorders share some symptoms, risk factors (such as prenatal exposure to maternal immune activation) and susceptibility loci in both common and rare structural variants. Interestingly, approximately 70% of individuals with ASD have at least one co-occurring psychiatric disorder (Konstantareas and Hewitt 2001, Lee, Magnusson et al. 2015, Mosner, Kinard et al. 2019).

Schizophrenia is a condition marked by positive symptoms, such as disturbances in thinking and perception (e.g., delusions and hallucinations), negative symptoms including alogia, avolition, anhedonia and flatted affect, and cognitive symptoms including impaired attention, verbal memory, and social cognition and interaction (AmericanPsychiatricAssociation 2013). The prodromal phase of schizophrenia typically precedes the onset of psychotic symptoms, and is identified by the presence of negative symptoms, attenuated psychotic symptoms, and loss of interest in work or social activities, as well as sleep disturbances (Organization 2019).

ASD are marked by deficits in social interaction and communication skills, varying levels of intellectual disability (often present from early life), and restricted interests and repetitive behaviors (Lord, Brugha et al. 2020). Importantly, some individuals with ASD exhibit cognitive strengths, including attention and memory to details and a strong drive to detect patterns (Baron-Cohen 2017).

The prevalence of schizophrenia and ASD is each approximately 1% worldwide (McGrath, Saha et al. 2008, Baxter, Brugha et al. 2015). The health, social and economic burden is substantial for the individuals that suffer from these disorders, their families and caregivers, and society as a whole (Baxter, Brugha et al. 2015, Charlson, Ferrari et al. 2018). Individuals with schizophrenia often struggle with daily functioning, social relationships, and independent living, and may experience discrimination, social isolation, and a decreased quality of life (Weittenhiller, Mikhail et al. 2021). Similarly, individuals with ASD have difficulties forming relationships and participating in social activities, which can result to social isolation and exclusion (Orsmond, Shattuck et al. 2013). Both disorders incur substantial healthcare costs, including therapy, medication, and special education/services, as well as loss of productivity for caregivers, reduced employment opportunities, lower wages, and decreased economic participation (Whiteford, Degenhardt et al. 2013, Baxter, Brugha et al. 2015).

Individuals diagnosed with schizophrenia have a significantly higher risk of premature mortality, with an average life expectancy around 15 years shorter than that of the general population (Hayes, Marston et al. 2017). This increased risk is due to several factors, including a higher incidence of suicide and an increased prevalence of physical illnesses that co-occur with schizophrenia (Hayes, Marston et al. 2017). Similarly, individuals with ASD have an estimated 3

times higher death rate than the general population, and mortality rates are associated with the level of intellectual disability, and comorbid medical conditions, such as cardiovascular disease (Bilder, Botts et al. 2013, Mouridsen 2013).

Sex/gender differences in schizophrenia and ASD

It is important to consider both sex and gender in the study of schizophrenia and ASD, as they can both influence the presentation and experience of these disorders. Studies have shown that the prevalence, age of onset, and severity and profile of symptoms of schizophrenia and ASD vary by sex/gender, but the underlying factors driving these differences are not clear (Abel, Drake et al. 2010, Ferri, Abel et al. 2018). Possible mediators may include sex hormones, genetics, differential structure or wiring of the brain, sociability, and social environment. Sex differences are important factors to study as they can inform treatment options and predict treatment responses (Abel, Drake et al. 2010, Ferri, Abel et al. 2018). An important consideration is that in the scientific literature, 'sex' and 'gender' are often used interchangeably. While it is difficult to disentangle the influence of each of these two variables, we recognize that 'sex' is a biological variable defined by sex chromosomes and sex steroid hormones, and 'gender' is a psychosocial construct determined by social and cultural factors (Rich-Edwards, Kaiser et al. 2018).

Schizophrenia is approximately 1.4 times more prevalent in males (McGrath, Saha et al. 2004). Schizophrenia onset occurs in males between 21-25 years old, and in females between 25-30, with another peak of onset in females after 45 years old (around menopause) (Gogtay, Vyas et al. 2011). Many psychiatric disorders, including schizophrenia, start in adolescence or early adulthood. A longitudinal study composed of 1037 individuals (52% male) from Dunedin New Zealand found that 73.9% of adults diagnosed with a mental disorder according to the DSM-IV

received a diagnosis before age 18, and 50% before age 15 (Kim-Cohen, Caspi et al. 2003). It is important to note that although the onset of schizophrenia is usually identified by the first psychotic episode, research suggests that there may be cognitive and other behavioural and movement problems present prior to that, and these early symptoms may differ between sexes (Rapoport, Giedd et al. 2012). Lastly, many studies have explored sex/gender in neuroanatomical and neurocognitive differences in schizophrenia. Males typically showed greater reductions in total brain volume, ventricular-brain ratio, frontal, and temporal lobe than females, as well as more decreases in white matter tissue (Haijma, Van Haren et al. 2013). Mendrek and colleagues described these sex/gender differences in a comprehensive review (Mendrek and Mancini-Marie 2016).

ASD is more prevalent in males than females, with males being 3-4 times more likely to be diagnosed (Demily, Poisson et al. 2017, Loomes, Hull et al. 2017). This discrepancy may be due to differences in how the symptoms are expressed, with females exhibiting fewer overt symptoms and masking social deficits more than males (Volkmar, Siegel et al. 2014, Bargiela, Steward et al. 2016). For example, males may act out with reactive aggression when challenged or frustrated, while girls tend to withdraw socially and react with emotional changes to their social adjustment difficulties (Bargiela, Steward et al. 2016). These factors may lead to misdiagnosis, or delayed diagnosis. While both males and females typically experience symptoms in early childhood, symptoms may not become fully apparent until later when social demands exceed limited capacities. Lai and colleagues described these sex/gender differences in a comprehensive review (Lai, Lombardo et al. 2015).

The neurodevelopmental hypothesis and the adolescent brain in schizophrenia and ASD

During adolescence, the brain undergoes significant structural and functional changes, such as the pruning of excess synapses and the formation of new connections (Fuhrmann, Knoll et al. 2015, Foulkes and Blakemore 2018). However, in individuals with schizophrenia and ASD, these changes in adolescence may be disrupted, leading to the emergence of symptoms. Research has identified brain abnormalities in individuals with schizophrenia and ASD, but there is substantial variability across patients (Alnaes, Kaufmann et al. 2019). These abnormalities are thought to reflect disruptions in early brain development that may contribute to the later emergence of NDD. The neurodevelopmental hypothesis of schizophrenia and ASD proposes that disturbances in early brain development, particularly during the prenatal period and early childhood contribute to the onset and progression of these disorders.

Individuals with schizophrenia typically have enlarged ventricles and reduced gray matter volume in several areas including the prefrontal cortex, temporal lobes, and hippocampus, that emerge before the onset of illness and to progress after exposure to antipsychotics (Fusar-Poli, Smieskova et al. 2013). Schizophrenia is also associated with abnormalities in white matter, consisting of fibers that connect different brain structures, thus can affect communication between brain regions, and disrupted functional connectivity, which contributes to cognitive and emotional symptoms of the disorder (Haijma, Van Haren et al. 2013, Van Erp, Walton et al. 2018). Lastly, disruption in various neurotransmitter systems is a documented characteristic of schizophrenia. Post-mortem studies find reduced dendritic spine density in schizophrenia results from either a failure to elaborate normal numbers of dendritic spines in early development, or from more rapid elimination of dendritic spines in adolescent development, or both (Moyer, Shelton et al. 2015). Individuals with ASD have differences in various brain structures (including the amygdala,

hippocampus, and cerebellum), neural connectivity, and abnormal neurotransmitter systems and brain activity.

The need for new treatments

Antipsychotic medications have been the primary treatment for schizophrenia for the past 6 decades, and while they provide reductions in positive symptoms and psychotic relapse, they often need to be combined with psychosocial interventions (Lally and MacCabe 2015). However, approximately 20-30% of patients may not experience any benefits from antipsychotic medications and may discontinue treatment due to intolerable adverse effects (Patel, Cherian et al. 2014, Faden 2019). Furthermore, current pharmacotherapy is often ineffective against negative symptoms of schizophrenia and cognitive impairment (Miyamoto, Duncan et al. 2005, Kahn, Sommer et al. 2015).

There are a variety of therapies available for managing ASD symptoms, including speech therapy, occupational therapy, and social skills training that are available to help manage the symptoms and improve quality of life. Medications can also be prescribed to treat specific symptoms such as anxiety and hyperactivity (Kabot, Masi et al. 2003, Aishworiya, Valica et al. 2022).

Despite extensive research, there is no singly unifying cause, and no cure for schizophrenia and ASD. This is in part due to the complex involvement of multiple interacting risk factors in their development. Prioritizing the discovery of efficient therapeutic options for schizophrenia and ASD is crucial, and this may involve exploring novel therapeutic targets and uncovering the complex mechanisms underlying the interaction of multiple risk factors.

Prenatal maternal immune activation (MIA)

Evidence for elevated risk for schizophrenia and ASD following prenatal infection

Maternal infection during pregnancy is a well-established risk factor for schizophrenia and ASD (Selemon and Zecevic 2015; Rapoport et al. 2005). Ecological epidemiological studies, which used naturally occurring epidemics, report an association between in utero exposure to maternal infection and offspring having an increased risk for schizophrenia. One of the earliest studies to report this association was conducted following the Spanish influenza epidemic of 1918-1919 (Kendell and Kemp 1989, Menninger 1994). Years later, during the 1957 influenza type A2 epidemic, a study conducted in Finland found that offspring in the second trimester of gestation during the epidemic had an increased risk of being hospitalized for schizophrenia later in life, compared to individuals born in the same month and city for the previous 6 years (Mednick, Machon et al. 1988). Similar findings were revealed when studying Danish populations (Mednick, Machon et al. 1990), and studies conducted in England and Wales reported an 88% increase in the risk developing schizophrenia in individuals born 5 months after the A2 influenza pandemic compared to 2 years prior (O'Callaghan, Sham et al. 1991).

Devastating events like these provide the opportunity to investigate the link between prenatal infection and increased prevalence of psychotic disorders. While some initial studies do not replicate this association, (Erlenmeyer-Kimling, Folnegovic et al. 1994, Susser, Lin et al. 1994, Morgan, Castle et al. 1997), a major limitation to consider if that they relied on maternal recall of infection based on the dates of the epidemic, thus were unable to precisely define the timing of exposure. Subsequent studies used large birth cohort with prospectively collected maternal serum specimens throughout pregnancy, such as the Prenatal Determinants of Schizophrenia (PDS) cohort (Susser, Schaefer et al. 2000), the Child Health and Development Studies (CHDS) cohort

(Berg, Christianson et al. 1988), and the Collaborative Perinatal Project (CPP) cohort (Klebanoff 2009). All these birth cohorts found that offspring from mothers who had an infection during pregnancy, which was serologically verified, had an increased risk for psychotic disorders including schizophrenia. Studies on the CHDS cohort specifically found a 3-fold increased risk when prenatal exposure to influenza occurred in the first half of pregnancy and a 7-fold risk during the first trimester (Brown, Begg et al. 2004).

While epidemiological studies exploring in utero exposure to maternal infection as a risk factor for NDDs have primarily focused on schizophrenia, growing evidence suggests that it also increases the risk of ASD in offspring (Atladottir, Thorsen et al. 2010, Lee, Magnusson et al. 2015). A study found that hospitalization for viral infection in the first trimester and for any infection in the second trimester was associated with a higher rate of ASD in offspring (Atladottir, Thorsen et al. 2010). Another study using the Danish National Birth Cohort found that influenza exposure during gestation was associated with a two-fold increase in the risk for infant ASD (Atladottir, Henriksen et al. 2012).

Apart from influenza, many other embryonic adversities have been associated with increased risk of schizophrenia and sometimes also ASD, including viruses (rubella, herpes simplex, measles, and polio) (Brown, Cohen et al. 2001), bacterial pathogens (Sorensen, Mortensen et al. 2009), parasites (*Toxoplasma gondii*) (Mortensen, Norgaard-Pedersen et al. 2007), maternal stress, malnutrition, and pregnancy related complications (Walder, Laplante et al. 2014, Vohr, Poggi Davis et al. 2017). These indicate that the maternal response, and not specific pathogens, is responsible for these effects observed in offspring.

In addition to embryonic adversities, early life adversities are also associated with increased risk of schizophrenia including obstetric complication, childhood infections,

psychosocial adversity, cannabis use, an urban environment, minority ethnicity and migrant status (Stilo and Murray 2019). Additionally, while maternal infection is primarily associated with schizophrenia and ASD, there have also been less frequent associations with other NDDs, such as attention deficit and hyperactivity disorder, cerebral palsy, and epilepsy (Knuesel, Chicha et al. 2014).

Modeling MIA in animals

Epidemiological studies that associate prenatal infection with NDDs such as schizophrenia and ASD are correlational. Although animal models cannot fully recapitulate the complexity of human psychiatric disorders or brain functions, they can provide valuable insight into the causal link and mechanisms involved. Animal models can also capture symptoms and neuroanatomical features that are observed in humans with NDDs, making them a useful tool for research (Meyer, Feldon et al. 2005).

The MIA model involves using an agent to simulate an infection in the maternal system. For example, polyriboinosinic-polyribocytidilic acid (poly IC) and lipopolysaccharide (LPS) are immune-activating agents that are widely used in the literature to stimulate maternal immune system (Meyer, Feldon et al. 2005, Depino 2015). Poly IC and LPS mimic bacterial-like and viral-like infections respectively without the need to use a pathogen, and they both induce activation of the innate immune response through TLR signaling pathways. LPS is a gram-negative bacterial cell wall component that mimics a bacterial infection by binding to toll-like receptor (TLR)4. Poly IC, on the other hand, is double-stranded RNA that is a by-product of viral replication and is recognized by pattern-recognition receptors including the TLR3 receptors and the retinoic acid inducible gene-I-like receptor I (RIG-I) (Zou, Kawai 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 cause an upregulation in proinflammatory cytokines and transcription factors such as nuclear factor kappa B (Zou, Kawai et al. 2013). TLR3 is expressed in the endoplasmic reticulum and delivered to endosomes where it recognizes the double stranded RNA and further engages many adaptor proteins and pro-inflammatory molecules (cytokines, chemokines, interferons, and nuclear factor kappa B) (Zou, Kawai et al. 2013). How the triggering of the maternal immune system specifically affects the fetus is still unknown (Minakova and Warner 2018). Poly IC not only causes an increase in cytokines in the maternal blood serum, but also leads to elevated levels of IL-1β, IL-6, IL-10, and TNFα protein and mRNA in the fetal circulation (including the brain) and amniotic fluid (Patterson 2009, Ratnayake, Quinn et al. 2013). It has been hypothesized that the elevated cytokines, in response to MIA, cross the placenta to affect the fetus, but it is also possible that the elevated production of cytokines comes from the placenta itself. These cytokines bind to specific cell-surface receptors, for example, on glial cells (including microglia) and neuronal cells and upon binding, they trigger a signaling cascade that leads to an increase in immune signaling molecules and changes in gene expression in the target cell (Meyer, Nyffeler et al. 2008). Additionally, cytokines could be indirectly affect the fetus by altering placental endocrine or immune functioning (Hsiao and Patterson 2011).

Interestingly, the structural and functional abnormalities observed in offspring exposed to MIA do not appear to be dependent on the specific immune-activating agents used, but rather on the overall maternal and fetal immune response (Smith, Li et al. 2007). Additionally, many studies have shown that MIA leads to a stronger immune response in male offspring compared to females (Zhang, Jing et al. 2019), and MIA can lead to sex-specific changes in microglial gene expression (Mattei, Ivanov et al. 2017). While poly IC has been shown to induce dose-dependent changes in

offspring, in the current thesis, we chose to use a dose of 5mg/kg, because it seems to be the lowest dose tested that reliably induces the whole spectrum of behavioural deficits (Meyer, Feldon et al. 2005, Meyer, Nyffeler et al. 2006). Additionally, poly IC was administered on embryonic day 9.5 (which corresponds to the middle-to-late first trimester in humans (Clancy, Darlington et al. 2001, Clancy, Finlay et al. 2007)) due to the high association between a viral infection in the first trimester and subsequent development of schizophrenia and ASD in offspring and since E9.5 is when microglia migrate and colonize the embryonic brain (discussed in more detail below) (Ginhoux, Lim et al. 2013).

Behavioral findings in MIA models

Studies in rodents have demonstrated that prenatal exposure to MIA results in a variety of behavioral phenotypes in offspring, ranging from increased repetitive behaviors, locomotion, anxiety and depression-like behaviors, and deficits in sensorimotor gating, sociability, latent inhibition, and cognition (learning and memory) (Reviewed here Meyer, Yee et al. 2007, Meyer 2014).

Sensorimotor gating is an automatic inhibitory mechanism to regulate sensory inputs by filtering out irrelevant sensory information in the central nervous system. This protective process allows the brain to gate out distracting stimuli and to appropriately react to relevant stimuli. While deficits in prepulse inhibition (PPI) have been observed in individuals with NDDs, including schizophrenia (Geyer, Krebs-Thomson et al. 2001, Perry, Minassian et al. 2007, Mena, Ruiz-Salas et al. 2016) and in ASD children but not adults (Cheng, Chan et al. 2018). Some MIA models using mice exhibits deficits in PPI when MIA was induced in early gestation [poly IC E9.5, at 5mg/kg (Delorme In review); poly IC E9.5, at 5mg/kg when tested in adolescence but not adulthood

(Guma, Bordignon et al. 2021); poly IC E9.5, at 10mg/kg (Li, Chen et al. 2021); poly IC E12.5, at 20mg/kg (Vigli, Palombelli et al. 2020); poly IC E9.5, at 5mg/kg (Hui, St-Pierre et al. 2018); poly IC E9.5, at 2mg/kg (Chang, Li et al. 2020); poly IC E9.5, at 20mg/kg but not 10mg/kg (Shi, Fatemi et al. 2003); poly IC E9.5, at 5mg/kg (Meyer, Nyffeler et al. 2008); poly IC E9.5, at 2mg/kg (Meyer, Murray et al. 2008)] and mid/late gestation [poly IC E15, at 5mg/kg (Zhang and van Praag 2015)]. Other studies do not find deficits in PPI [poly IC E12, at 20mg/kg (Zhao, Erickson et al. 2022); poly IC E15, at 2mg/kg (except a cohort from a specific breeder) (Kobayashi, Inaba et al. 2021); poly IC E17, at 5mg/kg (Meyer, Nyffeler et al. 2008)]. PPI deficits have also been observed for MIA in rat using prenatal poly IC [E9.5, 10mg/kg, deficits in adult but not adolescent MIA offspring) (Ding, Hu et al. 2019); E15, 4mg/kg (Gogos, Sbisa et al. 2020); poly IC E15, at 4mg/kg (Yee, Ribic et al. 2011)] and prenatal LPS [E15 and E18 but not E10, various doses (Fortier, Luheshi et al. 2007)]. Mice administered prenatal influenza virus (Shi, Fatemi et al. 2003) or IL-6 (Smith, Li et al. 2007) also show impairment in PPI.

Studies exploring anxiety-like phenotypes following prenatal poly IC (discussed here Quagliato, De Matos et al. 2021) report conflicting results. Some studies report increased anxiety-like behavior as assessed on the EPM test (E9.5, at 10mg/kg) (Li, Chen et al. 2021), while others report decreased anxiety-like behavior in adult (but not adolescent) offspring as measured by the thigmotaxis ratio in the open field test (daily between E12-17, at 5mg/kg) (Ozawa and et al. 2006), and others report no differences as measured by both EPM and open field (E12.5, at 20mg/kg) (Tartaglione, Villani et al. 2022). Of note, the study by Tartaglione and colleagues also does not report any differences on the open field test such as distance traveled (Tartaglione, Villani et al. 2022).

Reduced sociability is a common symptom of schizophrenia (Goldberg and Schmidt 2001) and ASD (Orsmond, Shattuck et al. 2013). Some MIA models exhibits deficits in social interaction [poly IC E9.5, at 5mg/kg (Hui, St-Pierre et al. 2018), 3 hits of poly IC E10.5, 12.5 and 14.5, 5mg/kg (Malkova, Yu et al. 2012); poly IC E13, at 20mg/kg (only at PND 40 and not 120) (Aavani, Rana et al. 2015); poly IC E12.5, at 20mg/kg (Tartaglione, Villani et al. 2022); poly IC E17, at 5mg/kg (Bitanihirwe, Peleg-Raibstein et al. 2010)], while others do not [poly IC E9.5, at 5mg/kg (Guma, Bordignon et al. 2021); poly IC E12.5, at 20mg/kg (Vigli, Palombelli et al. 2020)]. Interestingly, in a study by Zhao and colleagues, while social deficits were observed followed prenatal poly IC exposure and social deprivation, these effects were rescued by environmental enrichment [poly IC E12, at 20mg/kg (Zhao, Mohammed et al. 2021)]. Additionally, in a study by Smith and colleagues, not only were significant deficits in offspring sociability and PPI induced by administration of prenatal poly IC (E12.5, 20mg/kg), but the effects were reversed by co-administration of anti-IL-6, but not anti-IFNgamma (Smith, Li et al. 2007).

Cognitive impairments are a core feature of schizophrenia (Tripathi, Kar et al. 2018) and ASD (Banker and et al. 2021). Importantly, some individuals with ASD exhibit cognitive strengths, including attention and memory to details and a strong drive to detect patterns (Baron-Cohen 2017). Some MIA models using mice exhibits decreased cognitive-like behavior when MIA was induced in early gestation [poly IC E9.5, at 10mg/kg (Li, Chen et al. 2021)].

Other behaviors related to prenatal poly IC exposure include impaired including marble burying [poly IC E12.5, at 20mg/kg (Tartaglione, Villani et al. 2022)] and latent inhibition [poly IC E17, at 5mg/kg (Bitanihirwe, Peleg-Raibstein et al. 2010)].

Introduction to microglia

In 1913, Santiago Ramón y Cajal identified oligodendrocytes and microglia as supporting cells of the central nervous system (CNS) (Perez-Cerda, Sanchez-Gomez et al. 2015). Years later, Pío Del Río-Hortega, further characterized microglia and officially designated them as the only immune cells present in the brain parenchyma. The development of the brain and immune system are now known to be highly synchronized with microglia serving as a critical link between the two processes. Microglia play critical roles during various stages of brain development, such as migration and proliferation of neuronal progenitors, programmed cell death, and formation of synapses to establishment of neuronal circuits (Li and Barres 2018).

Microglia, which constitute 5–10% of total brain cells, can interact with neurons, astrocytes, and oligodendrocytes (Hoshiko, Arnoux et al. 2012). Unlike other cells in the brain, microglia are not generated in the brain. Instead, microglia are derived from mesodermal precursor cells in the embryonic yolk sac, which give rise to yolk sac macrophages (Li and Barres 2018). Microglial precursors then migrate and colonize the embryonic brain parenchyma (among other tissues) by embryonic day 9.5 (which is prior to the closure of the blood-brain-barrier) and receive instructive signals from the CNS environment that support their differentiation (Ginhoux, Greter et al. 2010, Kierdorf, Erny et al. 2013). By PND 14, microglia are mature and express adult-signature genes (Butovsky, Jedrychowski et al. 2014). In the adult brain, microglia are constantly monitoring brain activity to detect changes in brain homeostasis or harmful insults by extending and retracting their ramified processes (Hoshiko, Arnoux et al. 2012) and they have many pattern recognition receptors that function to sense and eliminate microbes invading the CNS parenchyma. Microglia maintain their CNS population by self-renewal (Ajami, Bennett et al. 2007).

Microglia play a crucial role in neuroinflammation. When microglia are stimulated by inflammatory cytokines (which can be a result of injury or infection) or TLR ligands, they increase production of reactive oxygen species and release pro-inflammatory cytokines and chemokines, which be toxic to mature neurons and oligodendrocytes (Pratt, Ni et al. 2013).

Microglia also play a role in modulating neuronal activity. Microglia are phagocytic; once they encounter a microbe, they rapidly recruit other microglia to the sites of damage and they engulf or phagocytose debris and unwanted/dying cells and they are involved in the recruitment and activation of peripheral immune cells infiltrating the infected CNS (Mariani and Kielian 2009). Microglia are also involved in synaptic pruning, a process by which unnecessary or dysfunctional synapses are eliminated during development and in certain pathological conditions. This process is important for shaping neural circuits and eliminating synapses that are no longer necessary (Stevens, Schafer et al. 2007, Paolicelli, Bolasco et al. 2011). Importantly, inflammatory perturbations to the brain during critical developmental stages can drive these cells to assume a more pathological role, rather than support neurodevelopment.

Microglial findings after MIA protocols in animals

It has been suggested that prenatal immune activation may directly affect microglia and alter their function in the developing brain. This could result in changes of developmental trajectories and lead to behavioral, cellular, and molecular deficits observed in MIA models (Knuesel, Chicha et al. 2014, Paolicelli and Ferretti 2017). Microglia anomalies in post-mortem brains has been observed in some individuals across many NDDs, including schizophrenia, ASD, depression and bipolar (Vargas, Nascimbene et al. 2005, Monji, Kato et al. 2009, Morgan, Chana et al. 2012).

The findings on MIA-induced microglial alterations in rodents are varied. Some studies find an increased microglia density, increase in expression of microglia "activation" markers (major histocompatibility complex II (MHCII), cluster of differentiation (CD) 68 or CD54), and changes in morphological appearance (Juckel, Manitz et al. 2011, Esslinger, Wachholz et al. 2016, Hui, St-Pierre et al. 2018). Other studies found no microglial alterations (Giovanoli, Notter et al. 2015, Giovanoli, Weber-Stadlbauer et al. 2016).

Not only is MIA exposure hypothesized to alter the microglial activity profile, but it is although though to prime microglia to respond differently to stimuli later in life. 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.

Limitations of MIA animal models

Although viral and bacterial mimics are frequently used, there are some limitations to consider. Firstly, poly IC and LPS do not recapitulate the full spectrum of immune responses typically elicited by exposure to infectious pathogens, such as influenza (Reisinger, Khan et al. 2015). As well, the use of poly IC and LPS has yielded inconsistent results due to methodological variability between studies. For example, the behavioral, cellular, and molecular outcomes are highly dependent on dose, route of administration, immunostimulant used, gestational timing of the agent, and the individual immune and thermogenic response of the mother (Mueller, Richetto et al. 2019). Other factors that may influence results include housing (enrichment), sex, age, strain, and species (Meyer 2019, Weber-Stadlbauer and Meyer 2019). Additionally, differences between manufacturers have been shown to affect the magnitude of the maternal immune response elicited (Careaga, Taylor et al. 2018).

Despite these limitations, there are clear benefits of using these immune stimulating agents compared to live pathogens, including no strict biosafety precautions are necessary, they are easily available, and you can control the immune response intensity and the duration of the immune response (Meyer and Feldon 2012). Importantly, evidence suggests that MIA induced by either poly IC or LPS presents various effects in offspring that account for several aspects of schizophrenia and ASD (e.g., epidemiology, pathophysiology, symptomatology, treatment) (Haddad, Patel et al. 2020).

Circadian rhythms

A brief overview of the circadian system

Circadian rhythms are ~24-hour cycles in behavior (e.g., sleep, mood) and physiology (e.g., certain hormones and genes). These biological rhythms are generated by the suprachiasmatic nucleus (SCN), a bilateral structure located in the anterior hypothalamus, above the optic chiasm, containing around 20,000 neurons (LeGates, Fernandez et al. 2014). Circadian rhythmicity is involved in regulating numerous activities in the body including sleep/wake cycles, body temperature, hormone production and release, metabolism, immune function and, cognition, mood and other behaviors (Reppert and Weaver 2002). Interestingly, virtually every cell in the body has circadian rhythms (Yoo, Yamazaki et al. 2004), and nearly half of all mammalian genes are expressed rhythmically in one or more tissues (Yan, Wang et al. 2008).

Life has evolved in an environment with predictable and rhythmic 24-hour cycles due to the rotation of earth on its axis. However, circadian rhythms are not simply passive responses to these 24-hour changes in the environment. They are endogenously generated, meaning that in the absence of external timing cues, organisms continue to exhibit circadian rhythms with a period of ~24 hours (Hastings, Maywood et al. 2019). Circadian rhythms are driven by a biological timekeeping system found in almost all organisms, from bacteria to humans (Patke, Young et al. 2020). Briefly, the molecular clock in mammals controls the expression of output "clock" genes through negative feedback loops that forms an autonomous oscillator and controls the activity and function of different cells and tissues (Dibner, Schibler et al. 2010). Interestingly, the rhythm machinery of the master clock is contained within single neurons in SCN (Welsh, Logothetis et al. 1995).

Although circadian rhythms persist in constant conditions, they need to be synchronized daily to external timing cues, called *zeitgebers*, to ensure a proper phase relationship with the solar day (Aschoff and Pohl 1978). Light exposure is the strongest *zeitgeber* (Hastings, Maywood et al. 2018) and is perceived by the retina. The retina contains a subset of cells, called intrinsically photosensitive retinal ganglion cells (ipRGCs). IpRGCs are non-image forming photoreceptors that express the blue-light sensitive photopigment melanopsin. IpRGCs in the retina send photic information to the SCN via the retinohypothalamic tract (RHT), which has monosynaptic fibers that terminate on neurons in the ventrolateral region of the SCN (Ebling 1996, Berson, Dunn et al. 2002). The neurons within the SCN produce circadian rhythms of neuronal firing and they synchronize cells throughout body through a variety of direct and indirect output pathways, including through neuronal connections, endocrine signals, body temperature rhythms, and indirect cues (Gachon, Nagoshi et al. 2004, Hastings, Maywood et al. 2018). The SCN can also generate a rhythmic output signal in the absence of external 24-hour signals, which are referred to as free running rhythms (Hastings, Maywood et al. 2018).

Interestingly, several secondary oscillators located in the brain and peripheral organs exist, including the liver and kidneys (Tahara and Shibata 2016). These secondary oscillators contain the rhythmically expression genes that makeup the intracellular SCN clock mechanism (Hastings, Maywood et al. 2018). But importantly, they require the orchestrated timing signals from the SCN to ensuring proper synchronization within the body and to the external environment.

Circadian rhythms are adaptive, in that they enable organisms to synchronize their physiological processes and behaviors with the solar day (Yerushalmi and Green 2009). This temporal organization of daily events allow the organism to anticipate changes in their environment and maintain optimal health. Being synchronized with the environment is particularly

important for survival and reproductive success, as well to be able to predict the availability of essential resources (Boden, Varcoe et al. 2013). Some factors that fluctuate in the environment and affect survival are light, humidity, temperature, mating selection, predator avoidance and prey availability (Vaze and Sharma 2013). By exploiting the temporal patterns in the external environment, organisms can optimize their energy metabolism, sleep/wake cycle, and hormonal regulation, which directly benefits their fitness. Taken together, circadian clocks exist throughout the body and rely on the SCN for synchronization within the body and with the external environment, which directly impacts health and fitness.

Consequences of environmental circadian disruption

In addition to light exposure, the timing of eating, exercise and social interaction can also function as *zeitgebers* (Sharma and Chandrashekaran 2005). Exposure to *zeitgebers* at inappropriate times of the day can disrupt circadian homeostasis and increase the risk for cardiovascular disease, cancer, diabetes, and obesity risk (Karatsoreos 2012, Evans and Davidson 2013, Vetter, Devore et al. 2015, Strohmaier, Devore et al. 2018). With the high demands of the current 24-hour society, many prioritize social and work schedules outside of the solar day, leading to circadian disruption (Roenneberg, Pilz et al. 2019). Circadian disruption can result from a variety of factors, including artificial light exposure at night (due to streetlights, buildings, and electronic devices before bed), exposure to dim daytime light from staying indoors, jetlag from traveling across time zones, "social jetlag" from keeping up with work, school or social activities, and shiftwork. Shiftwork can especially problematic because individuals are often forced to chronically work against their circadian rhythms (Costa 2010). Alarmingly, approximately 28%

of the Canadian labor force and 40% of the American labor force work mostly during non-standard times (Presser 2003, Williams 2008).

Circadian clock synchronization with the external environment has directly been linked to survival in rodents. For example, wildtype mice under non-24 hour LD cycles have a reduced lifespan (Park and et al. 2012). Increases in mortality were reported in Drosophila after chronic circadian disruption (Pittendrigh and Minis 1972) and aged mice after schedules of light exposure meant to mimic chronic jet lag (Davidson, Sellix et al. 2006). Additionally, hamsters that carry the circadian period mutation, *tau*, which reduces the circadian period to ~22 hours in *tau*/+ heterozygotes, had significantly decreased longevity when they were subjected to 24-hour cycles (Hurd and Ralph 1998). Finally, housing mice in 20-hour LD cycles resulted in weight gain, reduced dendritic length in neurons in the brain, and decreased cognitive flexibility (Karatsoreos, Bhagat et al. 2011). These studies demonstrate that the circadian clock's internal period must align with that of the environment for survival and optimal fitness. Circadian disruption also alters innate immune responses (Castanon-Cervantes, Wu et al. 2010), and affects mood-related behaviors and cognitive functions in mice (LeGates, Altimus et al. 2012, Fisk, Tam et al. 2018).

Overall, limiting exposure to *zeitgebers* at inappropriate times of the day can aid in reducing circadian disruption in the body, which can improve many aspects of health. However, the mechanisms by which environmental circadian disruption produces its effects are not well understood and prompt further research.

Sleep and circadian disturbances as a fundamental component of NDDs

Evidence of sleep/circadian rhythm disruption in NDDs

Disruptions in rest/activity rhythms, sleep behavior, and hormone rhythms have been reported in individuals with schizophrenia and ASD (Cohrs 2008, Wulff, Dijk et al. 2012), as well as dysregulated clock gene expression in individuals with schizophrenia (Johansson, Owe-Larsson et al. 2016) (**Fig. 1**) (modified from Delorme, Srivastava et al. 2020). These sleep disturbances precede prodromal psychotic symptoms in more than 77% of patients with schizophrenia (Tan and Ang 2001, Benson 2015), and some studies found youth at clinical high risk (CHR) for psychosis had more fragmented diurnal rhythms and later onset of nocturnal rest than controls, and the level of these disruptions predicted psychosis symptom severity 1 year later (Lunsford-Avery, Goncalves et al. 2017, Poe, Brucato et al. 2017). Interestingly, these disturbances in individuals with psychosis and schizophrenia have been documented for over a century, with "care for sleep" being recommended for treatment (Kraepelin, Barclay et al. 1919).

Examples of sleep disturbances include reduced slow-wave sleep, reduced total sleep time and efficiency, and increased rapid eye movement sleep latency, that persist after accounting for medication, social isolation, and the absence of a social routine (Chouinard, Poulin et al. 2004, Wulff and Joyce 2011, Zanini, Castro et al. 2013). Similarly, a meta-analysis of sleep parameters found greater sleep time and latency, longer time in bed, greater wake after sleep onset, and reduced motor activity in individuals with treated and remitted schizophrenia compared with controls (Meyer, Faulkner et al. 2020). Poorer sleep quality was associated with greater symptom severity in individuals with schizophrenia (Korenic, Klingaman et al. 2019, Laskemoen, Simonsen et al. 2019) and patients with disturbed circadian rhythms performed worse in cognitive tests compared with patients with normal circadian rhythms (Bromundt, Koster et al. 2011). Using

actigraphy recordings, severe disruptions in rest/activity rhythms and melatonin rhythms were observed in patients, including periods of phase advances and delays, and fragmented and irregular activity patterns compared with matched controls (Wulff, Dijk et al. 2012). These disturbances seem to persist throughout the lifespan, as older individuals with schizophrenia were found to spend more time in bed, had more fragmented sleep, and were awake more during the day compared with older nonpsychiatric individuals (Martin, Jeste et al. 2001, Martin, Jeste et al. 2005).

Individuals with chronic schizophrenia exhibited a loss of rhythmic mRNA expression of clock genes CRYPTOCHROME1 (CRY1) and PERIOD2 (PER2) in skin fibroblast cells, which were cultured ex vivo and subjected to a clock-synchronizing treatment (Johansson, Owe-Larsson et al. 2016). Additionally, blood mononuclear cells collected from patients experiencing their first episode of psychosis had decreased expression of CRY1, PER2, and CLOCK compared to controls (Johansson, Owe-Larsson et al. 2016). Using time of-death data from each subject, researchers found that the set of genes with daily rhythms in the dorsolateral prefrontal cortex of subjects with schizophrenia were distinct and had a different rhythmic pattern from those identified in healthy controls (Seney, Cahill et al. 2019). Further, distinct cellular pathways were enriched among genes of each of these rhythmic groups. The studies mentioned above may provide insight into the molecular underpinnings of circadian disturbances in schizophrenia. Certainly, further research is needed to measure additional biological markers of the circadian clock in individuals with NDDs over an extended period.

Individuals with ASD also experiences various sleep and circadian disturbances. Polysomnography studies report that children with ASD showed many abnormalities related to rapid eye movement sleep (Devnani and Hegde 2015) and sleep disturbances seem to be related

with the level of functioning in children with ASD, in that more disturbances were reported in individuals with lower functioning (Cohen, Conduit et al. 2014). Disruptions in the circadian hormone melatonin, and in glucocorticoid secretion have also been reported (Pinato, Galina Spilla et al. 2019).

Further research studying sleep and circadian disruptions is needed in individuals with NDDs to explore how these problems develop across the lifespan, how they vary in individuals with distinct phenotypic profiles, and to explore sex differences.

Sleep and circadian disturbances in animal models used to study NDDs

Given the prevalence of sleep and circadian disruption in individuals with NDDs, researchers studied if similar disturbances were observed in relevant animal models based on genetic risk factors of schizophrenia (full review here Delorme, Srivastava et al. 2020) and ASD (full review here Wintler, Schoch et al. 2020). These studies provided insight on link between NDD risk genes in animal models and sleep and circadian rhythm phenotypes. Interestingly, some NDD risk genes mutated in rodents that result in a disrupted sleep/circadian phenotype were highlighted in a multistage schizophrenia genome-wide association study (Schizophrenia Working Group of the Psychiatric Genomics Consortium 2014). Further, many of these genes or proteins (associated with NDDs that cause a disrupted sleep/circadian phenotype in animals) are involved in cellular functions, namely in neuronal communication and/or protein and vesicle trafficking, which is important because dysfunctional synaptic connectivity and function and abnormal neurotransmitter release are thought to be key factors in schizophrenia pathology (Frankle, Lerma et al. 2003, Harrison and Weinberger 2005). Some examples of these are SNAP-25, dysbindin-1 and Pallidin. Specifically, SNAP-25 which is a SNARE (soluble N-ethylmaleimidesensitive factor

attachment protein receptor) associated protein, involved in the docking of synaptic vesicles at the presynaptic terminal membrane, resulting in fusion and neurotransmitter release (Mohrmann, de Wit et al. 2010). Additionally, dysbindin-1 which is a SNARE-modulating presynaptic protein, essential for synaptic homeostasis (Dickman and Davis 2009). Dysbindin-1 has also been reported to modulate Snap-25 expression through a possible interaction with Snapin (Feng, Zhou et al. 2008), and it might regulate dopamine release in the central nervous system through its actions on the SNARE complex (Kumamoto, Matsuzaki et al. 2006). Dysbindin-1 and Pallidin also have similar mechanisms, as they are both subunits in the BLOC-1 stable protein complex (the biogenesis of lysosomerelated organelles complex-1). It was hypothesized that they exert their effects on phenotypes via affecting BLOC-1 stability (Lee, Wang et al. 2018).

Future studies should focus on exploring the mechanistic links with NDDs and disrupted sleep and circadian rhythms, and if dysfunction of vesicle trafficking and synaptic communication are part of the mechanism underlying this interaction.

Challenges of modeling schizophrenia and ASD in animals

A limitation of studying risk factors for NDDs in animal models is the difficulty in faithfully recapitulating the etiological factors and behavioral phenotypes that are often heterogeneous and uniquely human. It is widely accepted that the pathogenesis of NDDs is explained by a complex interaction between multiple interaction risk factors. A limitation of the studies summarized by us in Delorme and colleagues, and Wintler and colleagues, is that they relied on animal models with monogenic mutations, which likely have only a small impact on the pathology of NDDs. Studying interacting risk factors as an integrated system would thus be more appropriate, but also more complicated. Although no animal model can recapitulate the full

intricacies of NDDs, they are nonetheless an essential hypothesis-testing preclinical tool. Animal models can be genetically or pharmacologically engineered, and they offer a high level of experimental control that is necessary when exploring mechanistic links and testing therapeutic targets.

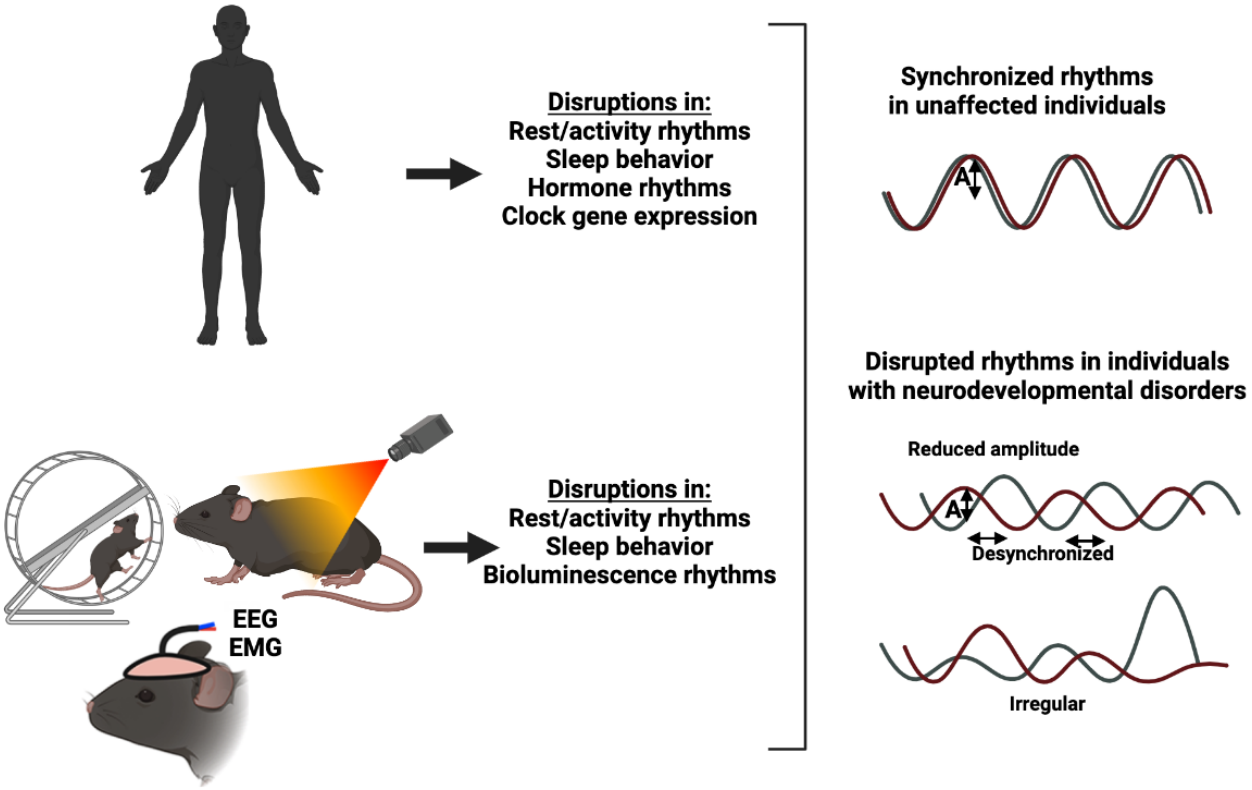


Fig. 1. Sleep and circadian rhythm disturbances are prevalent in individuals with NDDs, and related animal models. Specifically in humans, disruptions in rest/activity rhythms, sleep behavior, hormone rhythms and clock gene expression in various tissues have been reported. In related rodent models, disruptions in rest/activity rhythms and sleep behavior have also been reported, which were measured using wheel running, passive infrared sensors, and EEG and EEM, as well as disrupted bioluminescence rhythms. These disruptions can include a loss of circadian rhythmicity, fragmented rhythms, a decreased amplitude (A), and/or irregularity of rhythms. Made with BioRender.

Comparing sleep and circadian phenotypes across animal models

Firstly, distinguishing sleep versus circadian rhythms phenotypes is challenging. Sleep cycles and circadian rhythms are closely connected, making it difficult to separate the two, especially in animal models. Sleep is the most documented outputs of the circadian system. Briefly, sleep regulation involves a circadian component, where the SCN controls the activity of the brain's sleep and wake-promoting centers (Saper, Scammell et al. 2005), and a homeostatic component, where sleep pressure (need for sleep) increases with extended wakefulness and dissipates with sleep (Borbely 2009). In a stable environment, sleep is aligned with other circadian-regulated rhythms in the organism. However, a distinction between the two becomes apparent when either cycle is disrupted.

Major challenges when comparing sleep and circadian phenotypes in rodents across studies is the use of different assays and non-standardized experimental protocols for data collection and analysis. Firstly, when comparing between studies, it is critical to consider which assays were chosen to measure sleep and circadian rhythms when interpreting findings (Pritchett, Wulff et al. 2012). Sleep stages can be measured invasively through electroencephalography (EEG) and electromyography (EMG) or non-invasively through motion detection, where sleep is defined by a bout of immobility. Circadian rhythms can be measured through wheel-running assessment, bioluminescence recordings, and other markers, such as body temperature and clock gene expression. Secondly, it is important to standardize experimental conditions, such as wavelength and intensity of light and order of light challenges, and data analysis measures. For example, light exposure can be tested in the following order: standard lighting (12h of light and 12 h of dark), phase shifts (delay then advance), constant dark (DD), and constant light (LL) exposure being the

last condition. Standardized variables across studies would allow for more confident attribution of different phenotypes observed to the animal models.

Circadian rhythm disruption as a risk factor for NDDs

Evidence for circadian disturbances as a risk factor for NDDs

NDDs are complex disorders involving multiple risk factors that interact with each other in a particular way that results in the symptoms associated with these disorders (Davis, Eyre et al. 2016). Circadian disruption may be one such risk factor that interacts with other risk factors to exacerbate NDD-relevant behavioral and neurobiological abnormalities (**Fig. 2**) (modified from Delorme, Srivastava et al. 2020). One study that provided evidence for circadian disturbance as a risk factor for schizophrenia was done by Jones and colleagues. Briefly, they first assessed chronotype in participants, which is an individual's preferred timing of sleep and activity, influenced by biological clock, social environment, and age (Takahashi et al., 2018; Roenneberg et al., 2019). Generally, the more extreme the chronotype (i.e., preferring to wake up/go to sleep very early or very late), the greater the mismatch with social obligations and work schedules resulting in "social jetlag" (Wittmann et al., 2006). Social jetlag imposes a chronic disruption on the circadian system, and can be especially prominent for late chronotypes, leading to a greater sleep dept compared to early chronotypes (Roenneberg, Wirz-Justice et al. 2003, Roenneberg, Pilz et al. 2019). Interestingly, Jones and colleagues used mendelian randomization to infer causality between being an early chronotype and a reduced the risk of schizophrenia (Jones et al., 2019), an effect possibility mediated by chronic social jetlag.

There are a limited number of animal studies exploring circadian disruption as a risk factor for NDDs. For example, the Sandy (Dysbindin-1 mutant) mouse has behavioral abnormalities, including cognitive deficits, reminiscent of schizophrenia patients (Bhardwaj, Baharnoori et al. 2009, Cox, Tucker et al. 2009, Talbot 2009) and altered dopamine/D2 receptor signaling and neuronal excitability (Papaleo, Yang et al. 2012). Thus, our group used this mouse model and found that exposing Sandy mice to LL exposure (a condition that disrupts circadian rhythmicity) led to impaired prepulse inhibition, hyperlocomotion, and reduced anxiety-like behavior, compared to testing under a standard lighting condition (Bhardwaj, Stojkovic et al. 2015). It was hypothesized that circadian disturbances exacerbated preexisting connectivity impairments in the Sandy mice and unmasked schizophrenia-relevant behavioral deficits. Notably, 3 weeks of exposure to a standard light dark cycle partially rescue the observed behavioral deficits (Bhardwaj et al., 2015). This study highlights the potential role of circadian disruption in NDDs and suggests that interventions aimed at regulating circadian rhythms may be beneficial for mitigating deficits.

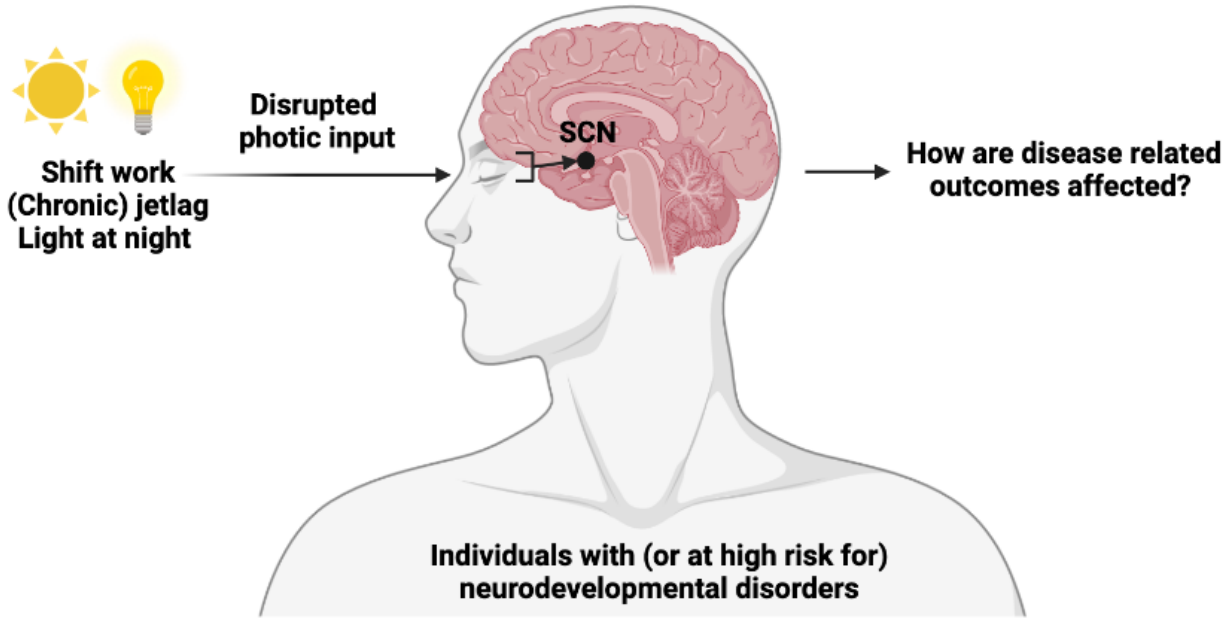


Fig. 2. Environmental circadian disruption as a risk factor for NDDs.

Disrupted photic input hits light-sensing retinal ganglion cells, which relay photic signals to the SCN. We hypothesize that disturbances to the circadian system, such as shift work, chronic jetlag and light at night, can interact with pre-existing risk factors within an individual with a neurodevelopmental disorder to affect disease related outcomes, including symptom severity. Individuals with, or at high risk for, neurodevelopmental disorders would benefit from limiting exposure to environments that can disturb circadian rhythms and restoring circadian rhythmicity may be a successful adjunctive therapy. Made with BioRender.

Constant light (LL) is a lighting condition that disrupts circadian rhythms

LL is a lighting condition that disrupts circadian rhythms and may exert its effects on the body in many ways. Intrinsically photoreceptive retinal ganglion cells (ipRGCs) transmit light information directly to the SCN via the RHT (LeGates, Fernandez et al. 2014) or indirectly via the geniculohypothalamic tract (GHT) from the intergeniculate leaflet of the thalamus (Pickard, Ralph et al. 1987). The SCN sends efferent projections to different brain regions; some regions with the densest innervation from the SCN include the subparaventricular zone, preoptic area, the bed nucleus of the stria terminalis, and the lateral septum, dorsomedial hypothalamus, and the arcuate nucleus (Watts, Swanson et al. 1987). Apart from the SCN, ipRGCs also directly innervate various cortical and limbic areas, including the amygdala and hippocampus (Schmidt, Do et al. 2011). Thus, one hypothesis is that LL exerts its effects through circuits involving the SCN, or circuits in other brain areas directly impinged upon by ipRGCs. LL exposure is known to affect metabolic systems (Coomans, Berg et al. 2013, Fonken and Nelson 2014), peripheral clock gene expression and circadian hormones (Sudo, Sasahara et al. 2003, Wideman and Murphy 2009), thus, another hypothesis is that LL exerts its effects by targeting peripheral tissues. Sleep amount and architecture are likely also affected by LL exposure, though we are unaware of studies that directly measured this. In this thesis, LL exposure was used to disrupt circadian rhythms and aimed to mimic light at night. LL is known to desynchronizes rhythms of cells of the SCN (Ohta, Yamazaki et al. 2005), and this long term desynchrony may disrupt SCN-driven rhythms in behavior and physiology (Dibner, Schibler et al. 2010).

Effect of LL on behavior

Measures of anxiety

LL exposure seems to have a direct impact on anxiety-like behavior and hormones related to stress. For example, reduced anxiety-like behavior was reported in rats exposed to 3 weeks LL (100-120 lux) starting at 6 weeks of age, mice exposed to 3 weeks of LL exposure starting at 9 weeks of age and hamsters exposed to 8 weeks in dim light at night (5 lux, 150 lux) starting at 11 weeks (Ma, Cao et al. 2007, Fonken, Finy et al. 2009, Bedrosian, Fonken et al. 2011). In the last two studies listed above, LL exposure also was shown to reduce corticosterone concentrations after 2 weeks in LL and no change at 6 weeks compared to controls (Fonken, Finy et al. 2009), and no change when measured during the light phase, when levels are known to be low (Bedrosian, Fonken et al. 2011). Other studies that assessed corticosterone levels after LL exposure had similar findings. Mice exposed to 3 weeks of LL (180 lux) starting at 10 weeks old had low levels of corticosterone at both CT1 and CT11 (Coomans, Berg et al. 2013), mice exposed to 3 weeks of LL (200 lux) starting at 10 weeks old showed no significant differences in total 24-hour corticosterone levels (Bhardwaj, Stojkovic et al. 2015) and finally, rats exposed to 3 weeks of LL exposure (300 lux) starting at 14 weeks old had a flattened and reduced corticosterone rhythm (Claustrat, Valatx et al. 2008). Interestingly, a study by Cissé and colleagues demonstrated that dim light at night exposure for 5 weeks (5 lux:130 lux) starting at 3 and 5 weeks old (i.e., juvenile and adolescent ages), induced more anxiety-like behavior (increased thigmotaxis behavior in open field test, but no differences in the elevated plus maze) in male mice but not females (Cisse, Peng et al. 2016). This study suggested that the effect of circadian disruption depended on age, since LL exposure during adulthood seems to reduce anxiety-like behaviors. However, a limitation of this study was that it did not include a group subjected to dim light at night at 8 weeks old, to show

opposite effects, or no effects compared to earlier disruption. The studies above suggest that LL directly affects anxiety-like behavior in rodents and its effects may depend on the developmental window during which circadian disruption occurs.

Prepulse inhibition of acoustic startle (PPI) and sociability

The few studies exploring the effect of environmental circadian disruption on sensory motor gating showed that LL had no effect on PPI (Bhardwaj, Stojkovic et al. 2015). Additionally, exposure to chronic jetlag (6-hour phase advance every 2 days for 4 weeks) did not affect PPI in males but reduced percent PPI in females, and reduced sociability in both males and females (Cloutier, Srivastava et al. 2022).

Cognitive-like behaviors

An early study exploring the effect of environmental circadian disruption on learning and memory, first trained rats on a passive avoidance memory task, then subjected rats to a light cycle that induces circadian disruption, including reversed day/night cycle, LL, or various phase shifts. Impairments were seen in retention performance only when the test (Fekete and et al. 1985). In a plus-maze discriminative avoidance task, 4 months old mice that were subjected to 35 days of LL exposure did not show any differences in memory performance compared to mice subjected to LD (Castro, Frussa-Filho et al. 2005).

Using the Morris water maze, some studies have shown that environmental circadian disruption impairs spatial memory. Rats exposed to 3 weeks LL (100-120 lux) starting at 6 weeks of age exhibited decreased latency to the escape platform for the first few training days, but spent significantly less time in the target quadrant on the test day than rats under 12:12LD (Ma, Cao et

al. 2007). In another study, mice exposed to 3 weeks of LL starting at 10 weeks old showed increase latency to the escape platform during some of the training days but it's unclear if mice were tested after the platform was removed (Fujioka, Fujioka et al. 2011). As well, in a study by Ling and colleagues, rats were first trained to locate the escape platform, then were subjected to 30 days of LL (400 or 800 lux). Rats under both LL intensities had a longer latency to the target area than the control group on the test day (Ling, Tian et al. 2009). Similarly in the water maze test, when rats are shifted by 3 hours a day for 6 days, researchers found no differences in acquisition training, but found impairments in the retention test, which occurred 7 and 17 days after the training. Thus, performance on the Morris water maze and other tests of cognitive function does seem to be impacted by LL or repeated phase shifts of the LD cycle.

Targeting sleep/circadian disruptions for therapeutic intervention

Despite the high prevalence of sleep and circadian disruptions in NDDs, which can significantly impact quality of life, they are often overlooked in the clinical management of these disorder. Preliminary findings on improving sleep quality and promoting a more regular circadian rhythm have been beneficial for individuals with schizophrenia. For example, the antipsychotic medication, paliperidone ER, improved sleep architecture and continuity in individuals with schizophrenia (Luthringer, Staner et al. 2007). The sleep-promoting drug ramelteon, a MT1/MT2 melatonin receptor agonist, as an add-on treatment for individuals with schizophrenia improved sleep and severity of positive and negative of symptoms, compared with individuals who received antipsychotics alone (haloperidol and risperidone) (Mishra, Maiti et al. 2020). Additionally, cognitive behavioral therapy for insomnia (CBT-I) may be an effective intervention for improving

sleep in individuals with schizophrenia with “classic severe insomnia”, denoted by short sleep duration, very poor sleep efficiency, and prolonged sleep onset latency (Chiu et al., 2018). CBT-I treatment and controlled light/dark exposure led to reductions in self-reported insomnia and earlier discharge compared to standard treatment alone in a population of males who were admitted to a psychiatric ward after experiencing an acute crisis (Sheaves, Freeman et al. 2018). Further research is needed to better understand sleep/circadian-promoting therapies on symptom severity. For example, promoting a proper phase relationship between the circadian system and environmental timing cues may improve NDD-related symptoms by strengthening and stabilizing circadian rhythmicity.

More research is needed to understand the interaction between NDDs, such as schizophrenia and ASD, and environmental circadian disruption. Although some groups have explored sleep and circadian disruption in individuals with NDDs, most studies were underpowered to explore sex differences, and they did not account for when individuals were exposed to light or dark, which would have acted as a measure of how much environmental circadian disruption the individuals were exposed to. Studies exploring sleep and circadian disruption in animal models based on NDDs only investigated genetic risk factors for the disorders. In this thesis, we aimed to explore circadian disruption in the MIA mouse model which is based on an environmental risk factor for NDDs. MIA is an important risk factor study given the widespread transmission of viral infections (e.g., influenza, COVID-19) that affects many pregnant individuals, and to our knowledge, disruption of sleep and circadian rhythms in this model is an area completed unexplored. Additionally, given that NDDs are multifactorial in nature, and that circadian disruption is ubiquitously experienced in our 24-hour society, we also sought to test the novel hypothesis that environmental circadian disruption would be a risk factor for NDDs. Only a

few studies have directly tested this hypothesis, but not in the MIA mouse model. In this thesis, the following aims were investigated that underly **Chapters 1, 2, 3**.

Chapter 1: How prenatal exposure to maternal immune activation (MIA) alters circadian rhythm parameters in a sex specific way.

Chapter 2: How prenatal exposure to MIA and circadian disruption during adulthood synergistically impact behavior and microglia in a sex specific way; and

Chapter 3: how behavior, microglia, and transcriptomics are impacted in MIA if the exposure to circadian disruption occurs during juvenility/adolescence.

CHAPTER 1: ALTERED CIRCADIAN RHYTHMS IN A MOUSE MODEL OF NEURODEVELOPMENTAL DISORDERS BASED ON PRENATAL MATERNAL IMMUNE ACTIVATION

Tara C. Delorme[1,2], Lalit K. Srivastava[2,3] and Nicolas Cermakian[2,3]

[1] Integrated Program in Neuroscience, McGill University, Montréal, Québec, H3A 2B4.

[2] Douglas Mental Health University Institute, Montréal, Québec, H4H 1R3.

[3] Department of Psychiatry, McGill University, Montréal, Québec, H3A 1A1.

Published: https://doi.org/10.1016/j.bbi.2020.12.030

1.0. Abstract

Individuals with neurodevelopmental disorders, such as schizophrenia and autism spectrum disorder, exhibit various sleep and circadian rhythm disturbances that often persist and worsen throughout the lifespan. To study the interaction between circadian rhythm disruption and neurodevelopmental disorders, we utilized a mouse model based on prenatal maternal immune activation (MIA). We hypothesized that MIA exposure would lead to impaired circadian locomotor activity rhythms in adult mouse offspring. We induced MIA by injecting pregnant dams with polyinosinic:polycytidylic acid (poly IC) at embryonic day 9.5, then aged resulting offspring to adulthood. We firstly confirmed that poly IC injection in pregnant dams elevated plasma levels of pro- and anti-inflammatory cytokines and chemokines. We then placed adult offspring in running wheels and subjected them to various lighting conditions. Overall, poly IC-exposed male offspring exhibited altered locomotor activity rhythms, reminiscent of individuals with neurodevelopmental disorders. In particular, we report increased (subjective) day activity across 3 different lighting conditions: 12 h of light, 12 h of dark (12:12LD), constant darkness (DD) and constant light. Further data analysis indicated that this was driven by increased activity in the beginning of the (subjective) day in 12:12LD and DD, and at the end of the day in 12:12LD. This effect was sex-dependent, as female poly IC-exposed offspring showed overall much milder alterations in locomotor activity rhythms than saline-exposed offspring. We also confirmed that the observed behavioral impairments in adult poly IC-exposed offspring were not due to differences in maternal behavior. These data further our understanding of the link between circadian rhythm disruption and neurodevelopmental disorders and may have implications for mitigating risk to the disorder and/or informing the development of circadian-based therapies.

Keywords: circadian rhythms; neurodevelopmental risk factor; schizophrenia; autism spectrum disorder; interaction; disrupted activity rhythms; prenatal immune challenge; poly IC; wheel running; mouse model

1.1. Introduction

Neurodevelopmental disorders, such as schizophrenia (SCZ) and autism spectrum disorder (ASD), are chronic and debilitating disorders, each with a worldwide prevalence of around 1% (McGrath, Saha et al. 2008, Baron-Cohen, Scott et al. 2009). SCZ is typically diagnosed in late adolescence or early adulthood and is characterized by hallucinations, delusions, lack of emotional reactivity and impairments in learning and attention (Tandon, Gaebel et al. 2013). ASD is typically diagnosed before the age of 3 and is characterized by deficits in social interaction and communication and stereotyped repetitive behaviors (American Psychiatric Association 2013). Interestingly, both of these psychiatric disorders have common genetic and environmental risk factors, and overlapping symptoms, such as disruptions in sleep and circadian rhythms.

Circadian rhythms are endogenous cycles that occur with a periodicity of ~24 h. They are governed by a master clock, located in the hypothalamic suprachiasmatic nucleus, and are also influenced by clocks in other brain regions and peripheral tissues (Hastings, Maywood et al. 2018). Circadian clocks have to be periodically synchronized to cyclic signals in the environment, such as the light-dark cycle. The most documented behavior that follows a circadian cycle is the sleep-wake cycle. Sleep and circadian rhythms control a variety physiological and behavioral processes, thus disruptions in these systems have widespread and detrimental effects.

Strikingly, disrupted sleep and circadian rhythms are exhibited in up to 80% of individuals with SCZ (Cohrs 2008, Delorme, Srivastava et al. 2020) and ASD (Couturier, Speechley et al.

2005, Souders, Mason et al. 2009). Various clinical findings suggest that sleep and circadian rhythm disturbances are a characteristic feature of SCZ (Kaskie, Graziano et al. 2017, Cosgrave, Wulff et al. 2018) and ASD (Missig, McDougle et al. 2020, Wintler, Schoch et al. 2020), potentially involved in the development of the disorders. In both disorders, these disturbances generally appear at an early age and persist throughout the lifespan (Martin, Jeste et al. 2005, Goldman, Richdale et al. 2012). In SCZ specifically, sleep disturbances often precede prodromal psychotic symptoms (Tan and Ang 2001), and in both disorders, poorer sleep quality correlated with greater symptom severity (Korenic, Klingaman et al. 2019, Laskemoen, Simonsen et al. 2019). Many individuals with SCZ or ASD exhibited shifted and/or blunted 24 h melatonin profiles, a circadian hormone with a primary role in the regulation of the sleep-wake cycle (Wiggs and Stores 2004, Tordjman, Anderson et al. 2012, Wulff, Dijk et al. 2012). In addition, reports suggest that individuals with SCZ also have alterations in circadian gene expression in various tissues (Johansson, Owe-Larsson et al. 2016, Seney, Cahill et al. 2019). Overall, the topic of sleep and circadian rhythms in individuals with SCZ and ASD is a rapidly growing field, yet the distinct biological mechanism leading to these disruptions is unknown. One avenue to study this interaction is by utilizing an animal model based on prenatal infection, which is a known neurodevelopmental risk factor for SCZ and ASD.

Epidemiological studies have reported an association between maternal infection and an offspring's subsequent risk of developing SCZ (Brown 2012) or ASD (Lee, Magnusson et al. 2015). Maternal immune activation (MIA) is a well-documented prenatal risk factor for neurodevelopmental disorders, whereby the maternal immune system is triggered by an infectious or infectious-like stimulus. Notably, maternal viral infection during the first trimester increases the offspring's risk of developing SCZ by 7-fold (Brown, Begg et al. 2004) and ASD by 2-fold

(Atladottir, Thorsen et al. 2010, Lee, Magnusson et al. 2015). The end of the first trimester in primate gestation corresponds to early gestation (E9.5) in rodents (Clancy, Darlington et al. 2001). Research in humans and animal studies have shown that MIA during gestation exerts long-term changes on offspring brain development and behavior (Knuesel, Chicha et al. 2014, Estes and McAllister 2016, Boulanger-Bertolus, Pancaro et al. 2018, Brown and Meyer 2018, Guma, Plitman et al. 2019). In humans, these changes have been implicated in the physical and psychiatric health of the offspring, while in rodents, MIA leads to behavioral deficits reminiscent of SCZ and ASD.

In the context of SCZ and ASD, various studies have characterized sleep and circadian rhythm disturbances in genetic animal models (Delorme, Srivastava et al. 2020, Wintler, Schoch et al. 2020). To our knowledge, no group has explored these disturbances in a neurodevelopmental animal model. We hypothesized that prenatal infection contributes to the disrupted circadian phenotype observed in patients with neurodevelopmental disorders. In the current study, we induced MIA in mice by using the analog of viral double-stranded RNA, polyinosinic:polycytidylic acid (poly IC). Poly IC is recognized primarily by Toll-like receptor 3 (Alexopoulou, Holt et al. 2001), and upon binding, it stimulates the production and release of many pro-inflammatory cytokines and chemokines (Meyer, Nyffeler et al. 2006). Poly IC injection leads to an inflammatory response in pregnant dams (Cunningham, Campion et al. 2007) and chronic changes in brain (frontal cortex, cingulate cortex and hippocampus) and blood cytokines in offspring at postnatal days 0, 7, 14, 30 and 60 (Garay, Hsiao et al. 2013). Long term neuroinflammation was also reported in offspring (Hui, St-Pierre et al. 2018). We first validated our MIA model by assessing maternal plasma levels of several cytokines and chemokines following poly IC injection in pregnant dams. We then measured wheel running behavior in adult poly IC or saline-exposed offspring as a measure of an inherent disruption of the circadian system.

Finally, we gathered evidence that the disrupted circadian phenotype was not due to differences in maternal behavior.

1.2. Methods

1.2.1. Maternal immune activation (MIA) model

Male and female C57BL/6J mice were ordered from Jackson Laboratory (product number: 000664) at 8 weeks old. Mice were placed in ventilated cages under a standard laboratory lighting condition of 12 h of light: 12 h of dark (12:12LD) for 2 weeks to acclimate to their surroundings. Three days prior to mating, soiled male bedding was placed in the female cages to increase the likelihood of copulation. Mice were mated overnight, and females were assessed between Zeitgeber Time (ZT) 0 and ZT 1 (i.e. from 0 h to 1 h after lights on) for the presence of a vaginal plug, which denoted embryonic day 0.5 (E0.5). On E9.5, to simulate viral infection, pregnant dams were intraperitoneally injected with poly IC dissolved in double-distilled water based on body weight (5 mg/kg; lot 1, 086M4045V [data in main paper], and lot 2, 096M4023V [data in Supplementary figures]; Sigma-Aldrich, St. Louis, MO, USA). A control group was injected with sterile saline solution. Dams were left to deliver their litters naturally.

For the first cohort, 16 female breeders were mated with 8 male breeders: 5 out of 10 poly IC-injected dams gave birth (average litter size: 5.8, total male offspring used=14, total female offspring used=6) and 4 out of 6 saline injected dams gave birth (average litter size: 7, total male offspring used=10, total female offspring used=5). For the second cohort, 18 female breeders were mated with 9 male breeders: 6 out of 10 poly IC-injected dams gave birth (average litter size: 6.8, total male offspring used=12, total female offspring used=6) and 6 out of 8 saline-injected dams gave birth (average litter size: 7.4, total male offspring used=12, total female offspring used=6).

Litters of poly IC- and saline-exposed dams did not differ in size (cohort 1: p=.4358, cohort 2: p=.5009).

At postnatal day 21, pups were weaned, weighed, and tagged. Two to three sex-matched littermates were group housed and offspring were aged to adulthood (8 weeks old) before testing. To minimize the effect of litter, we mated enough mice to ensure that groups contained a minimum of 4 litters for each experimental condition. We also analyzed data using a mixed effects model, which is well suited to deal with litter effects, and we visually confirmed that the significant effects that we reported were not driven by a cluster of mice from the same litter. All excess offspring were euthanized, and after the conclusion of testing, all mice were euthanized. Animal use was in accordance with the guidelines of the Canadian Council of Animal Care and was approved by the McGill University Animal Care Committee.

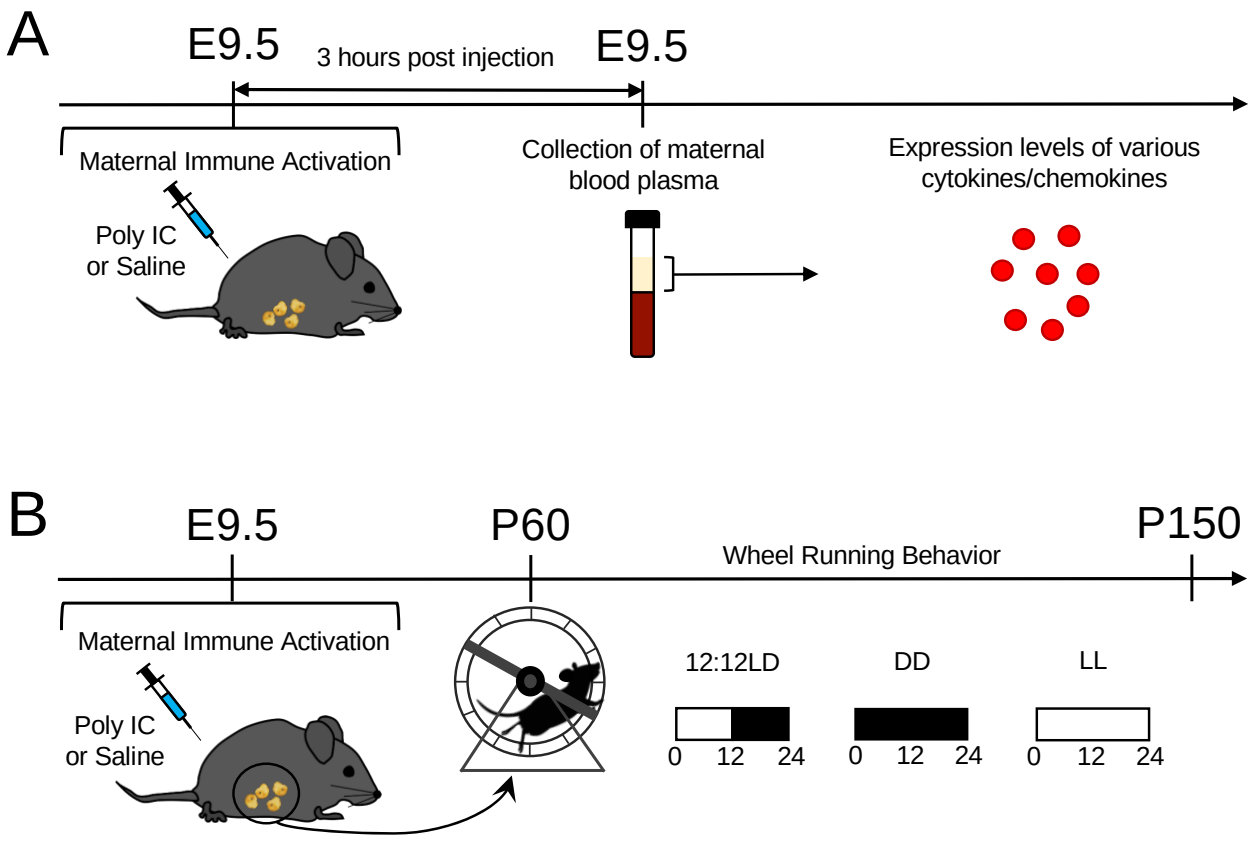


Fig. 1.1. Schematic for the maternal immune activation protocol. On E9.5, pregnant dams were intraperitoneally injected with poly IC (5 mg/kg) or saline. (A) Confirmation of inflammatory response in pregnant dams injected with poly IC. Pregnant dams were anesthetized 3 h after poly IC or saline injection and blood was collected. Plasma was tested for levels of pro- and anti-inflammatory cytokines and chemokines. (B) Another cohort of pregnant dams delivered their litters naturally after poly IC or saline injection. Resulting offspring were placed in running wheels at adulthood. Circadian locomotor activity was recorded under different lighting conditions: 12 h of light, 12 h of dark (12:12LD), constant darkness (DD) and constant light (LL).

1.2.2. Verification of inflammatory response in pregnant dams after poly IC injection

The following protocol is depicted in **Fig. 1.1A**. Pregnant dams were anesthetized 3 h after poly IC injection and trunk blood was collected, using heparin (10 µL) as an anticoagulant. Pregnancy was confirmed via dissection. Blood samples were centrifuged at 4°C, and the supernatant was stored at -80°C, before being processed for levels of various pro- and anti-inflammatory cytokines and chemokines by Eve Technologies (Calgary, AB, Canada). The Mouse Cytokine Array Proinflammatory Focused 10-plex (MDF10) was used, for the following biomarkers: IL-1β, IL-2, IL-4, IL-6, IL-10, IL-12p70, MCP-1 and TNFα. Concentration values were provided by the company, and as per their instructions, samples that were below the detectable range were designated as 0 pg/mL. GM-CSF and IFNγ were also part of the Array but the data are not reported because most samples were close to or below the minimum detectable concentration.

1.2.3. Wheel-running activity under different lighting conditions

The following protocol is depicted in **Fig. 1.1B**. Mice were individually housed in running wheel cages, which were placed in light-proof ventilated cabinets. Light was controlled via an external timer, with "lights on" between 150 and 200 lux. After a 2-week acclimation and entrainment period, mice were exposed to 3 lighting conditions, each for 3 weeks: 12:12LD, constant darkness (DD) and constant light (LL) (n=10–12 per group for male offspring and n=5-6 per group for female offspring). Wheel running data was collected to evaluate the effect of MIA on circadian behaviors. 12:12LD is a condition where external timing cues (the light:dark cycle) guide entrainment. Exposure to DD allows for the assessment of free-running rhythms without the influence of light as a timing cue. Exposure to LL has been shown to weaken the suprachiasmatic

nucleus neuronal network (Ohta, Yamazaki et al. 2005), thus, may uncover pre-existing network connectivity impairments in the poly IC-exposed mice. Running wheel activity data was collected and analyzed using ClockLab software, version 6 (Actimetrics, Wilmette, IL, USA). The analysis was carried out on the last 10 days of wheel-running recordings for each condition. We calculated various circadian locomotor activity variables, including circadian period (tau; calculated using a chi-square periodogram), duration of the active period (*alpha*; denoted by the numbers of hours between activity onset and offset), total amount of daily activity and percent day activity relative to total daily activity. In constant conditions (DD and LL), the subjective night was defined to be from the beginning of activity onset until half a period later, and the rest of the circadian cycle was defined as the subjective day. Analyses for non-parametric variables included interdaily stability, which quantifies the synchronization to the 24h light-dark cycle, intradaily variability, which quantifies rhythm fragmentation, and relative amplitude, which quantifies the robustness of the rhythm. A detailed analysis of activity bouts was also done, where a bout is defined as a sustained period of activity. Namely, we calculated the number of counts per bout of activity, the duration of activity bouts, the number of activity bouts per day and the peak rate of activity (defined as the peak number of counts per min). Onset variability was defined as the temporal relation between the internal circadian clock and the light-dark cycle. It was calculated as the difference between the onset of activity and the time of lights off. In constant conditions (DD and LL), the onset variability was computed by calculating the deviation of the observed activity onset from the predicted activity onset. The predicted activity onset was calculated using the free-running period, which assumes no day-to-day variability. Lastly, the 24 h profiles were created by averaging the total counts from a 10-day period over the 24 h day for each mouse, then calculating group averages. In the constant conditions, the 24 h profiles were created similarly to those in the

12:12LD condition except that the group averages were created by aligning profiles at the onset of activity for each mouse.

1.2.4. Maternal behavior

The following protocol is depicted in **Supplementary Fig. 1.1**. Postnatal maternal behavior was video-recorded in the home cage for 24 hours between postnatal day 4 and 5. Each dam (n=4-7) was observed in the home cage for four 72-min observation periods, at the following times: the beginning of the light phase (starting at ZT 0.5) and the dark phase (starting at ZT 12), and in the middle of the light phase (starting at ZT 4) and the dark phase (starting at ZT 16). Within the observation period, the behavior of each dam was scored every minute (72 observations per session, total of 288 observations (epochs) per dam in a day). Three variables were recorded during both the day and night: out of nest behavior, in nest behavior and nest building. A more detailed analysis of maternal behavior is presented in the Supplementary figures.

1.2.5 Statistics

Data were analyzed and graphed using Prism (GraphPad, version 8). Maternal cytokine and chemokine expression levels, and postnatal maternal behavior data were analyzed using an independent samples t-test between poly IC and saline-injected pregnant dams. Data that did not pass the Shapiro-Wilk normality test were analyzed by Mann-Whitney U test, and data with unequal variances (assessed by an *F*-test) were analyzed by an independent samples t-test with Welsh's correction. Wheel-running data were analyzed using a mixed-effects analysis with Geisser-Greenhouse correction, and Sidak's *post-hoc* tests, to determine interactions between lighting condition (12:12LD, DD and LL) and group (poly IC or saline exposure). If no interaction

was found, then main effects were explored. To further analyze our data, we performed an independent samples t-test (with Welsh's correction, or Mann-Whitney U test when appropriate) between poly IC or saline-exposed adult offspring at the beginning and end of the (subjective) day. Males and females were assessed separately. Individual data points represent data from individual mice, bars show mean ± SEM. $p<0.05$ was considered statistically significant.

1.3. Results

1.3.1. Verification of inflammatory response in pregnant dams after poly IC injection

Various cytokines and chemokines are known to be acutely elevated in blood plasma of pregnant dams following poly IC injection (Meyer, Nyffeler et al. 2006, Arrode-Bruses and Bruses 2012). We confirmed that the lot of poly IC used induced an inflammatory response in pregnant dams (n=4–5 per group). We sacrificed pregnant dams 3 h post poly IC or saline injection on E9.5 and quantified blood levels of pro- and anti-inflammatory cytokines and chemokines (see experimental timeline in **Fig. 1.1A**). Poly IC-injected dams had a significant elevation in blood plasma expression of IL-6 (p =.0137), IL-1β (p <.0001), IL-10 (p =.0001), TNFα (p =.0159), IL-12(p70) (p =.0159) and MCP-1 (p =.0190) compared to controls, and a trending difference in IL-2 (p =.0642) and IL-4 (p =.0714) (**Fig. 1.2A-H**). When we replicated this experiment, we used a second lot of poly IC. In our second lot, similar differences were observed compared to our control group, although we generally observed about a 2-fold reduction in cytokine and chemokine levels compared to our first lot (**Supplementary Fig. 1.2A-H**). Overall, we confirmed that both lots of poly IC induced an inflammatory immune response in pregnant dams compared to a saline injection.

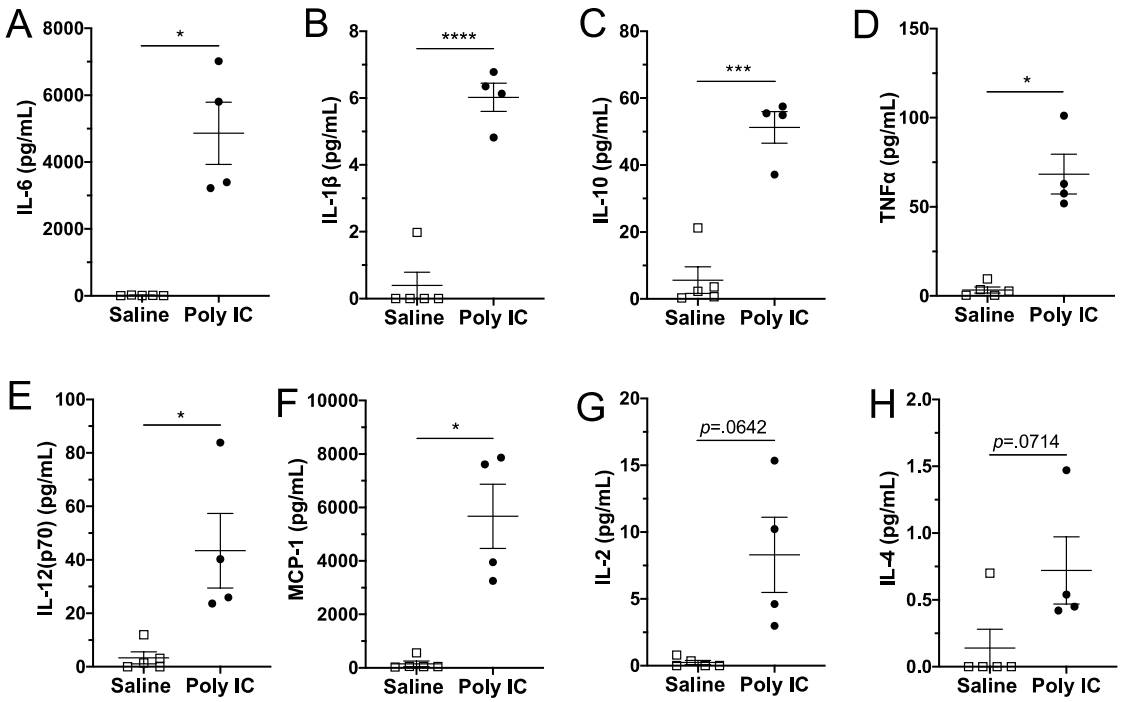


Fig. 1.2. Pro- and anti-inflammatory cytokine and chemokine expression levels in plasma of pregnant dams injected with poly IC (lot: 086M4045V) (n=4) or saline (n=5). Expression levels of the following cytokine and chemokines: (A) IL-6, (B) IL-1β, (C) IL-10, (D) TNFα, (E) IL-12(p70), (F) MCP-1, (G) IL-2 and (H) IL-4. Samples that were below the detectable range were designated as 0 pg/mL. Individual data points represent independent dams and data were represented as mean ± SEM. An independent samples t-test (with Welsh's correction, or Mann-Whitney U test when appropriate) was used, ****p<.0001, ***p<.001, and *p<.05. This experiment was repeated with the second lot of poly IC; results shown in Supplementary Fig. 2.2.

1.3.2. Wheel-running activity under different lighting conditions

We explored if circadian locomotor activity rhythms were altered in the MIA mouse model using *in utero* poly IC exposure at E9.5 (see experimental timeline in **Fig. 1.1B**). In this experiment, poly IC and saline–exposed mice (n=10-12 male offspring per group, n=5-6 female offspring per group) were subjected to different lighting conditions (12:12LD, DD and LL). Representative actograms for each lighting condition are shown for saline-exposed animals (**Fig. 1.3A-C,** males; **Fig. 1.4A-C**, females) and for poly IC-exposed animals (**Fig. 1.3D-F**, males; **Fig. 1.4D-F**, females). Activity profiles, with the average daily activity for all mice in each group, are also shown for each lighting condition (**Fig. 1.5A-C**, males; **Fig. 1.6A-C**, females). The **Tables** list all analyzed parameters. In the next sections, we describe the analyses for the first cohort of mice, then compare it to the results obtained in the second cohort to confirm our findings. Males are presented first, followed by females.

1.3.2.1. Circadian Locomotor Activity Variables

We report a group x lighting condition interaction trending significance for circadian period [$F_{(2, 62)} = 2.878$, $p = .0638$], and *post-hoc* analysis revealed a longer period in poly IC-exposed males under DD ($p = .0190$) (**Fig. 1.3G**). Overall, poly IC-exposed males had a significantly longer *alpha*, which is defined by the duration between the onset and offset of activity, than controls [Group, $F_{(1, 62)}=7.126$, $p = .0097$], without a group x lighting condition interaction. *Post-hoc* analysis revealed a longer *alpha* in poly IC-exposed males under 12:12LD ($p = .0001$) and DD ($p = .0422$) (**Fig. 1.3H**). Interestingly, poly IC-exposed males had more (subjective) day activity counts across lighting conditions than controls [Group, $F_{(1, 62)} = 35.90$, $p < .0001$]. *Post-hoc* analysis revealed more activity in poly IC-exposed males during 12:12LD (p

=.0085), DD (p =.0042) and LL (p =.0461) (**Fig. 1.3I**). Similarly, poly IC-exposed males had significantly more percent (subjective) day activity than controls [$F_{(1, 62)}$ = 25.30, p <.0001], without a group x lighting condition interaction. *Post-hoc* analysis revealed increased activity in poly IC-exposed males during 12:12LD (p =.0041), DD (p =.0340) and LL (p =.0153) (**Fig. 1.3J**). Males showed no group x lighting condition interactions or group differences in onset variability, (subjective) night activity counts and total activity (**Table 1.1**).

Interestingly, when we repeated this experiment with a different lot of poly IC, we reproduced the difference between groups on (subjective) day activity counts [Group, $F_{(1, 64)}$ = 12.34, p =.0008], without a group x lighting condition interaction. *Post-hoc* analysis revealed increased activity in poly IC-exposed males during 12:12LD (p =.0041), but not DD (p =.1395) or LL (p =.1402) (**Supplementary Fig. 1.3I**). We surprisingly did not see a difference between groups on percent (subjective) day activity [Group, $F_{(1, 22)}$ = 1.245, p =.2765] (**Supplementary Fig. 1.3J**), but this is likely due to the trending difference we found in (subjective) night activity counts [Group, $F_{(1, 22)}$ = 3.745, p =.0659] and total activity [Group, $F_{(1, 22)}$ = 4.646, p =.0423] (**Supplementary Table 1.1**).

In females, we did not find a group x lighting condition interaction nor any group differences for circadian locomotor activity variables **(Fig. 1.4G-J and Table 1.2)**. When we repeated this experiment with a different lot of poly IC, we found a trend for poly IC-exposed females to have more percent (subjective) day activity [$F_{(1, 30)}$ = 3.602, p =.0674] and (subjective) day activity counts [$F_{(1, 10)}$ = 4.052, p =.0718], but no differences in other circadian locomotor activity variables (**Supplementary Fig. 1.4G-J and Supplementary Table 1.2).**

Overall, females exposed to *in utero* poly IC did not show as severe alterations as their male counterparts. Poly IC-exposed males primarily showed increased (subjective) day activity

counts across the 3 different lighting conditions, which was consistent in both cohorts. In the first cohort, we also report a longer period in DD, an extended *alpha*, and increased percent (subjective) day activity in poly IC-exposed mice compared to controls.

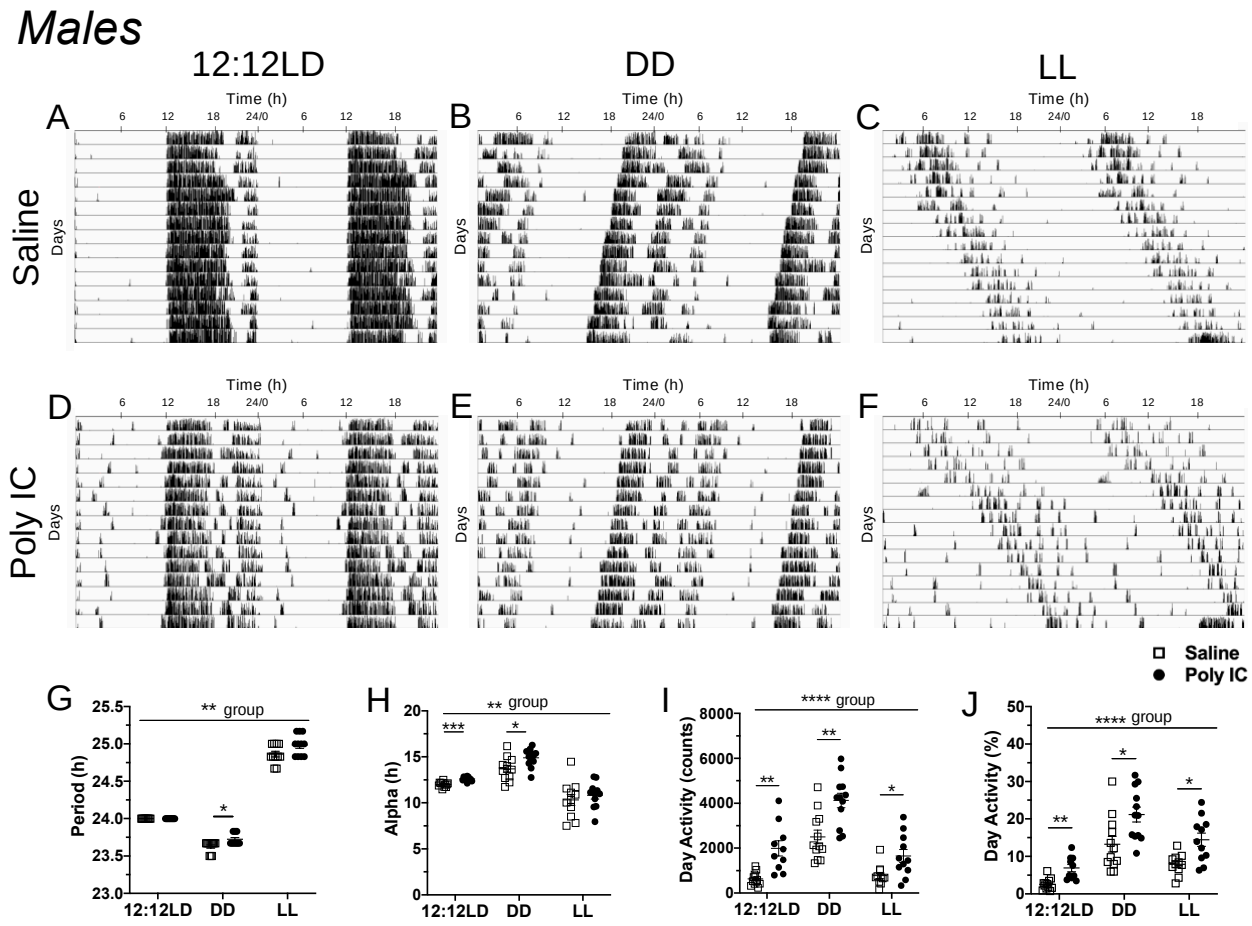


Fig. 1.3. Circadian locomotor activity variables in male offspring. Representative actograms of (A-C) saline-exposed (n=11-12 from 4 litters) and (D-F) poly IC-exposed (n=10-12 from 5 litters) male offspring under (A, D) 12:12LD, (B, E) DD and (C, F) LL. Actograms depict circadian locomotor activity, whereby days are vertically stacked one on the other, time (in hours) is shown across the x-axis, and data are double plotted to facilitate visualization. The last 15 days of each condition are shown. Individual data points represent independent mice. Circadian locomotor activity variables were analyzed in the last 10 days of each condition, including (G) period (h), (H) *alpha* (h), (I) day activity (counts) and (J) day activity (%). Values are mean ± SEM. A mixed-effects analysis (factors: lighting condition, group) was used with Geisser-Greenhouse correction and Sidak's *post-hoc* tests. If no interaction was found, main effects were explored and presented

in the graph. ****p<.0001, ***p <.001, **p <.01 and *p <.05. This experiment was repeated with the second lot of poly IC; results shown in Supplementary Fig. 1.3.

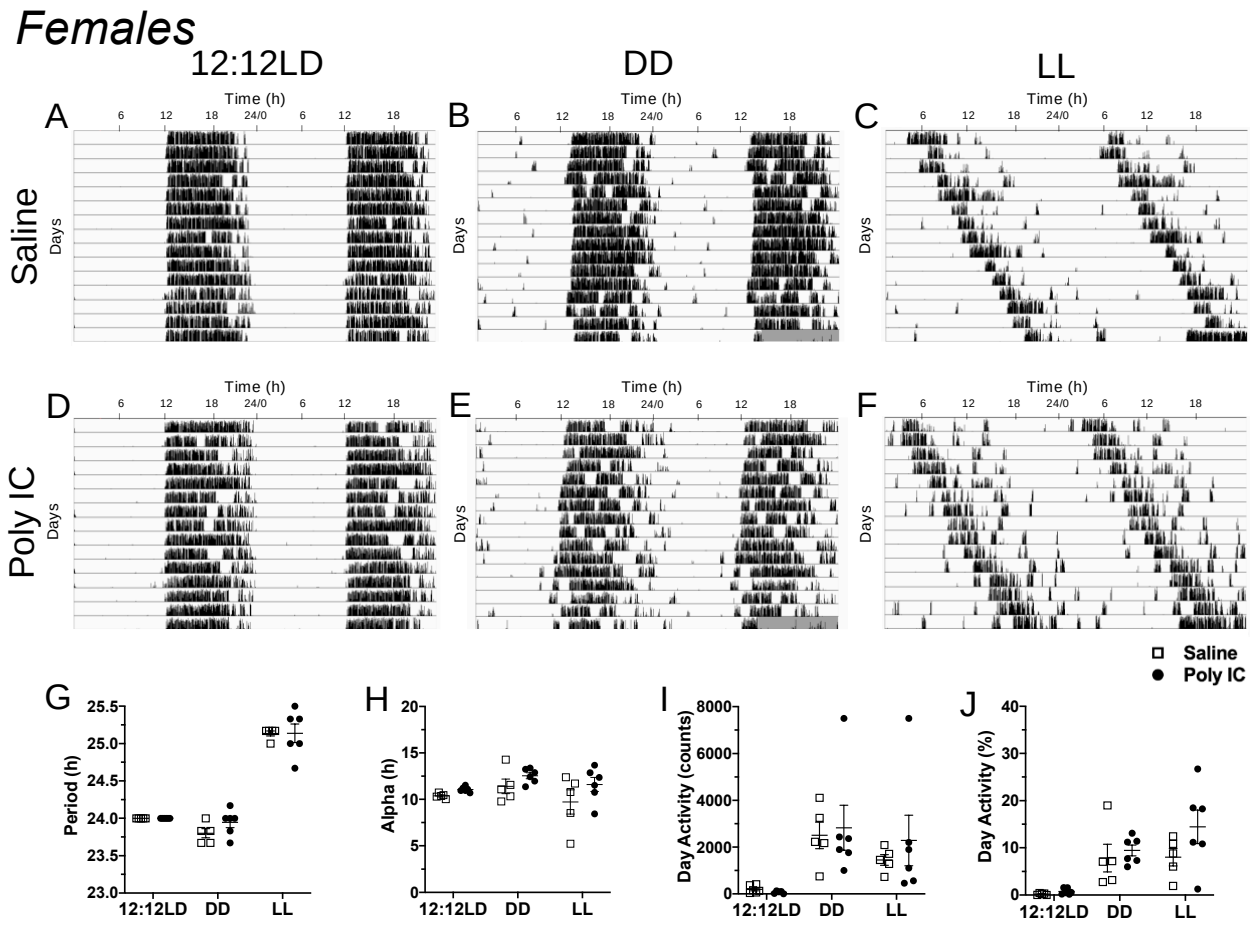


Fig. 1.4. Circadian locomotor activity variables in female offspring. Representative actograms of (A-C) saline-exposed (n=5 from 4 litters) and (D-F) poly IC-exposed (n=6 from 5 litters) female offspring under (A, D) 12:12LD, (B, E) DD and (C, F) LL. Actograms depict circadian locomotor activity, whereby days are vertically stacked one on the other, time (in h) is shown across the x-axis, and data are double plotted to facilitate visualization. The last 15 days of each condition are shown. Individual data points represent independent mice. Circadian locomotor activity variables were analyzed in the last 10 days of each condition, including (G) period (h), (H) *alpha*, (I) day activity (counts) and (J) day activity (%). Values are mean ± SEM. A mixed-effects analysis (factors: lighting condition, group) was used with Geisser-Greenhouse correction. No significant group differences were found. This experiment was repeated with the second lot of poly IC; results shown in Supplementary Fig. 2.4.

Table 1.1. Summary of wheel running parameters from poly IC- and saline-exposed male offspring.

	12:12LD		DD		LL	
	Saline (n=12)	Poly IC (n=10)	Saline (n=12)	Poly IC (n=12)	Saline (n=11)	Poly IC (n=11)
Period (h)	24.0 ± 0.00	24.0 ± 0.00	23.6 ± 0.02	23.7 ± 0.02	24.9 ± 0.04	25.0 ± 0.04
Alpha (h)	12.0 ± 0.08	12.6 ± 0.08	13.7 ± 0.36	14.9 ± 0.28	10.3 ± 0.60	10.8 ± 0.43
(Subjective) Day Activity (counts)	627 ± 84	1992 ± 339	2499 ± 306	4123 ± 321	781 ± 138	1652 ± 286
(Subjective) Night Activity (counts)	26081 ± 1509	26181 ± 1243	18276 ± 1563	16265 ± 1301	9489 ± 1151	9087 ± 1339
Total Activity (counts)	26707 ± 1457	28174 ± 1434	20564 ± 1486	20180 ± 1263	10515 ± 1341	10494 ± 1468
(Subjective) Day Activity (%)	2.54 ± 0.43	6.88 ± 0.97	13.25 ± 2.09	21.18 ± 1.98	7.89 ± 0.83	14.43 ± 1.80
Intradaily Variability	0.481 ± 0.028	0.578 ± 0.029	0.812 ± 0.107	1.08 ± 0.083	0.892 ± 0.080	1.074 ± 0.101
Relative Amplitude	0.997 ± 0.001	0.986 ± 0.004	0.968 ± 0.012	0.970 ± 0.010	0.977 ± 0.011	0.975 ± 0.014
Interdaily Stability	0.834 ± 0.019	0.858 ± 0.013	N/A	N/A	N/A	N/A
Onset Variability	0.18 ± 0.02	0.18 ± 0.02	2.27 ± 0.21	2.22 ± 0.33	2.72 ± 0.40	2.76 ± 0.48
Number of Bouts	66.0 ± 4.79	73.0 ± 5.68	125.5 ± 11.03	128.5 ± 9.05	132.7 ± 6.65	147.1 ± 5.75
Average Bout Length (minutes)	84.6 ± 5.93	82.9 ± 7.95	52.9 ± 6.14	49.4 ± 4.41	27.8 ± 4.13	24.7 ± 3.77
Average Counts per Bout	4187 ± 438	4023 ± 534	2509 ± 308	2290 ± 253	1117 ± 223	1010 ± 176
Average Peak Rate	69.69 ± 2.09	71.41 ± 3.33	72.46 ± 2.34	74.11 ± 1.61	67.73 ± 1.82	69.67 ± 2.57

Notes: Values represent mean ± SEM. N/A denotes parameters that could not be assessed in constant conditions. 12:12LD, 12 h of light, 12 h of dark; DD, constant dark; LL, constant light.

Table 1.2. Summary of wheel running parameters from poly IC- and saline-exposed female offspring.

	12:12LD		DD		LL	
	Saline (n=5)	Poly IC (n=6)	Saline (n=5)	Poly IC (n=6)	Saline (n=5)	Poly IC (n=6)
Period (h)	24.0 ± 0.00	24.0 ± 0.00	23.8 ±0.06	23.9 ± 0.07	25.1 ± 0.03	25.1 ± 0.12
Alpha (h)	10.4 ± 0.12	11.1 ± 0.12	12.1 ± 0.47	11.9 ± 0.77	9.7 ± 1.30	11.6 ± 0.76
(Subjective) Day Activity (counts)	66 ± 24	193 ± 64	2825 ± 958	2500 ± 566	2285 ± 1081	1450 ± 227
(Subjective) Night Activity (counts)	31928 ± 2142	26558 ± 915	28133 ± 2536	26451 ± 2025	10721 ± 3487	11254 ± 1324
Total Activity (counts)	31994 ± 2151	26751 ± 873	30958 ± 2859	28951 ± 2366	11786 ± 4001	13237 ± 1542
(Subjective) Day Activity (%)	0.20 ± 0.08	0.75 ± 0.26	8.94 ± 2.31	8.45 ± 1.73	8.00 ± 1.86	14.45 ± 3.55
Intradaily Variability	0.297 ± 0.046	0.405 ± 0.022	0.423 ± 0.028	0.527 ± 0.063	0.831 ± 0.156	1.059 ± 0.115
Relative Amplitude	0.999 ± 0.001	0.999 ± 0.001	0.988 ± 0.010	0.979 ± 0.005	0.969 ± 0.023	0.897 ± 0.057
Interdaily Stability	0.863 ± 0.014	0.867 ± 0.021	N/A	N/A	N/A	N/A
Onset Variability	0.33 ± 0.06	0.29 ± 0.07	2.46 ± 0.64	1.95 ± 0.38	1.55 ± 0.43	1.91 ± 0.54
Number of Bouts	77.6 ± 6.31	106.2 ± 6.12	88.8 ± 10.26	119.0 ± 5.56	98.6 ± 12.00	149.0 ± 8.67
Average Bout Length (minutes)	107.1 ± 13.44	76.6 ± 4.70	97.8 ± 18.34	66.2 ± 3.15	34.2 ± 8.19	25.72 ± 3.08
Average Counts per Bout	6045 ± 702	3602 ± 204	5516 ± 1094	3196 ± 280	1618 ± 473	1116 ± 149
Average Peak Rate	69.40 ± 3.20	63.85 ± 1.86	72.12 ± 1.06	69.50 ± 2.73	65.55 ± 6.58	67.91 ± 2.35

Notes: Values represent mean ± SEM. N/A denotes parameters that could not be assessed in constant conditions. 12:12LD, 12 h of light, 12 h of dark; DD, constant dark; LL, constant light.

1.3.2.2. Daily Activity Profiles

To further analyze our data, daily activity profiles were created by plotting average running activity per group from ZT 0-24 in 12:12LD, and CT 0-24 in DD and LL. When observing the first 3 h of the (subjective) day, male poly IC exposed offspring exhibited increased activity in 12:12LD (p =.0031) and DD (p =.0067), but not LL (**Fig. 1.5D-F**). Additionally, poly IC-exposed males also showed increased activity in the last 1.5 h of the day in 12:12LD (p <.0001) but not in DD or LL (**Fig. 1.5G-I**). In the second cohort, we similarly found an increase in activity in the first h of the (subjective) day under 12:12LD (p =.0090) and DD (p =.0068), but not LL (**Supplementary Fig. 1.5D-F**). These differences were not observed in females, due to the lack of day activity counts in the poly IC-exposed females (**Fig. 1.6D-I**). Overall, these analyses complemented the ANOVAs we conducted, by suggesting that the differences between groups in 12:12LD and DD are mainly driven by increased activity in the beginning of the (subjective) day.

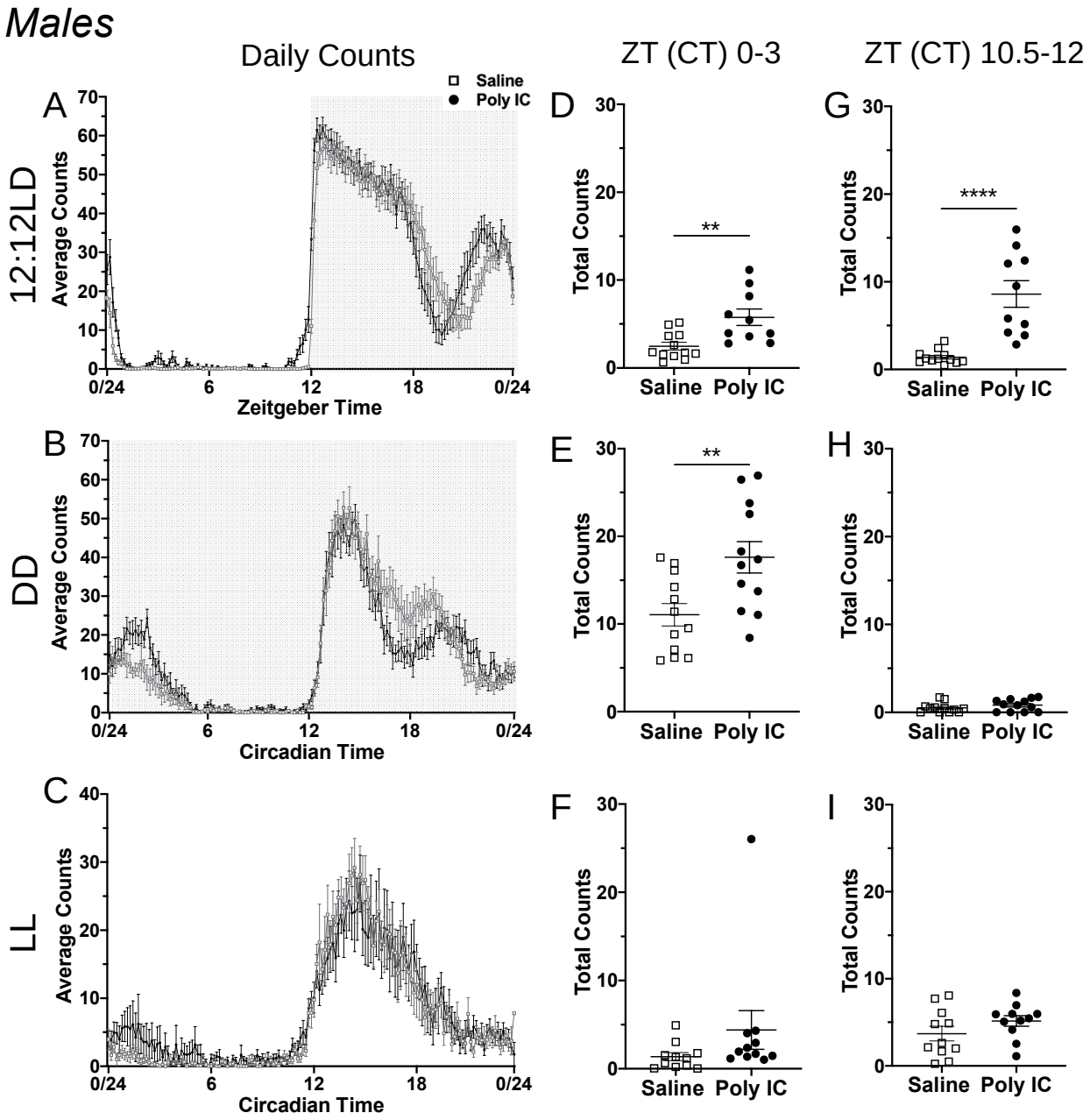


Fig. 1.5. Daily activity profiles averaged across all saline-exposed (n=11-12 from 4 litters) and poly IC-exposed (n=10-12 from 5 litters) male offspring, under (A) 12:12LD, (B) DD and (C) LL. Poly IC-exposed mice are depicted as black circles connected by a black line and saline-exposed mice as white squares connected by a gray line. Analysis of total activity counts between groups at the beginning (ZT (CT) 0-3) and end (ZT (CT) 10.5-12) of the (subjective) day in (D, G) 12:12LD, (E, H) DD and (F, I) LL respectively. An independent samples t-test

(with Welsh's correction, or Mann-Whitney U test when appropriate) was used, ****p <.0001 and **p <.01. This experiment was repeated with the second lot of poly IC; results shown in Supplementary Fig. 1.5.

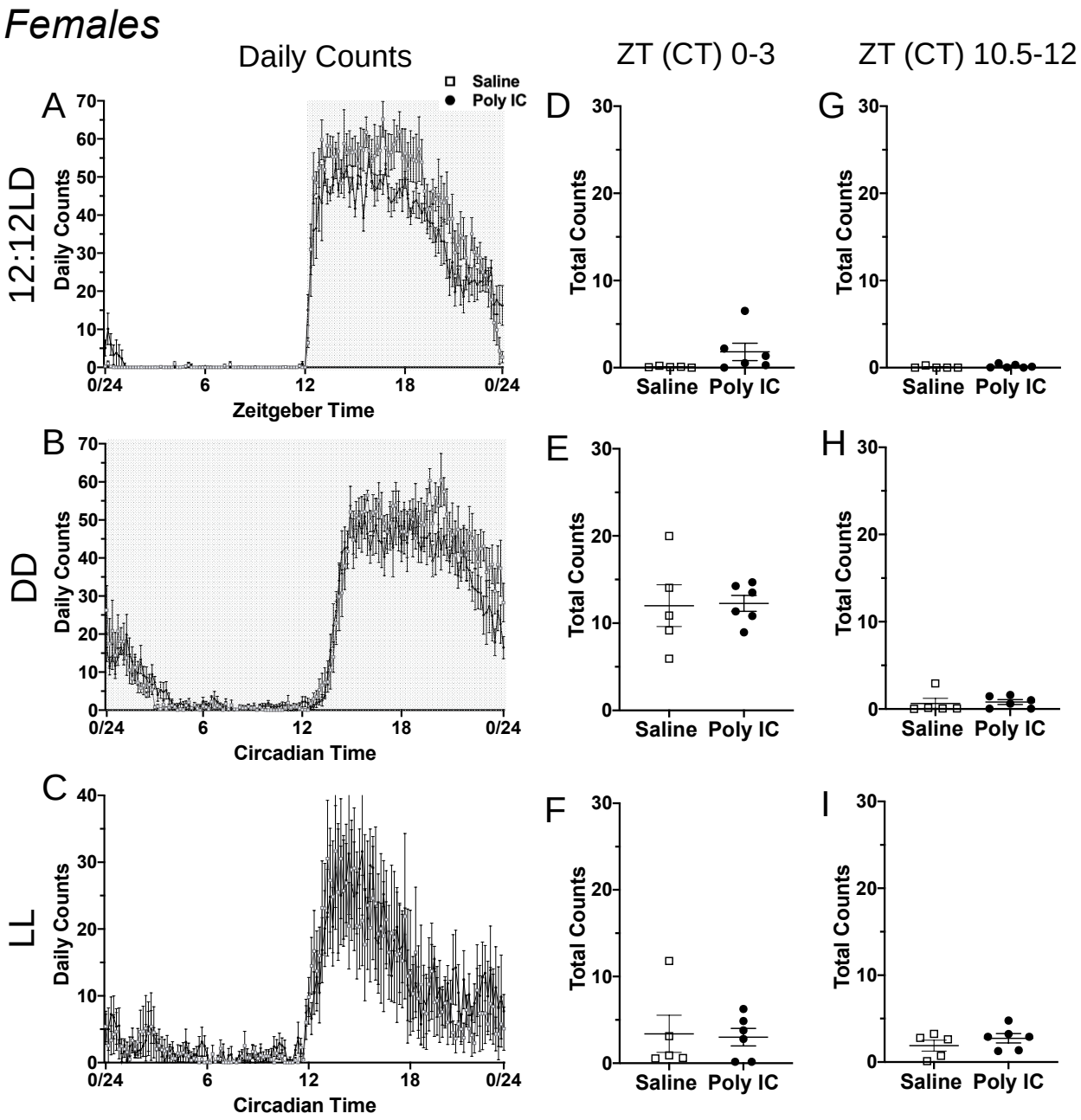


Fig. 1.6. Daily activity profiles averaged across all saline-exposed (n=5 from 4 litters) and poly IC-exposed (n=6 from 5 litters) female offspring, under (A) 12:12LD, (B) DD and (C) LL. Poly IC-exposed mice are depicted as black circles connected by a black line and saline-exposed mice as white squares connected by a gray line. Analysis of total activity counts between groups at the beginning (ZT (CT) 0-3) and end (ZT (CT) 10.5-12) of the (subjective) day in (D, G) 12:12LD,

(E, H) DD and (F, I) LL respectively. An independent samples t-test (with Welsh's correction, or Mann-Whitney U test when appropriate) was used. No significant group differences were found. This experiment was repeated with the second lot of poly IC; results shown in Supplementary Fig. 1.6.

1.3.2.3. Non-parametric parameters and Analysis of Activity Bouts

When exploring non-parametric rest-activity parameters in males, poly IC-exposed male mice had overall greater intradaily variability scores than controls [$F_{(1, 22)}$ = 6.621, p =.017], without a group x lighting condition interaction (**Fig. 1.7A)**. No group differences were found in scores of relative amplitude (**Fig. 1.7B**). In the second cohort, no differences were found in non-parametric variables (**Supplementary Fig. 1.7A-B**). As well, no differences were found in onset variability scores in either cohort (**Fig. 1.7C, Supplementary Fig. 1.7C).**

In the first cohort of male mice, we did not find any differences in the bout analysis **(Fig. 1.7D, Table 1.1)**. However, in the second cohort, there was a trend for poly IC-exposed male mice to have a greater average bout length [$F_{(1, 22)}$ = 3.286, p =.0836], and a significantly greater average peak rate [$F_{(1, 22)}$ = 4.568, p =.0439] than controls (**Supplementary Fig. 1.7D, Supplementary Table 1.1**).

In females, poly IC-exposed mice had a trend for overall higher intradaily variability scores [$F_{(1, 9)}$ = 4.27, p =.0687], without a poly IC x lighting condition interaction (**Fig. 1.7E**). No differences were found in scores of relative amplitude (**Fig. 1.7F**). In the second cohort, no differences were found on non-parametric measures in females, nor in onset variability scores in either cohort (**Fig. 1.7G, Supplementary Fig. 1.7E-G**).

For the analysis of activity bouts in females, we report no group x lighting condition interactions, but poly IC-exposed mice had increased number of bouts [Group, $F_{(1, 9)}$ = 24.11, p =.0008], decreased average bout length [Group, $F_{(1, 9)}$ = 6.878, p =.0277] and decreased counts per bout [Group, $F_{(1, 9)}$ = 12.03, p =.0071] than controls (**Fig. 1.7H and Table 1.2**). These differences were not seen in females of the second cohort (**Supplementary Fig. 1.7H and Supplementary Table 1.2**).

Overall, in the first cohort, poly IC-exposed males had greater intradaily variability scores than controls, and in the second cohort, poly IC-exposed males showed a trend for greater average bout length and had a greater average peak rate than controls. Poly IC-exposed females showed a trend for greater intradaily variability scores, and significantly increased number of bouts, decreased average bout length and decreased counts per bout compared to controls in the first cohort, which was not observed in the second cohort.

1.3.2.4. Evaluating sex differences

To confirm that the effects of MIA on wheel-running activity are sex dependent, circadian activity variables were analyzed using a three-way ANOVA with the following factors: sex (male, female), group (poly IC, saline exposure) and lighting condition (12:12LD, DD, LL) (Supplementary Table 3). We explored the group ×sex interaction and the main effect of sex. Notably, in the first cohort, there was a significant group ×sex interaction on (subjective) day activity (counts) ($p = .0430$), number of bouts ($p = .0297$) and average counts per bout ($p = .0132$). As well, there was a significant effect of sex on the majority of the parameters we tested including period (h) ($p < .0001$), alpha (h) ($p = .0002$), total activity ($p = .0003$), (subjective) day activity (%) ($p = .0002$), and intradaily variability ($p = .0003$). In the second cohort, we were able to reproduce the main effects of sex in all of the aforementioned parameters, except period (h), alpha (h) and (subjective) day activity (%). Data collected from the daily activity profiles were analyzed with two-way ANOVAs with the following factors: sex (male, female) and group (poly IC, saline exposure) (Supplementary Table 4). Notably, there was a main effect of sex under 12:12LD at the beginning of day in cohort 1 ($p = .0004$) and cohort 2 ($p = .0276$). In the first cohort only, we also observed a significant group ×sex interaction at the end of the day activity under 12:12LD ($p = .0014$). Overall,

the group ×sex interactions found in the first cohort support poly IC exposure having a sex-specific effect on wheel-running parameters. Additionally, the many statistically significant effects of sex in both cohorts supports sex differences across group and lighting conditions.

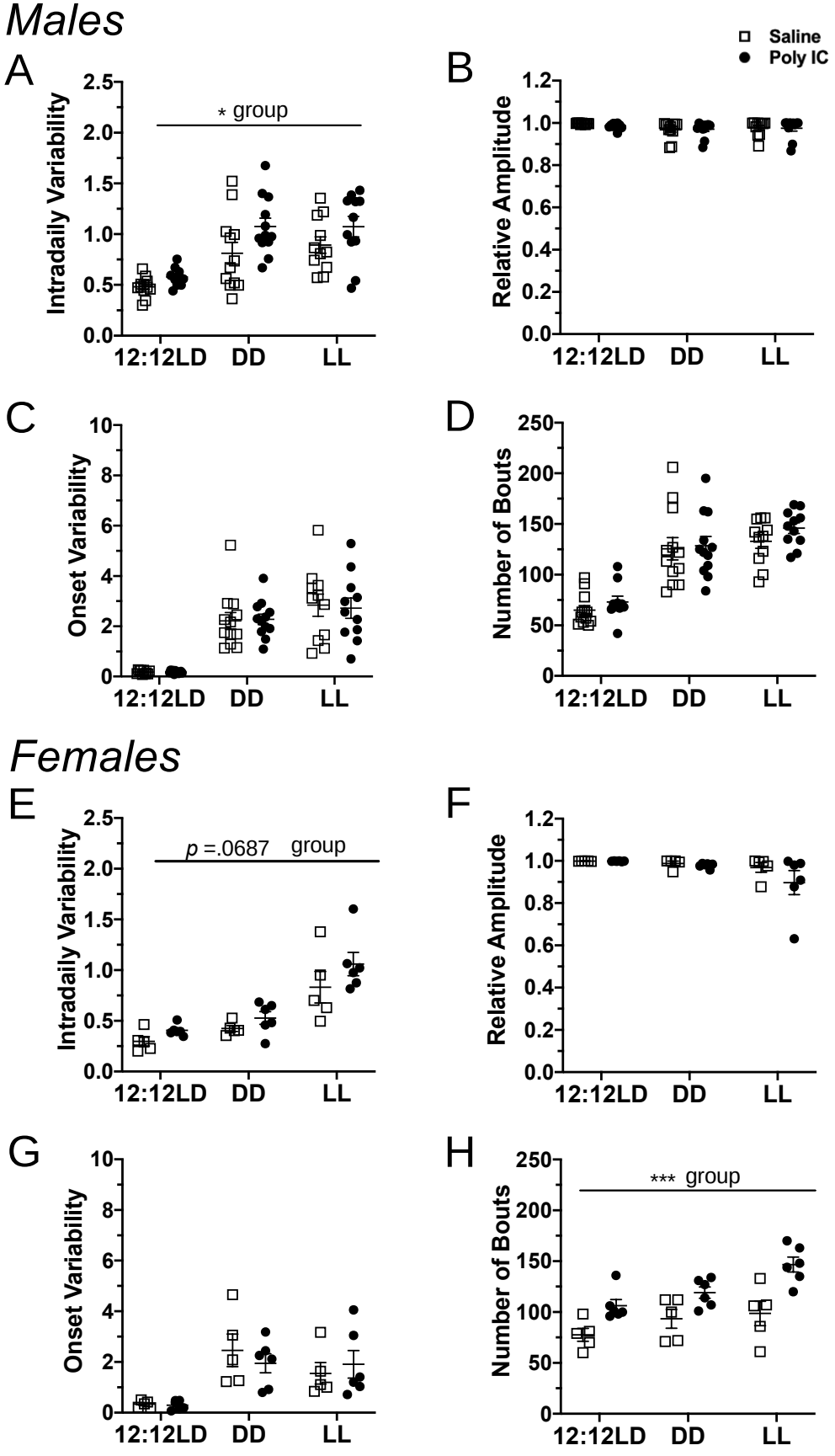

Males
A
Intradaily Variability
* group
12:12LD
DD
LL
B
Saline
Poly IC
Relative Amplitude
C
Onset Variability
D
Number of Bouts
Females
E
Intradaily Variability
p =.0687 group
F
Relative Amplitude
G
Onset Variability
H
Number of Bouts
*** group

Fig. 1.7. Activity rhythm characteristics and analysis of activity bouts. Analysis was done for (A-D) male offspring (saline-exposed n=11-12 from 4 litters; poly IC-exposed n=10-12 from 5 litters) and (E-H) female offspring (saline-exposed n=5 from 4 litters; poly IC-exposed n=6 from 5 litters) separately for the following measures: (A, E) intradaily variability, (B, F) relative amplitude, (C, G) onset variability and (D, H) number of bouts. Values are mean ± SEM. A mixed-effects analysis (factors: lighting condition, group) was used with Geisser-Greenhouse correction. If no interaction was found, main effects were explored and presented in the graph. ***p <.001 and *p <.05. This experiment was repeated with the second lot of poly IC; results shown in Supplementary Fig. 1.7.

1.3.3. Maternal behavior of poly IC versus saline injected dams

It is known that mother-pup interactions have direct long-term effects in adult offspring (Walker, Deschamps et al. 2004). However, it is unknown whether a single poly IC injection during gestation (E9.5) alters maternal behavior. We aimed to explore postnatal maternal behavior in poly IC-injected dams compared to controls (protocol depicted in **Supplementary Fig. 1.1**). **Fig. 1.8** presents a general overview of maternal behavior, which we divided into 3 general categories: out of nest behavior, in nest behavior and nest building behavior. Maternal behavior did not differ between groups for out of nest behavior (day: p=.9084; night p=.4189), in nest behavior (day: p=.6567; night p=.5942) or nest building behavior (day: p=.2620; night p=.5976) during the day or night (**Fig. 1.8**). When maternal behavior was explored in more detail, there were still no differences between groups for grooming behavior (day: p=.4790; night p=.9639), wandering (day: p=1798; night p=.8622), nest rustling (day: p=.4339; night p=.8354), nest still (day: p=.7559; night p=.4681), eating (day: p=4345; night p=.5259) and drinking (day: p=.4579; night p=.2860) (**Supplementary Fig. 1.8**). Overall, these data imply that the differences that we observed in offspring behaviors are not due to differences in maternal behavior.

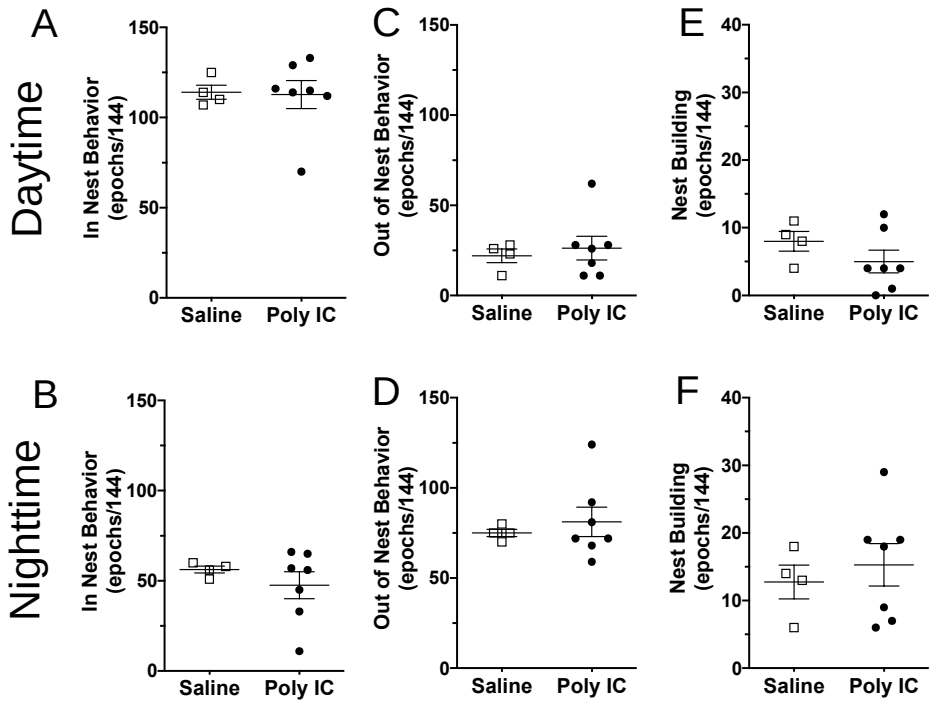


Fig. 1.8. Maternal behavior of saline and poly IC-injected dams. Dams were observed between postnatal day 4 to 5 during both the (A, C, E) daytime and the (B, D, F) nighttime and scored for maternal behavior. Dams were scored on (A-B) in nest behavior, (C-D) out of nest behavior and (E-F) nest building. Individual data points represent independent dams and data were represented as mean ± SEM. An independent samples t-test (with Welsh's correction, or Mann-Whitney U test when appropriate) was used. No significant group differences were found.

1.4. Discussion

Circadian rhythm disruption is a prominent feature in schizophrenia (SCZ) and autism spectrum disorder (ASD), thus there has been a growing interest in studying the role circadian rhythms play in these neurodevelopmental disorders. Here, we report that adult male offspring exposed *in utero* to poly IC exhibited altered circadian running behavior under a standard laboratory lighting condition (12:12LD), constant darkness (DD) and constant light (LL) compared to saline-exposed controls. In particular, poly IC-exposed male mice exhibited more (subjective) day activity than controls. This was driven mainly by increased activity in the beginning of the (subjective) day in 12:12LD and DD, and at the end of the day in 12:12LD. These were sex-dependent differences, as female offspring exposed to poly IC showed much fewer disturbances than males. Our data contributes to the current literature by showing that MIA, a risk factor for neurodevelopmental disorders, leads to alterations in circadian rhythms in mice, which has repeatedly been observed in individuals with SCZ and ASD. Additionally, circadian rhythms under different lighting conditions have only been characterized in animal models with mutations in risk genes for SCZ and ASD. Thus, our data can serve as a baseline for future experiments that aim to integrate both genetic and environmental risk factors for SCZ and ASD in animal models.

A number of sleep and circadian rhythm disruptions have been reported in genetic animal models used to study SCZ and ASD (Delorme, Srivastava et al. 2020, Wintler, Schoch et al. 2020). Interestingly, the models display somewhat different sleep and circadian rhythms disruptions from one another. Phenotypes include decreased sleep time, more fragmented sleep and circadian activity, and increased free-running period. Some studies have even found a heterogeneity of disruption, whereby a subset of SCZ and ASD animals exhibit more severe disruptions than their SCZ and ASD counterparts. In our study using a neurodevelopmental risk factor for SCZ and ASD,

there are parallels in the disruptions we found to those reported in genetic animal models. For example, similar to our findings, other groups have also reported increased (subjective) day activity in 12:12LD (*Snap-25* mutant mice (Oliver, Sobczyk et al. 2012)), DD (*Pallidin* mutant mice (Lee, Wang et al. 2018)) and LL (*Dysbindin-1* mutant mice (Bhardwaj, Stojkovic et al. 2015)). Also, similar to Maple and colleagues (*Egr3* mutant mice), we found an extended *alpha* under 12:12LD (Maple, Rowe et al. 2018). These parallels are possibly outlining a common mechanism through which factors that contribute to SCZ and ASD lead to disrupted circadian rhythms. Overall, each animal model captures important features of SCZ and ASD-related sleep and circadian disruptions, and the differences observed between models may speak to the heterogeneity of sleep and circadian disruptions observed in humans affected by SCZ and ASD. For example, some patients exhibit highly irregular 24 h patterns, while others exhibit delayed activity rhythms and even free-running rhythms (Wulff, Dijk et al. 2012). A metanalysis comparing sleep parameters in psychiatric patients found that SCZ patients exhibited longer total sleep time, greater sleep latency and poorer sleep continuity (Meyer, Faulkner et al. 2020). Therefore, future studies should examine sleep differences in the MIA model, to explore if the increase (subjective) daytime running activity that we observed is due to disturbed sleep initiation and continuity. To our knowledge, only one prior study has reported behavioral rhythm alterations in the MIA model: Missig and colleagues who tested the combined effects of prenatal poly IC (E12.5) and post-natal lipopolysaccharide (LPS) treatment (postnatal day 9) (Missig, Mokler et al. 2018). Using wireless telemetry transmitters, they reported that male mice exposed to prenatal poly IC only had more general home cage activity during the light phase under 12:12LD at 7 but not 12 weeks of age. Although we administered poly IC earlier in gestation (E9.5), these findings are consistent with ours because we found more wheel running activity during the light phase in

poly IC-exposed male mice. However, we showed that the effect is also seen under free-running conditions (DD, LL), and is sex-dependent (the previous report was only in males).

In this report, we provided evidence that both poly IC lots used induced an immune response in pregnant dams. Consistent with existing literature, we reported an increase in several pro- and anti-inflammatory cytokines and chemokines, including IL-6, 1L-1β, IL-10, MCP-1 and TNFα, following poly IC injection in pregnant dams (Meyer, Nyffeler et al. 2006, Arrode-Bruses and Bruses 2012). Interestingly, in the literature, IL-6 was shown to be critical in the adverse effects of *in utero* poly IC exposure. Namely, a single administration of IL-6 in a pregnant dam (but not IFNγ) led to similar deficits to those observed in the MIA model, and coadministration of anti-IL-6 antibody normalized MIA-induced changes (Smith, Li et al. 2007). In our study, although IL-6 expression levels in maternal plasma from both lots of poly IC were significantly higher than controls, there was a 2-fold reduction in the second lot compared to the first. Perhaps this contributed to the milder effects on behavior observed in the second cohort. Further, varying potencies of poly IC per lot appear to be common in the literature (Meyer, Nyffeler et al. 2006, Mueller, Richetto et al. 2019). To further support our main findings in both cohorts of mice, we performed additional analyses, where we added cohort as a factor and performed 3-way ANOVAs on the data from the wheel running parameters. We consistently found a main effect of group for increased (subjective) day running, with no cohort x group interaction (data not shown). Thus, irrespective of the lot of poly IC, we do observe an increase in (subjective) day activity counts in both cohorts.

Intriguingly, the effects that we observed were sex-dependent. Namely, female offspring showed overall much milder effects of *in utero* poly IC exposure than males. This finding is consistent with reports of individuals with SCZ (Ochoa, Usall et al. 2012) and ASD (Ferri, Abel

et al. 2018), where incident rate, age of onset and course of the disorder are all affected in a sex-dependent manner. An association has been reported between increased maternal cytokine levels during pregnancy and psychosis in adult offspring (Buka, Tsuang et al. 2001, Brown, Hooton et al. 2004, Allswede, Buka et al. 2016). However, exposure to different prenatal maternal cytokine levels may contribute to the development of psychosis in a sex-dependent manner. For example, in humans, when maternal serum was collected early in the third trimester, researchers found that higher levels of maternal IL-6 were more prevalent in male offspring with SCZ than male controls, and lower levels of maternal TNFα levels were more found among female individuals with SCZ than female controls (Goldstein, Cherkerzian et al. 2014). In rodent models of prenatal infection, sex-differences are apparent in various SCZ and ASD-like behaviors (Xuan and Hampson 2014, Carney 2019, Gogos, Sbisa et al. 2020), and across gene expression and cellular correlates (Hui, St-Pierre et al. 2018, Braun, Carpentier et al. 2019). Thus, immunological processes may affect fetal brain development in a sex-dependent manner that perhaps leads to the sex differences observed in circadian behavior.

Despite altered sleep and circadian rhythms being documented in genetic animal models of SCZ (Delorme, Srivastava et al. 2020) and ASD (Wintler, Schoch et al. 2020), and reported here in a neurodevelopmental model, the biological mechanisms that underly these alterations are largely unknown. Further, it is largely unknown how MIA specifically affects the circadian system, or how circadian rhythm disruption affects outcomes in offspring exposed to MIA. To our knowledge, the only data to test this were those of Spisska and colleagues, who simulated maternal infection using LPS in rats and measured clock gene expression in the suprachiasmatic nucleus during postnatal development. Differences were mainly observed in *Nr1d1* expression, which was arrhythmic at P3 and had a significantly increased amplitude at P20 compared to controls. As well,

in the night, pineal glands from LPS-treated animals had higher activity of alkylamine-N-acetyltransferase (AA-NAT), the rate-limiting enzyme in melatonin synthesis (Spisska, Pacesova et al. 2020). Overall, the higher amplitude of *Nr1d1* expression and AA-NAT were hypothesized to be an adaptive feature of the clock against immune perturbations (Spisska, Pacesova et al. 2020). For future studies, a possible mechanism that may be of interest involves altered synaptic transmission and connectivity, which have been suggested as key factors in the pathogenesis of SCZ and ASD (Yin, Chen et al. 2012, Fromer, Pocklington et al. 2014, Guang, Pang et al. 2018), and have also been linked to the circadian system (Hannou, Roy et al. 2020). MIA is also known to affect synaptic development and function in offspring (Oh-Nishi, Obayashi et al. 2010, Coiro, Padmashri et al. 2015). Moreover, in genetic animal models of SCZ and ASD that exhibit sleep and circadian disruptions, many of the susceptibility genes encode proteins that are implicated in neuronal communication, and/or protein and vesicle trafficking (Oliver, Sobczyk et al. 2012, Bhardwaj, Stojkovic et al. 2015, Pritchett, Jagannath et al. 2015, Jaaro-Peled, Altimus et al. 2016, Lee, Wang et al. 2018, Maple, Rowe et al. 2018, Ingiosi, Schoch et al. 2019, Delorme, Srivastava et al. 2020). Altogether, further research is needed to uncover the biological mechanisms linking prenatal maternal inflammation to altered circadian rhythms.

A longstanding hypothesis states that neurodevelopmental disorders result from complex interactions between genetic and environmental risk factors that alter the development of brain structures and proper brain development and function. Future studies should explore a complementary angle to study the role of circadian rhythms play in neurodevelopmental disorders. There are various negative health and behavioral consequences observed when the sleep or circadian system is disrupted in healthy subjects or in laboratory animals. Emerging studies on the effects of sleep and circadian disruption (induced through shiftwork or jetlag) on mental and

physical health are becoming more numerous (Karatsoreos 2012, Foster, Peirson et al. 2013). However, such studies are largely correlative, and the underlying mechanisms are unknown. Due to the prevalence of sleep and circadian disruption observed in patients with SCZ or ASD, one could wonder what impacts environmentally disrupting circadian rhythms would have on the course of these disorders, especially in genetically vulnerable individuals. Such findings would highlight a role for sleep and circadian rhythms as a causal risk factor for SCZ and ASD, and not only a characteristic trait of the disorder.

Discovering more efficient therapeutic options for individuals with SCZ or ASD may rely on exploring novel risk factors and uncovering the complex mechanisms in which these risk factors interact to jointly exert their effect. The development of circadian-based therapies in conjunction with other therapies may be a promising avenue of research.

1.5. Acknowledgments

The authors thank the members of the Cermakian and Srivastava laboratories for helpful discussions. They also thank Nicola M. Ludin, Katherine Lord, William Ozell-Landry and Geneviève Dubeau Laramée for help with mouse handling and data analyses. This work was supported by grants from the Canadian Institutes of Health Research [PJT-153299] and Velux Stiftung [Project 927]. TD was supported by graduate scholarships from the Schizophrenia Society of Canada Foundation, the Canadian College of Neuropsychopharmacology, and the Fonds de Recherche du Québec – Santé.

1.6. References

Alexopoulou, L., Holt, A.C., Medzhitov, R., Flavell, R.A., 2001. Recognition of double-stranded RNA and activation of NF-kappaB by Toll-like receptor 3. Nature 413, 732-738.

Allswede, D.M., Buka, S.L., Yolken, R.H., Torrey, E.F., Cannon, T.D., 2016. Elevated maternal cytokine levels at birth and risk for psychosis in adult offspring. Schizophr Res 172, 41-45.

Arrode-Bruses, G., Bruses, J.L., 2012. Maternal immune activation by poly I:C induces expression of cytokines IL-1beta and IL-13, chemokine MCP-1 and colony stimulating factor VEGF in fetal mouse brain. J Neuroinflammation 9, 83.

American Psychiatric Association, 2013. Diagnostic and statistical manual of mental disorders (5th ed.). https://doi.org/10.1176/appi.books.9780890425596.dsm01.

Atladottir, H.O., Thorsen, P., Ostergaard, L., Schendel, D.E., Lemcke, S., Abdallah, M., Parner, E.T., 2010. Maternal infection requiring hospitalization during pregnancy and autism spectrum disorders. J Autism Dev Disord 40, 1423-1430.

Baron-Cohen, S., Scott, F.J., Allison, C., Williams, J., Bolton, P., Matthews, F.E., Brayne, C., 2009. Prevalence of autism-spectrum conditions: UK school-based population study. Br J Psychiatry 194, 500-509.

Bhardwaj, S.K., Stojkovic, K., Kiessling, S., Srivastava, L.K., Cermakian, N., 2015. Constant light uncovers behavioral effects of a mutation in the schizophrenia risk gene Dtnbp1 in mice. Behav Brain Res 284, 58-68.

Boulanger-Bertolus, J., Pancaro, C., Mashour, G.A., 2018. Increasing Role of Maternal Immune Activation in Neurodevelopmental Disorders. Front Behav Neurosci 12, 230.

Braun, A.E., Carpentier, P.A., Babineau, B.A., Narayan, A.R., Kielhold, M.L., Moon, H.M., Shankar, A., Su, J., Saravanapandian, V., Haditsch, U., Palmer, T.D., 2019. "Females Are Not Just 'Protected' Males": Sex-Specific Vulnerabilities in Placenta and Brain after Prenatal Immune Disruption. eNeuro 6.

Brown, A.S., 2012. Epidemiologic studies of exposure to prenatal infection and risk of schizophrenia and autism. Dev Neurobiol 72, 1272-1276.

Brown, A.S., Begg, M.D., Gravenstein, S., Schaefer, C.A., Wyatt, R.J., Bresnahan, M., Babulas, V.P., Susser, E.S., 2004a. Serologic evidence of prenatal influenza in the etiology of schizophrenia. Arch Gen Psychiatry 61, 774-780.

Brown, A.S., Hooton, J., Schaefer, C.A., Zhang, H., Petkova, E., Babulas, V., Perrin, M., Gorman, J.M., Susser, E.S., 2004b. Elevated maternal interleukin-8 levels and risk of schizophrenia in adult offspring. Am J Psychiatry 161, 889-895.

Brown, A.S., Meyer, U., 2018. Maternal Immune Activation and Neuropsychiatric Illness: A Translational Research Perspective. Am J Psychiatry 175, 1073-1083.

Buka, S.L., Tsuang, M.T., Torrey, E.F., Klebanoff, M.A., Wagner, R.L., Yolken, R.H., 2001. Maternal cytokine levels during pregnancy and adult psychosis. Brain Behav Immun 15, 411-420.

Carney, R.S.E., 2019. Does Prenatal Exposure to Maternal Inflammation Causes Sex Differences in Schizophrenia-Related Behavioral Outcomes in Adult Rats? eNeuro 6.

Clancy, B., Darlington, R.B., Finlay, B.L., 2001. Translating developmental time across mammalian species. Neuroscience 105, 7-17.

Cohrs, S., 2008. Sleep disturbances in patients with schizophrenia : impact and effect of antipsychotics. CNS Drugs 22, 939-962.

Coiro, P., Padmashri, R., Suresh, A., Spartz, E., Pendyala, G., Chou, S., Jung, Y., Meays, B., Roy, S., Gautam, N., Alnouti, Y., Li, M., Dunaevsky, A., 2015. Impaired synaptic development in a maternal immune activation mouse model of neurodevelopmental disorders. Brain Behav Immun 50, 249-258.

Cosgrave, J., Wulff, K., Gehrman, P., 2018. Sleep, circadian rhythms, and schizophrenia: where we are and where we need to go. Curr Opin Psychiatry 31, 176-182.

Couturier, J.L., Speechley, K.N., Steele, M., Norman, R., Stringer, B., Nicolson, R., 2005. Parental perception of sleep problems in children of normal intelligence with pervasive developmental disorders: prevalence, severity, and pattern. J Am Acad Child Adolesc Psychiatry 44, 815-822.

Cunningham, C., Campion, S., Teeling, J., Felton, L., Perry, V.H., 2007. The sickness behaviour and CNS inflammatory mediator profile induced by systemic challenge of mice with synthetic double-stranded RNA (poly I:C). Brain Behav Immun 21, 490-502.

Delorme, T.C., Srivastava, L.K., Cermakian, N., 2020. Are Circadian Disturbances a Core Pathophysiological Component of Schizophrenia? J Biol Rhythms 35, 325-339.

Estes, M.L., McAllister, A.K., 2016. Maternal immune activation: Implications for neuropsychiatric disorders. Science 353, 772-777.

Ferri, S.L., Abel, T., Brodkin, E.S., 2018. Sex Differences in Autism Spectrum Disorder: a Review. Curr Psychiatry Rep 20, 9.

Foster, R.G., Peirson, S.N., Wulff, K., Winnebeck, E., Vetter, C., Roenneberg, T., 2013. Sleep and circadian rhythm disruption in social jetlag and mental illness. Prog Mol Biol Transl Sci 119, 325-346.

Fromer, M., Pocklington, A.J., Kavanagh, D.H., Williams, H.J., Dwyer, S., Gormley, P., Georgieva, L., Rees, E., Palta, P., Ruderfer, D.M., Carrera, N., Humphreys, I., Johnson, J.S., Roussos, P., Barker, D.D., Banks, E., Milanova, V., Grant, S.G., Hannon, E., Rose, S.A., Chambert, K., Mahajan, M., Scolnick, E.M., Moran, J.L., Kirov, G., Palotie, A., McCarroll, S.A., Holmans, P., Sklar, P., Owen, M.J., Purcell, S.M., O'Donovan, M.C., 2014. De novo mutations in schizophrenia implicate synaptic networks. Nature 506, 179-184.

Garay, P.A., Hsiao, E.Y., Patterson, P.H., McAllister, A.K., 2013. Maternal immune activation causes age- and region-specific changes in brain cytokines in offspring throughout development. Brain Behav Immun 31, 54-68.

Gogos, A., Sbisa, A., Witkamp, D., van den Buuse, M., 2020. Sex differences in the effect of maternal immune activation on cognitive and psychosis-like behaviour in Long Evans rats. Eur J Neurosci 52, 2614-2626.

Goldman, S.E., Richdale, A.L., Clemons, T., Malow, B.A., 2012. Parental sleep concerns in autism spectrum disorders: variations from childhood to adolescence. J Autism Dev Disord 42, 531-538.

Goldstein, J.M., Cherkerzian, S., Seidman, L.J., Donatelli, J.A., Remington, A.G., Tsuang, M.T., Hornig, M., Buka, S.L., 2014. Prenatal maternal immune disruption and sex-dependent risk for psychoses. Psychol Med 44, 3249-3261.

Guang, S., Pang, N., Deng, X., Yang, L., He, F., Wu, L., Chen, C., Yin, F., Peng, J., 2018. Synaptopathology Involved in Autism Spectrum Disorder. Front Cell Neurosci 12, 470.

Guma, E., Plitman, E., Chakravarty, M.M., 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. Neurosci Biobehav Rev 104, 141-157.

Hannou, L., Roy, P.G., Ballester Roig, M.N., Mongrain, V., 2020. Transcriptional control of synaptic components by the clock machinery. Eur J Neurosci 51, 241-267.

Hastings, M.H., Maywood, E.S., Brancaccio, M., 2018. Generation of circadian rhythms in the suprachiasmatic nucleus. Nat Rev Neurosci 19, 453-469.

Hui, C.W., St-Pierre, A., El Hajj, H., Remy, Y., Hebert, S.S., Luheshi, G.N., Srivastava, L.K., Tremblay, M.E., 2018. Prenatal Immune Challenge in Mice Leads to Partly Sex-Dependent Behavioral, Microglial, and Molecular Abnormalities Associated with Schizophrenia. Front Mol Neurosci 11, 13.

Ingiosi, A.M., Schoch, H., Wintler, T., Singletary, K.G., Righelli, D., Roser, L.G., Medina, E., Risso, D., Frank, M.G., Peixoto, L., 2019. Shank3 modulates sleep and expression of circadian transcription factors. Elife 8.

Jaaro-Peled, H., Altimus, C., LeGates, T., Cash-Padgett, T., Zoubovsky, S., Hikida, T., Ishizuka, K., Hattar, S., Mongrain, V., Sawa, A., 2016. Abnormal wake/sleep pattern in a novel gain-of-function model of DISC1. Neurosci Res 112, 63-69.

Johansson, A.S., Owe-Larsson, B., Hetta, J., Lundkvist, G.B., 2016. Altered circadian clock gene expression in patients with schizophrenia. Schizophr Res 174, 17-23.

Karatsoreos, I.N., 2012. Effects of circadian disruption on mental and physical health. Curr Neurol Neurosci Rep 12, 218-225.

Kaskie, R.E., Graziano, B., Ferrarelli, F., 2017. Schizophrenia and sleep disorders: links, risks, and management challenges. Nat Sci Sleep 9, 227-239.

Knuesel, I., Chicha, L., Britschgi, M., Schobel, S.A., Bodmer, M., Hellings, J.A., Toovey, S., Prinssen, E.P., 2014. Maternal immune activation and abnormal brain development across CNS disorders. Nat Rev Neurol 10, 643-660.

Korenic, S.A., Klingaman, E.A., Wickwire, E.M., Gaston, F.E., Chen, H., Wijtenburg, S.A., Rowland, L.M., 2019. Sleep quality is related to brain glutamate and symptom severity in schizophrenia. J Psychiatr Res 120, 14-20.

Laskemoen, J.F., Simonsen, C., Buchmann, C., Barrett, E.A., Bjella, T., Lagerberg, T.V., Vedal, T.J., Andreassen, O.A., Melle, I., Aas, M., 2019. Sleep disturbances in schizophrenia spectrum and bipolar disorders - a transdiagnostic perspective. Compr Psychiatry 91, 6-12.

Lee, B.K., Magnusson, C., Gardner, R.M., Blomstrom, A., Newschaffer, C.J., Burstyn, I., Karlsson, H., Dalman, C., 2015. Maternal hospitalization with infection during pregnancy and risk of autism spectrum disorders. Brain Behav Immun 44, 100-105.

Lee, F.Y., Wang, H.B., Hitchcock, O.N., Loh, D.H., Whittaker, D.S., Kim, Y.S., Aiken, A., Kokikian, C., Dell'Angelica, E.C., Colwell, C.S., Ghiani, C.A., 2018. Sleep/Wake Disruption in a Mouse Model of BLOC-1 Deficiency. Front Neurosci 12, 759.

Maple, A.M., Rowe, R.K., Lifshitz, J., Fernandez, F., Gallitano, A.L., 2018. Influence of Schizophrenia-Associated Gene Egr3 on Sleep Behavior and Circadian Rhythms in Mice. J Biol Rhythms 33, 662-670.

Martin, J.L., Jeste, D.V., Ancoli-Israel, S., 2005. Older schizophrenia patients have more disrupted sleep and circadian rhythms than age-matched comparison subjects. J Psychiatr Res 39, 251-259.

McGrath, J., Saha, S., Chant, D., Welham, J., 2008. Schizophrenia: a concise overview of incidence, prevalence, and mortality. Epidemiol Rev 30, 67-76.

Meyer, N., Faulkner, S.M., McCutcheon, R.A., Pillinger, T., Dijk, D.J., MacCabe, J.H., 2020. Sleep and Circadian Rhythm Disturbance in Remitted Schizophrenia and Bipolar Disorder: A Systematic Review and Meta-analysis. Schizophr Bull.

Meyer, U., Nyffeler, M., Engler, A., Urwyler, A., Schedlowski, M., Knuesel, I., Yee, B.K., Feldon, J., 2006. The time of prenatal immune challenge determines the specificity of inflammation-mediated brain and behavioral pathology. J Neurosci 26, 4752-4762.

Missig, G., McDougle, C.J., Carlezon, W.A., Jr., 2020. Sleep as a translationally-relevant endpoint in studies of autism spectrum disorder (ASD). Neuropsychopharmacology 45, 90-103.

Missig, G., Mokler, E.L., Robbins, J.O., Alexander, A.J., McDougle, C.J., Carlezon, W.A., Jr., 2018. Perinatal Immune Activation Produces Persistent Sleep Alterations and Epileptiform Activity in Male Mice. Neuropsychopharmacology 43, 482-491.

Mueller, F.S., Richetto, J., Hayes, L.N., Zambon, A., Pollak, D.D., Sawa, A., Meyer, U., Weber-Stadlbauer, U., 2019. Influence of poly(I:C) variability on thermoregulation, immune responses and pregnancy outcomes in mouse models of maternal immune activation. Brain Behav Immun 80, 406-418.

Ochoa, S., Usall, J., Cobo, J., Labad, X., Kulkarni, J., 2012. Gender differences in schizophrenia and first-episode psychosis: a comprehensive literature review. Schizophr Res Treatment 2012, 916198.

Oh-Nishi, A., Obayashi, S., Sugihara, I., Minamimoto, T., Suhara, T., 2010. Maternal immune activation by polyriboinosinic-polyribocytidilic acid injection produces synaptic

dysfunction but not neuronal loss in the hippocampus of juvenile rat offspring. Brain Res 1363, 170-179.

Ohta, H., Yamazaki, S., McMahon, D.G., 2005. Constant light desynchronizes mammalian clock neurons. Nat Neurosci 8, 267-269.

Oliver, P.L., Sobczyk, M.V., Maywood, E.S., Edwards, B., Lee, S., Livieratos, A., Oster, H., Butler, R., Godinho, S.I., Wulff, K., Peirson, S.N., Fisher, S.P., Chesham, J.E., Smith, J.W., Hastings, M.H., Davies, K.E., Foster, R.G., 2012. Disrupted circadian rhythms in a mouse model of schizophrenia. Curr Biol 22, 314-319.

Pritchett, D., Jagannath, A., Brown, L.A., Tam, S.K., Hasan, S., Gatti, S., Harrison, P.J., Bannerman, D.M., Foster, R.G., Peirson, S.N., 2015. Deletion of Metabotropic Glutamate Receptors 2 and 3 (mGlu2 & mGlu3) in Mice Disrupts Sleep and Wheel-Running Activity, and Increases the Sensitivity of the Circadian System to Light. PLoS One 10, e0125523.

Seney, M.L., Cahill, K., Enwright, J.F., 3rd, Logan, R.W., Huo, Z., Zong, W., Tseng, G., McClung, C.A., 2019. Diurnal rhythms in gene expression in the prefrontal cortex in schizophrenia. Nat Commun 10, 3355.

Smith, S.E., Li, J., Garbett, K., Mirnics, K., Patterson, P.H., 2007. Maternal immune activation alters fetal brain development through interleukin-6. J Neurosci 27, 10695-10702.

Souders, M.C., Mason, T.B., Valladares, O., Bucan, M., Levy, S.E., Mandell, D.S., Weaver, T.E., Pinto-Martin, J., 2009. Sleep behaviors and sleep quality in children with autism spectrum disorders. Sleep 32, 1566-1578.

Spisska, V., Pacesova, D., Mikova, H., Pohanova, P., Telensky, P., Novotny, J., Bendova, Z., 2020. Prenatal exposure to lipopolysaccharide induces changes in the circadian clock in the SCN and AA-NAT activity in the pineal gland. Brain Res 1743, 146952.

Tan, H.Y., Ang, Y.G., 2001. First-episode psychosis in the military: a comparative study of prodromal symptoms. Aust N Z J Psychiatry 35, 512-519.

Tandon, R., Gaebel, W., Barch, D.M., Bustillo, J., Gur, R.E., Heckers, S., Malaspina, D., Owen, M.J., Schultz, S., Tsuang, M., Van Os, J., Carpenter, W., 2013. Definition and description of schizophrenia in the DSM-5. Schizophr Res 150, 3-10.

Tordjman, S., Anderson, G.M., Bellissant, E., Botbol, M., Charbuy, H., Camus, F., Graignic, R., Kermarrec, S., Fougerou, C., Cohen, D., Touitou, Y., 2012. Day and nighttime excretion of 6-sulphatoxymelatonin in adolescents and young adults with autistic disorder. Psychoneuroendocrinology 37, 1990-1997.

Walker, C.D., Deschamps, S., Proulx, K., Tu, M., Salzman, C., Woodside, B., Lupien, S., Gallo-Payet, N., Richard, D., 2004. Mother to infant or infant to mother? Reciprocal regulation of responsiveness to stress in rodents and the implications for humans. J Psychiatry Neurosci 29, 364-382.

Wiggs, L., Stores, G., 2004. Sleep patterns and sleep disorders in children with autistic spectrum disorders: insights using parent report and actigraphy. Dev Med Child Neurol 46, 372-380.

Wintler, T., Schoch, H., Frank, M.G., Peixoto, L., 2020. Sleep, brain development, and autism spectrum disorders: Insights from animal models. J Neurosci Res 00, 1-13.

Wulff, K., Dijk, D.J., Middleton, B., Foster, R.G., Joyce, E.M., 2012. Sleep and circadian rhythm disruption in schizophrenia. Br J Psychiatry 200, 308-316.

Xuan, I.C., Hampson, D.R., 2014. Gender-dependent effects of maternal immune activation on the behavior of mouse offspring. PLoS One 9, e104433.

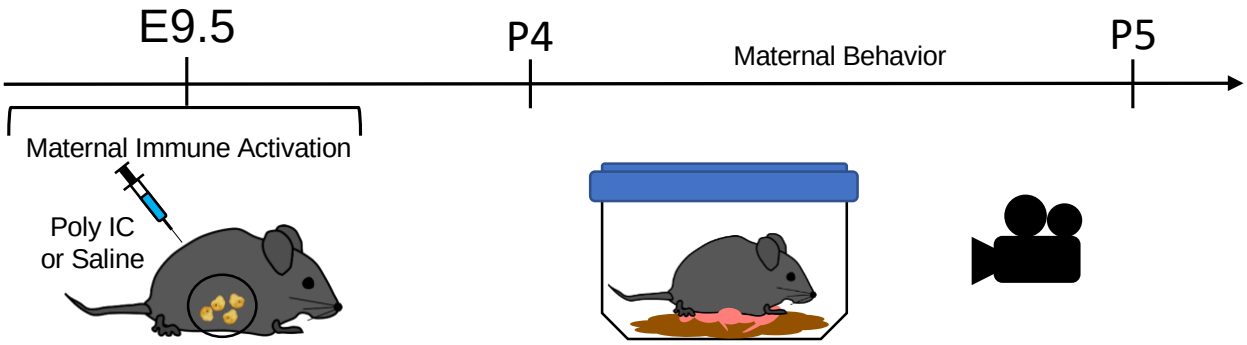
Yin, D.M., Chen, Y.J., Sathyamurthy, A., Xiong, W.C., Mei, L., 2012. Synaptic dysfunction in schizophrenia. Adv Exp Med Biol 970, 493-516

CHAPTER 1: ALTERED CIRCADIAN RHYTHMS IN A MOUSE MODEL OF NEURODEVELOPMENTAL DISORDERS BASED ON PRENATAL MATERNAL IMMUNE ACTIVATION

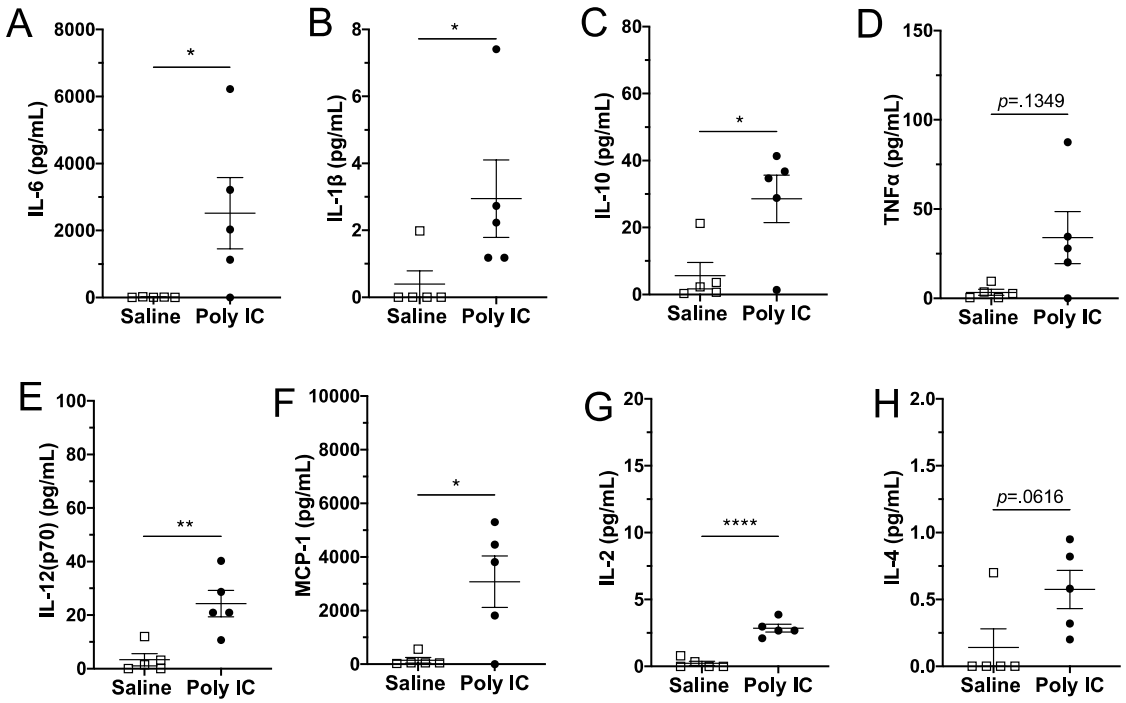
Tara C. Delorme, Lalit K. Srivastava, and Nicolas Cermakian

Supplementary Material

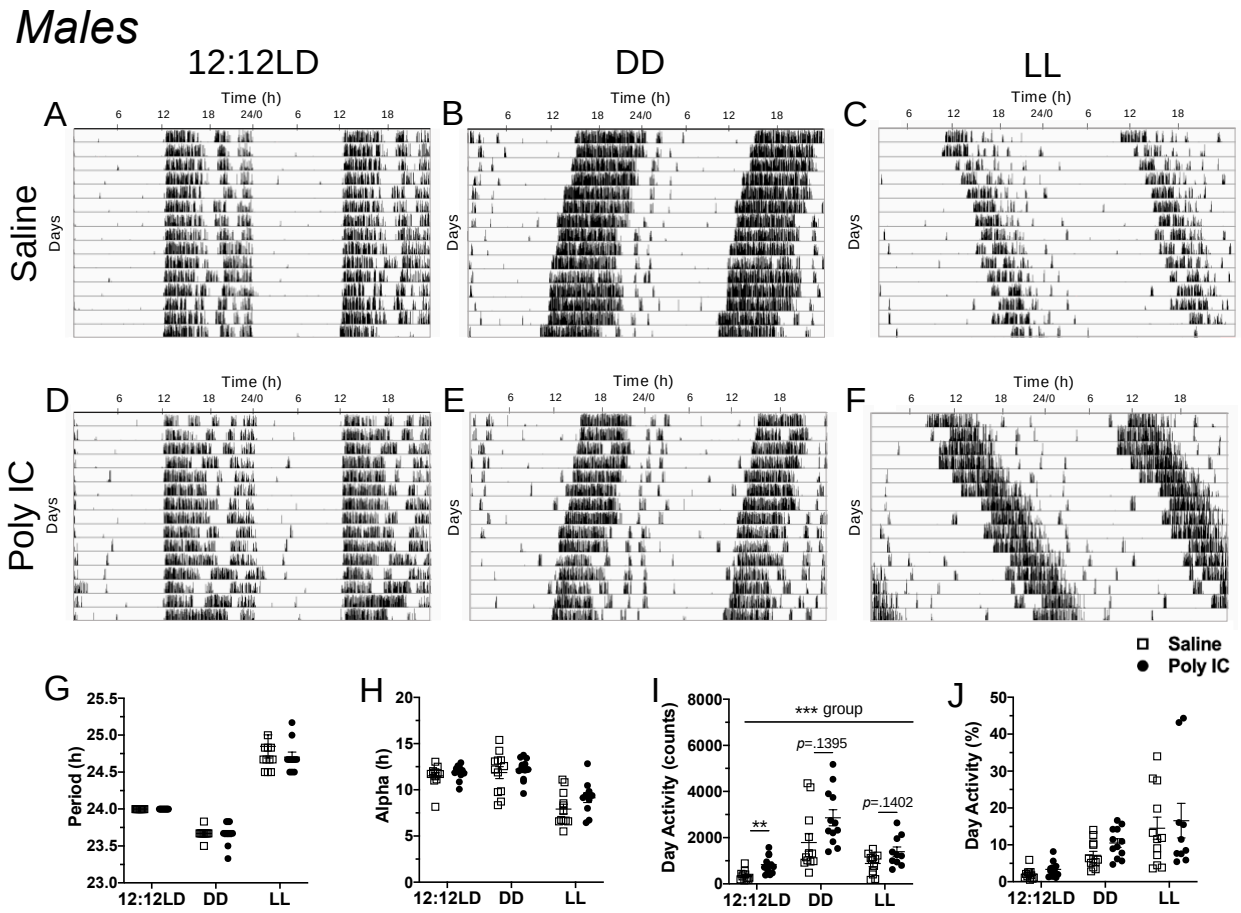
Published: https://doi.org/10.1016/j.bbi.2020.12.030



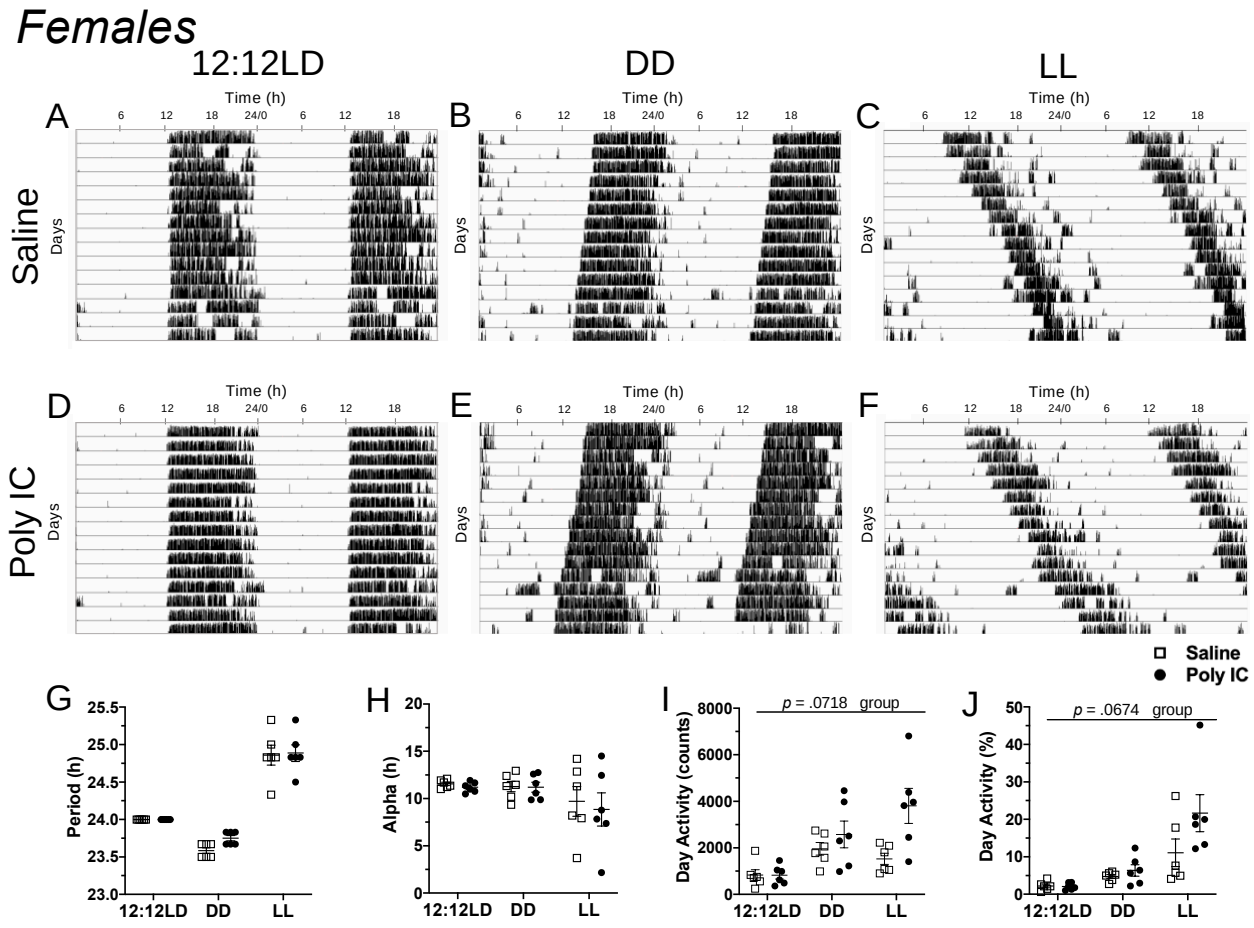
Supplementary Fig. 1.1. Schematic for scoring maternal behavior protocol. On E9.5, pregnant dams were intraperitoneally injected with poly IC (5 mg/kg) or saline. Pregnant dams delivered their litters naturally and maternal behavior was video recorded and scored between postnatal day 4-5.



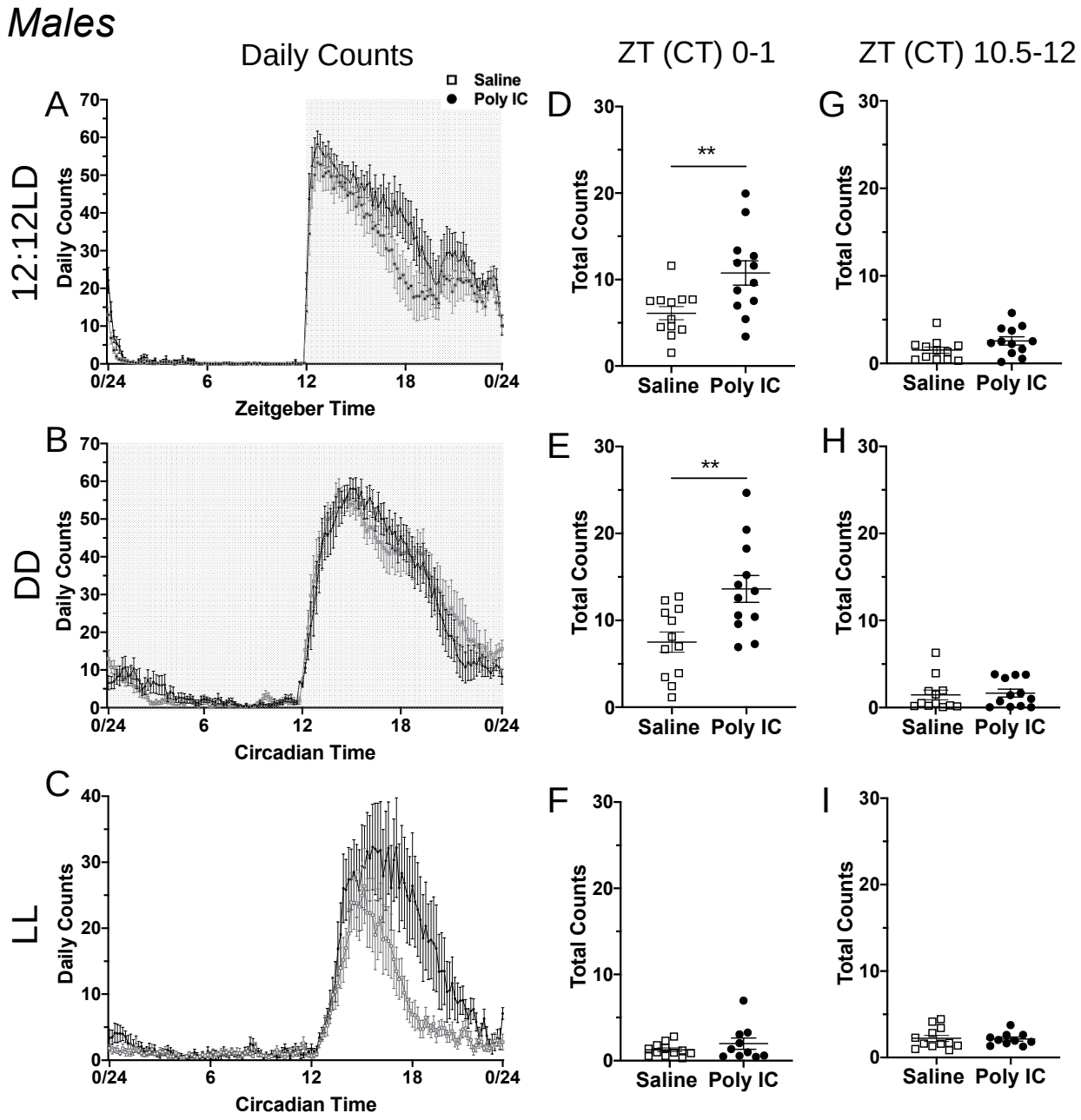
Supplementary Fig. 1.2. Pro- and anti-inflammatory cytokine and chemokine expression levels in plasma of pregnant dams injected with poly IC (lot: 096M4023V) (n=5) or saline (n=5). Expression levels of the following cytokine and chemokines: (A) IL-6, (B) IL-1β, (C) IL-10, (D) TNFα, (E) IL-12(p70), (F) MCP-1, (G) IL-2, (H) IL-4. Samples that were below the detectable range were designated as 0 pg/mL. Individual data points represent independent dams and data were represented as mean ± SEM. An independent samples t-test (with Welsh's correction, or Mann-Whitney U test when appropriate) was used, ****$p<.0001$, **$p<.01$ and *$p<.05$.



Supplementary Fig. 1.3. Circadian locomotor activity variables in male offspring. Representative actograms of (A-C) saline-exposed (n=12 from 6 litters) and (D-F) poly IC-exposed male offspring (n=10-12 from 6 litters) under (A, D) 12:12LD, (B, E) DD and (C, F) LL. Actograms depict circadian locomotor activity, whereby days are vertically stacked one on the other, time (in hours) is shown across the x-axis, and data are double plotted to facilitate visualization. The last 15 days of each condition are shown. Individual data points represent independent mice. Circadian locomotor activity variables were analyzed in the last 10 days of each condition, including (G) period (h), (H) *alpha* (h), (I) day activity (counts) and (J) day activity (%). Values are mean ± SEM. A mixed-effects analysis (factors: lighting, group) was used with Geisser-Greenhouse correction and Sidak's *post-hoc* tests. If no interaction was found, main effects were explored and presented in the graph. ***p <.001.

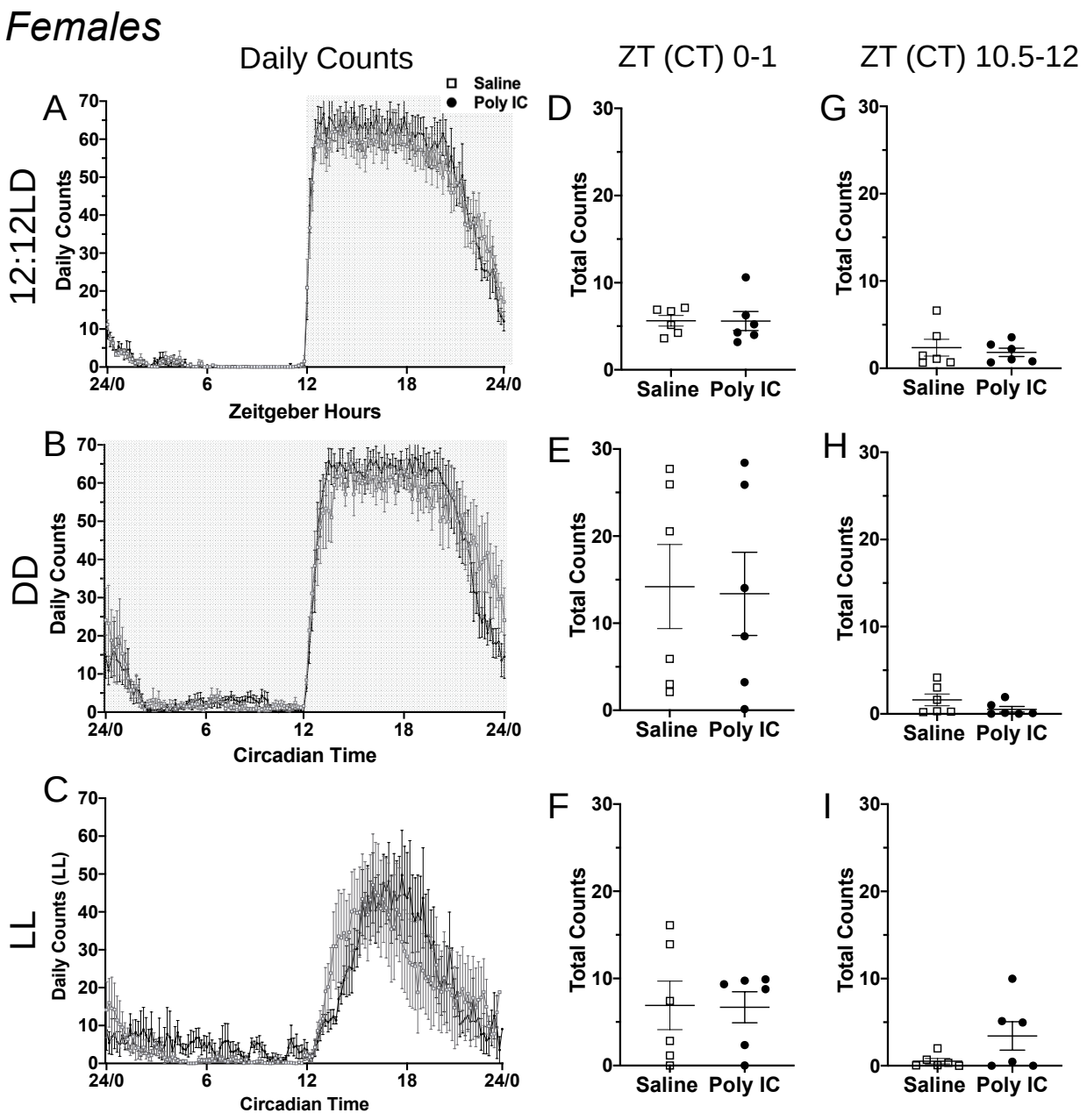


Supplementary Fig. 1.4. Circadian locomotor activity variables in female offspring. Representative actograms of (A-C) saline-exposed (n=6 from 6 litters) and (D-F) poly IC-exposed (n=6 from 6 litters) female offspring under (A, D) 12:12LD, (B, E) DD and (C, F) LL. Actograms depict circadian locomotor activity, whereby days are vertically stacked one on the other, time (in h) is shown across the x-axis, and data are double plotted to facilitate visualization. The last 15 days of each condition are shown. Individual data points represent independent mice. Circadian locomotor activity variables were analyzed in the last 10 days of each condition, including (G) period (h), (H) *alpha*, (I) day activity (counts) and (J) day activity (%). Values are mean ± SEM. A mixed-effects analysis (factors: lighting condition, group) was used with Geisser-Greenhouse correction. No significant group differences were found.



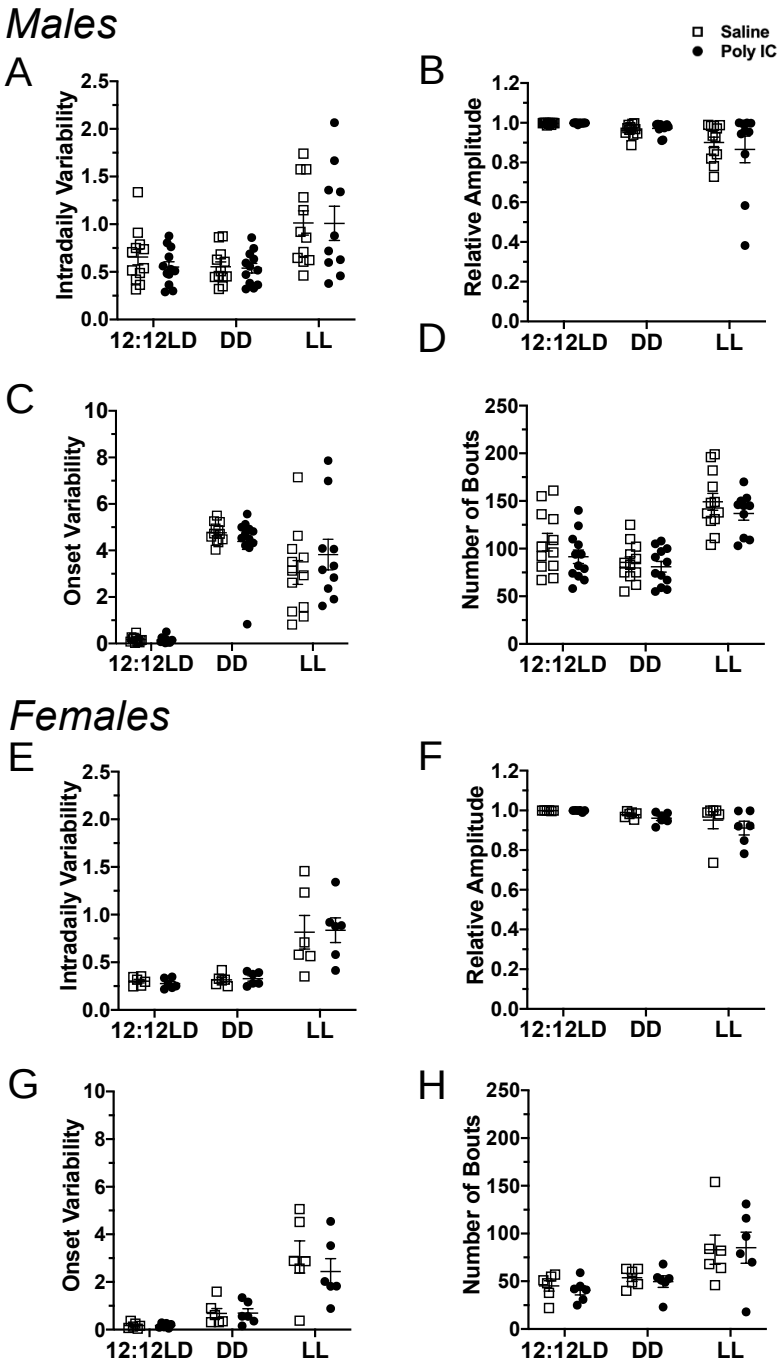
Supplementary Fig. 1.5. Daily activity profiles averaged across all saline-exposed (n=12 from 6 litters) and poly IC-exposed (n=10-12 from 6 litters) male offspring, under (A) 12:12LD, (B) DD and (C) LL. Poly IC-exposed mice are depicted as black circles connected by a black line and saline-exposed mice as white squares connected by a gray line. Analysis of total activity counts between groups at the beginning (ZT (CT) 0-1) and end (ZT (CT) 10.5-12) of the (subjective)

day in (D, G) 12:12LD, (E, H) DD and (F, I) LL respectively. An independent samples t-test (with Welsh's correction, or Mann-Whitney U test when appropriate) was used, $^{**}p < .01$.



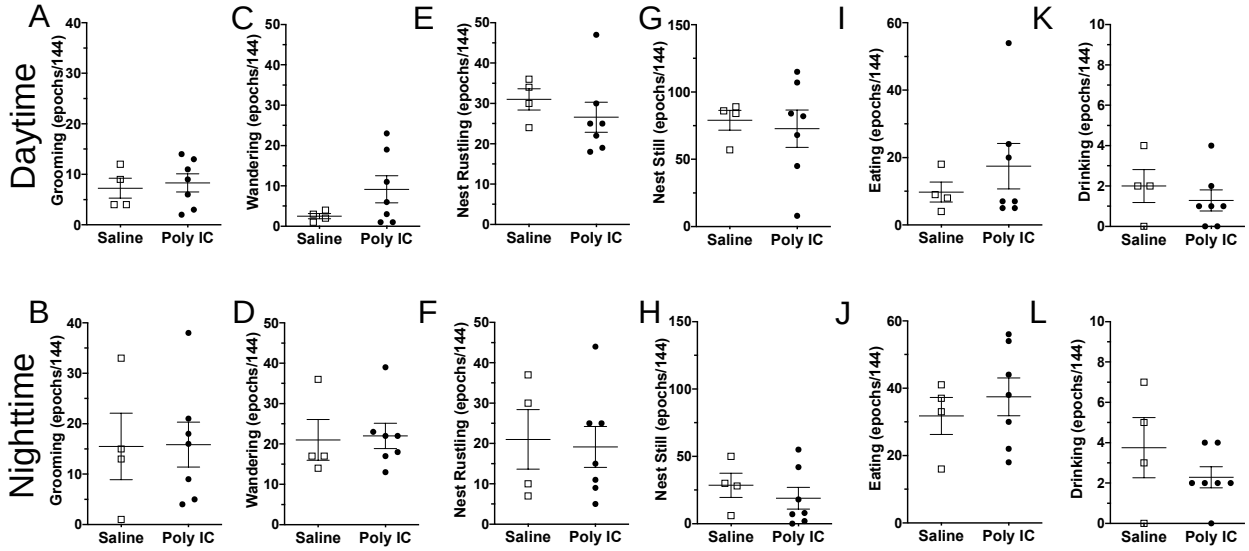
Supplementary Fig. 1.6. Daily activity profiles averaged across all saline-exposed (n=6 from 6 litters) and poly IC-exposed (n=6 from 6 litters) female offspring, under (A) 12:12LD, (B) DD and (C) LL. Poly IC-exposed mice are depicted as black circles connected by a black line and saline-exposed mice as white squares connected by a gray line. Analysis of total activity counts between groups at the beginning (ZT (CT) 0-1) and end (ZT (CT) 10.5-12) of the (subjective) day in (D, G) 12:12LD, (E, H) DD and (F, I) LL respectively. An independent samples t-test

(with Welsh's correction, or Mann-Whitney U test when appropriate) was used. No significant group differences were found.



Supplementary Fig. 1.7. Activity rhythm characteristics and analysis of activity bouts. Analysis was done for (A-D) male offspring (saline-exposed n=12 from 6 litters; poly IC-exposed n=10-12 from 6 litters) and (E-H) female offspring (saline-exposed n=6 from 6 litters; poly IC-exposed n=6 from 6 litters) separately for the following measures: (A, E) intradaily variability, (B, F)

relative amplitude, (C, G) onset variability and (D, H) number of bouts. Values are mean ± SEM. A mixed-effects analysis (factors: lighting condition, group) was used with Geisser-Greenhouse correction. No significant group differences were found.



Supplementary Fig. 1.8. Maternal behavior of saline and poly IC-injected dams. Dams were observed between postnatal day 4 to 5 during both the (A, C, E, G, I, K) daytime and the (B, D, F, H, J, L) nighttime and scored for maternal behavior. Dams were scored on (A-B) grooming behavior, (C-D) wandering, (E-F) nest rustling, (G-H) nest still, (I-J) eating and (K-L) drinking. Individual data points represent independent dams and data were represented as mean ± SEM. An independent samples t-test (with Welsh's correction, or Mann-Whitney U test when appropriate) was used. No significant group differences were found.

Supplementary Table 1.1. Summary of wheel running parameters from poly IC and saline-exposed male offspring from the second cohort.

	12:12LD		DD		LL	
	Saline (n=12)	Poly IC (n=12)	Saline (n=12)	Poly IC (n=12)	Saline (n=12)	Poly IC (n=10)
Period (h)	24.0 ± 0.00	24.0 ± 0.00	23.7 ± 0.02	23.7 ± 0.04	24.8 ± 0.16	24.7 ± 0.07
Alpha (h)	11.5 ± 0.35	11.9 ± 0.23	11.9 ± 0.64	12.2 ± 0.35	7.9 ± 0.52	9.1 ± 0.53
(Subjective) Day Activity (counts)	350 ± 61	827 ± 110	1789 ± 375	2857 ± 349	876 ± 129	1392 ± 204
(Subjective) Night Activity (counts)	19826 ± 2695	26313 ± 2434	22527 ± 2097	25431 ± 2314	6695 ± 1164	11019 ± 2244
Total Activity (counts)	20175 ± 2725	27140 ± 2414	24316 ± 2271	28288 ± 2411	7571 ± 1207	12411 ± 2295
(Subjective) Day Activity (%)	2.07 ± 0.41	3.37 ± 0.58	7.19 ± 1.07	10.50 ± 1.17	14.53 ± 3.00	16.56 ± 4.68
Intradaily Variability	0.656 ± 0.082	0.551 ± 0.055	0.553 ± 0.053	0.539 ± 0.050	1.013 ± 0.127	1.009 ± 0.179
Relative Amplitude	0.997 ± 0.001	0.998 ± 0.001	0.963 ± 0.009	0.972 ± 0.008	0.901 ± 0.026	0.866 ± 0.067
Interdaily Stability	0.816 ± 0.032	0.867 ± 0.023	N/A	N/A	N/A	N/A
Onset Variability	0.17 ± 0.04	0.15 ± 0.04	4.76 ± 0.12	4.39 ± 0.35	3.06 ± 0.51	3.72 ± 0.60
Number of Bouts	106.8 ± 9.28	91.4 ± 7.08	85.5 ± 5.80	80.9 ± 5.58	149.1 ± 8.92	136.8 ± 6.94
Average Bout Length (minutes)	51.5 ± 7.60	70.7 ± 6.77	60.9 ± 6.37	68.3 ± 7.84	19.3 ± 2.04	28.6 ± 4.42
Average Counts per Bout	2359 ± 459	3481 ± 517	3078 ± 401	3585 ± 545	711 ± 116	1329 ± 270
Average Peak Rate	65.44 ± 3.06	72.15 ± 2.30	70.53 ± 2.78	71.94 ± 2.19	59.10 ± 3.13	69.92 ± 4.24

Notes: Values represent mean ± SEM. N/A denotes parameters that could not be assessed in constant conditions. 12:12LD, 12 h of light, 12 h of dark; DD, constant dark; LL, constant light.

Supplementary Table 1.2. Summary of wheel running parameters from poly IC and saline-exposed female offspring from the second cohort.

	12:12LD		DD		LL	
	Saline (n=6)	Poly IC (n=6)	Saline (n=6)	Poly IC (n=6)	Saline (n=6)	Poly IC (n=6)
Period (h)	24.0 ± 0.00	24.0 ± 0.00	23.6 ± 0.04	23.8 ± 0.04	24.9 ± 0.13	24.9 ± 0.11
Alpha (h)	11.6 ± 0.17	11.2 ± 0.22	11.3 ± 0.55	10.9 ± 0.55	9.7 ± 1.60	9.1 ± 2.12
(Subjective) Day Activity (counts)	829 ± 226	826 ± 168	1957 ± 270	2573 ± 576	1526 ± 237	3805 ± 752
(Subjective) Night Activity (counts)	36594 ± 2359	38308 ± 2531	39126 ± 3188	39003 ± 2818	19326 ± 5668	17773 ± 3864
Total Activity (counts)	37423 ± 2451	39134 ± 2630	41083 ± 3367	41576 ± 2796	20852 ± 5734	21577 ± 4481
(Subjective) Day Activity (%)	2.19 ± 0.5	2.08 ± 0.4	4.74 ± 0.5	6.37 ± 1.5	11.08 ± 3.7	21.65 ± 4.9
Intradaily Variability	0.302 ± 0.018	0.277 ± 0.022	0.315 ± 0.024	0.329 ± 0.028	0.815 ± 0.176	0.836 ± 0.130
Relative Amplitude	0.999 ± 0.001	0.998 ± 0.002	0.979 ± 0.007	0.961 ± 0.012	0.950 ± 0.043	0.911 ± 0.035
Interdaily Stability	0.876 ± 0.030	0.928 ± 0.010	N/A	N/A	N/A	N/A

Onset Variability	0.19 ± 0.06	0.15 ± 0.04	0.68 ± 0.21	0.69 ± 0.19	3.05 ± 0.68	2.56 ± 0.65
Number of Bouts	46.3 ± 3.29	43.0 ± 4.95	53.7 ± 3.95	49.7 ± 6.00	83.0 ± 15.27	85.2 ± 16.30
Average Bout Length (minutes)	109.7 ± 14.68	117.7 ± 13.35	98.1 ± 9.18	107.97 ± 18.90	47.4 ± 16.71	42.51 ± 5.48
Average Counts per Bout	6425 ± 746	6992 ± 875	5912 ± 684	6745 ± 1328	2783 ± 1184	2317 ±322
Average Peak Rate	72.87 ± 3.32	62.49 ± 4.88	66.04 ± 3.23	67.47 ± 4.52	65.45 ± 4.03	80.29 ± 5.47

Notes: Values represent mean ± SEM. N/A denotes parameters that could not be assessed in constant conditions. 12:12LD, 12 h of light, 12 h of dark; DD, constant dark; LL, constant light.

Supplementary Table 1.3. Summary of three-way ANOVAs to explore sex differences in circadian locomotor variables.

	Cohort 1				Cohort 2			
	Group x Sex Interaction		Main Effect of Sex		Group x Sex Interaction		Main Effect of Sex	
	F	*p*-value	F	*p*-value	F	*p*-value	F	*p*-value
Period (h)	0.21	0.6540	58.38	0.0001	1.16	0.2900	0.35	0.5600
Alpha (h)	0.61	0.4400	18.22	0.0002	1.40	0.2458	0.07	0.7891
(Subjective) Day Activity (counts)	4.45	0.0430	4.58	0.0403	0.41	0.5279	7.0	0.0125
(Subjective) Night Activity (counts)	1.45	0.2385	24.71	0.0001	1.18	0.2847	35.46	0.0001
Total Activity (counts)	2.04	0.1636	16.46	0.0003	0.97	0.3327	35.50	0.0001
(Subjective) Day Activity (%)	2.47	0.1196	15.45	0.0002	0.35	0.5592	0.43	0.5172
Relative Amplitude	1.39	0.2469	0.48	0.4917	0.10	0.7576	0.90	0.3488
Intradaily Variability	0.09	0.7636	16.40	0.0003	0.09	0.7632	9.44	0.0043
Onset Variability	0.04	0.8365	2.36	0.1277	0.51	0.4765	44.69	0.0001
Number of Bouts	5.20	0.0297	0.77	0.3861	0.46	0.4997	56.16	0.0001
Average Bout Length (minutes)	3.59	0.0674	7.55	0.0099	0.22	0.6427	22.13	0.0001
Average Counts per Bout	6.91	0.0132	11.50	0.0019	0.18	0.6775	25.37	0.0001
Average Peak Rate	0.04	0.8365	2.36	0.1277	0.84	0.3654	0.08	0.7726

Notes. Results from three-way ANOVAs for circadian locomotor variables, highlighting the group x sex interactions and main effects of sex analysis. ANOVA factors include sex (male, female), group (poly IC, saline exposure) and lighting condition (12 h of light, 12 h of dark (12:12LD), constant dark (DD) and constant light (LL)).

Supplementary Table 1.4. Summary of two-way ANOVAs to explore sex differences in data collected from daily profiles.

	Cohort 1				Cohort 2			
	Group x Sex Interaction		Main Effect of Sex		Group x Sex Interaction		Main Effect of Sex	
	F	*p*-value	F	*p*-value	F	*p*-value	F	*p*-value
Beginning of Day Activity (12:12LD)	0.96	0.3355	15.77	0.0004	3.66	0.0646	5.33	0.0276
Beginning of Subjective Day Activity (DD)	2.98	0.0942	1.46	0.2360	1.65	0.2089	1.42	0.2419
Beginning of Subjective Day Activity (LL)	0.91	0.3470	0.03	0.8588	0.13	0.7133	16.88	0.0003
End of Day Activity (12:12LD)	12.47	0.0014	23.60	0.0001	1.98	0.1693	0.01	0.9314
End of Subjective Day Activity (DD)	0.07	0.7930	0.03	0.8644	1.23	0.2767	0.72	0.4071
End of Subjective Day (LL)	0.14	0.7073	7.06	0.0127	4.91	0.0345	0.09	0.7623

Notes. Results from two-way ANOVA for data collected from daily activity profiles, highlighting the group x sex interactions and main effects of sex analysis. ANOVA factors include sex (male, female), group (poly IC, saline exposure). 12:12LD, 12 h of light, 12 h of dark; DD, constant dark; LL, constant light.

CHAPTER 2: BEHAVIORAL AND CELLULAR RESPONSES TO CIRCADIAN DISRUPTION AND PRENATAL IMMUNE ACTIVATION IN MICE

Preface

In Chapter 1, we first validated the lots of poly IC used and confirmed that they significantly elevated blood plasma levels of several pro- and anti-inflammatory cytokine and chemokines. Due to practical constraints, two lots of poly IC were used, and interestingly, this seemed to reveal an effect where the level of circadian impairments varied depending on the prenatal poly IC dose. We then wanted to see if we could recapitulate the circadian disruptions observed in individuals with NDDs. We induced MIA in pregnant dams and gave the adult male and female offspring access to running wheels. We subjected mice to 3 different lighting condition: standard lighting, constant dark and constant light. We found that MIA in males led to altered patterns of circadian locomotion, including increased (subjective) day activity, while females exhibited a milder phenotype. Given that schizophrenia and ASD are considered multifactorial in nature and the prevalence of light at night in modern society, we next sought to explore the effects of exposure to a second risk factor, environmental circadian disruption during adulthood.

CHAPTER 2: BEHAVIORAL AND CELLULAR RESPONSES TO CIRCADIAN DISRUPTION AND PRENATAL IMMUNE ACTIVATION IN MICE

Tara C. Delorme, BSc[1,2], William Ozell-Landry, BSc[1], Nicolas Cermakian, PhD[1,3,*] and Lalit K. Srivastava, PhD[1,3,*]

[1] Douglas Mental Health University Institute, Montréal, Québec, Canada, H4H 1R3.

[2] Integrated Program in Neuroscience, McGill University, Montréal, Québec, Canada, H3A 2B4.

[3] Department of Psychiatry, McGill University, Montréal, Québec, Canada, H3A 1A1.

*Co-corresponding authors

Published: https://doi.org/10.1038/s41598-023-34363-w

2.0. Abstract

Most individuals with neurodevelopmental disorders (NDDs), including schizophrenia and autism spectrum disorders, experience disruptions in sleep and circadian rhythms. Epidemiological studies indicate that exposure to prenatal infection increases the risk of developing NDDs. We studied how environmental circadian disruption contributes to NDDs using maternal immune activation (MIA) in mice, which models prenatal infection. Pregnant dams were injected with viral mimetic poly IC (or saline) at E9.5. Adult poly IC- and saline-exposed offspring were subjected to 4 weeks of each of the following: standard lighting (LD1), constant light (LL) and standard lighting again (LD2). Behavioral tests were conducted in the last 12 days of each condition. Poly IC exposure led to significant behavioral differences, including reduced sociability (males only) and deficits in prepulse inhibition. Interestingly, poly IC exposure led to reduced sociability specifically when males were tested after LL exposure. Mice were exposed again to either LD or LL for 4 weeks and microglia were characterized. Notably, poly IC exposure led to increased microglial morphology index and density in dentate gyrus; an effect attenuated by LL exposure. Our findings highlight interactions between circadian disruption and prenatal infection, which has implications in informing the development of circadian-based therapies for individuals with NDDs.

2.1. Introduction

Neurodevelopmental disorders (NDDs), such as schizophrenia (SCZ) and autism spectrum disorders (ASD), are multifactorial in nature, whereby multiple interacting risk factors are required to trigger disease onset and contribute to symptomatology (Cheroni, Caporale et al. 2020, Schmitt, Falkai et al. 2022). These risk factors are thought to pathologically disrupt normal brain

development (Fatemi and Folsom 2009). Studying how multiple risk factors for NDDs interact with each other, rather than studying single risk factors, is necessary and becoming a recurrent theme in research (Davis, Eyre et al. 2016). One such risk factor is prenatal infection, which has been strongly associated with an offspring's risk of developing NDDs (Kwon, Choi et al. 2022, Massrali, Adhya et al. 2022). Exposure to prenatal infection in the first trimester of pregnancy has been associated with an elevated risk of offspring to develop SCZ (Brown, Begg et al. 2004) and ASD (Lee, Magnusson et al. 2015).

Prenatal infection is believed to act as a primer for disease, and in combination with other risk factors may result in the full display of symptoms (Rapoport, Giedd et al. 2012). Prenatal infection can be modelled in animals using a maternal immune activation (MIA) protocol (Haddad, Patel et al. 2020), where an infection is simulated in pregnant dams using viral mimetic polyinosinic:polycytidylic acid (poly IC). MIA results in behavioral deficits related to NDDs (Murray, Davies et al. 2017) (e.g., reduced sociability (Meyer 2014)), and cellular dysfunction (e.g., altered microglia properties (Hui, St-Pierre et al. 2018)). Microglia are immune surveillant cells that colonize the fetal brain (Mendes and Majewska 2021) and remain in the central nervous system (CNS) until adulthood. Microglia contribute to sculpting neural circuits by pruning synapses and remodeling circuits during normal brain development and disease (Salter and Stevens 2017, Madore, Yin et al. 2020). Given that the alterations in microglia following MIA persist until adulthood (Hui, St-Pierre et al. 2018), they are expected to play key roles in NDDs (Mondelli, Vernon et al. 2017).

A less commonly discussed perturbation in the context of NDDs is the disruption of circadian rhythms. Circadian rhythms are daily (~24 hour) cycles in behavior (e.g., sleep, mood) and physiology (e.g., certain hormones and genes). These rhythms are generated through clock

mechanisms present in most mammalian cells (Patke, Young et al. 2020). To maintain synchrony with our environment, these endogenously generated rhythms need to be 'reset' daily by cyclic cues. The strongest rhythmic environmental cue is light exposure (Hastings, Maywood et al. 2018). Disruption to the circadian timing system, most commonly through the inappropriate exposure to light, can be detrimental to our mental and physical health, especially if experienced chronically (Karatsoreos, Bhagat et al. 2011, Walker, Walton et al. 2020). For example, most shift workers regularly experience circadian disruption (Boivin, Boudreau et al. 2022), and are more at risk for cardiovascular disease (Puttonen, Harma et al. 2010), cancer (Costa, Haus et al. 2010), sleep disorders (Drake, Roehrs et al. 2004) and negative mental health outcomes (Brown, Martin et al. 2020).

Not only do about 80% of individuals with SCZ and ASD exhibit various disruptions in sleep, rest/activity rhythms, daily hormone rhythms and circadian clock gene expression (Krakowiak, Goodlin-Jones et al. 2008, Wulff, Dijk et al. 2012, Johansson, Owe-Larsson et al. 2016), but sleep is also inversely correlated with the severity of psychosis (Tandon, Shipley et al. 1992). Circadian disturbances are similarly reported in animal models based on genetic risk factors for SCZ (Delorme, Srivastava et al. 2020) and ASD (Wintler, Schoch et al. 2020), and using an MIA protocol in mice (Delorme, Srivastava et al. 2021). In many individuals with NDDs, circadian disturbances precede the onset of psychosis (Tandon, Shipley et al. 1992, Boivin 2000), which supports a role for circadian disruption as a risk factor for NDDs. This hypothesis is reinforced in a genetic mouse model for SCZ, where SCZ-related behaviors worsened after altered light exposure (Bhardwaj, Stojkovic et al. 2015, Cloutier, Srivastava et al. 2022). If circadian disruption is a risk factor for NDDs, individuals with NDDs may be vulnerable to the effects of environmental circadian disruption (e.g., shift work), in ways that affects the expression of symptoms

experienced. This is especially problematic given that approximately 28% of the Canadian labor force and 40% of the American labor force work mostly during non-standard times (Presser 2003, Williams 2008).

In this study, we aimed to explore if circadian disruption caused by inappropriate light exposure is a risk factor for NDDs and if MIA (first risk factor in-utero) combined with exposure to circadian disruption (second risk factor in adulthood) would synergistically induce behavioral and microglial deficits in offspring. To explore this, we used an MIA protocol in mice and characterized behavior before and after the mice were exposed to constant light (LL) in adulthood and then characterized microglia after re-exposure to LL or standard lighting. Sex differences are observed in the prevalence, age of onset, and severity and profile of symptoms of individuals with SCZ and ASD (Abel, Drake et al. 2010, Ferri, Abel et al. 2018). Specifically, SCZ is approximately 1.4 times more prevalent in males (McGrath, Saha et al. 2004), and ASD is 3-4 times more likely to be diagnosed in males than females (Demily, Poisson et al. 2017, Loomes, Hull et al. 2017). Sex differences are also apparent in rodent models of MIA, where males tend to exhibit stronger behavioral and cellular phenotypes than females (Haida, Al Sagheer et al. 2019) and MIA has been shown to lead to sex-specific changes in microglial gene expression (Mattei, Ivanov et al. 2017). For these reasons, we have incorporated female mice in our experiments when feasible.

2.2. Materials and Methods

For detailed materials and methods, see the Supplementary Information.

2.2.1. Animals

Animal use was in accordance with the guidelines of the Canadian Council of Animal Care and was approved by the McGill University Animal Care Committee. Authors complied with the ARRIVE guidelines. Further details are in Supplementary Methods.

2.2.2. Maternal immune activation (MIA) protocol

MIA was performed as previously described (Delorme, Srivastava et al. 2021). On embryonic day 9.5, pregnant dams were intraperitoneally injected with poly IC dissolved in double-distilled water based on body weight (5 mg/kg; lot 1: 086M4045V; Sigma-Aldrich, St. Louis, MO, USA) or sterile saline solution. Experiments were replicated using a second lot of poly IC (lot 2: 096M4023V). We have previously shown that these lots of poly IC induced an immune response in pregnant dams (Delorme, Srivastava et al. 2021). Litters of poly IC- and saline-exposed dams did not differ in number of pups (**Supplementary Fig. 2.1A-B**). Further details are in Supplementary Methods.

2.2.3. Experimental timeline

The experimental protocol is depicted in **Fig. 2.1.** Baseline behavior was assessed by subjecting mice to behavioral testing in the last 12 days of a 4-week exposure to standard lighting (LD1). Mice were tested once again, but this time in the last 12 days of a 4-week exposure to LL, a lighting condition known to disrupt circadian rhythms. Finally, to 'rescue' LL-induced deficits, we tested mice a third time in the last 12 days of a 4-week exposure to standard lighting again (LD2). After the conclusion of behavioral testing, male mice were placed back into either regular lighting (LD) or LL for 4 more weeks before their brains were harvested for

immunohistochemistry. At this stage, due to space constraints, female poly IC- and saline-exposed mice were euthanized and not used for subsequent experiments. Further details are in Supplementary Methods.

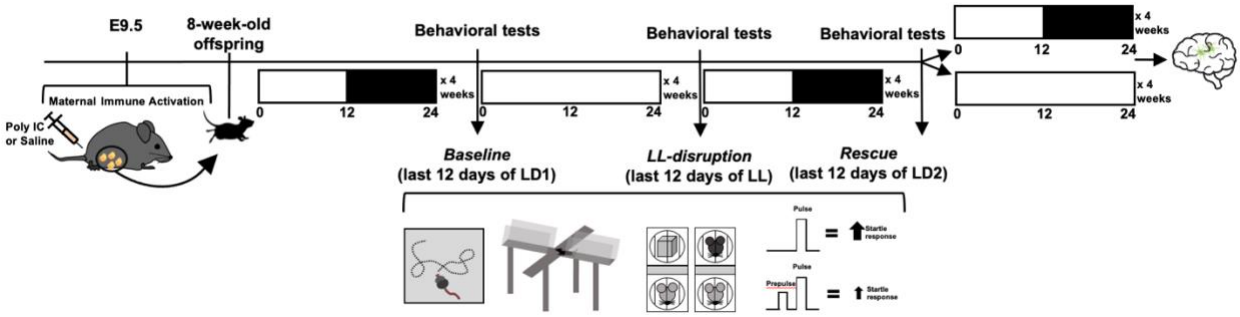


Fig. 2.1. Experimental timeline. On E9.5 pregnant dams were intraperitoneally injected with poly IC or saline. Poly IC- and saline-exposed offspring were aged to adulthood and were successively subjected to 4 weeks of each of the following conditions: standard lighting (LD1), constant light (LL) and standard lighting again (LD2). Behavioral tests were conducted in the last 12 days of each condition. Mice underwent the open field test, the elevated plus maze, the three-chamber social interaction test and prepulse inhibition of acoustic startle. After the last behavioral test, poly IC- and saline-exposed males were placed back into standard lighting (LD) or LL for 4 weeks before their brains were harvested for immunohistochemistry.

2.2.4. Behavioral outcomes

Detailed information for each behavioral test is in Supplementary Methods.

2.2.4.1. *Open field test*

The open field test was used to assess spontaneous locomotor activity and anxiety-like behavior (Seibenhener and Wooten 2015). Measures such as horizontal activity (number of horizontal beam breaks), total distance traveled and thigmotaxis (time spent in the outer edges of the apparatus divided by time spent in the center) were analyzed (Valle 1970).

2.2.4.2. *Elevated plus maze (EPM) test*

Anxiety-like behavior was assessed using the elevated plus maze (EPM) (Walf and Frye 2007). The apparatus consisted of a plus-shaped maze. Two opposing arms (closed arms) were enclosed by 10 cm high walls, while the other two opposing arms (open arms) did not have walls. See EPM formula below.

$$\textbf{EPM formula} = \frac{\text{Time spent in open arms (seconds)}}{\text{Time spent in open arms (seconds)} + \text{Time spent in closed arms (seconds)}} \times 100$$

2.2.4.3. *Three-chamber social interaction test*

Social preference and social memory were assessed using the three chamber social interaction test (Kaidanovich-Beilin, Lipina et al. 2011). In the social preference phase, a mouse that our experimental mouse had never interacted with before, called stranger 1, was placed under one of the wire containers, and an object under the other wire container. See social preference formula below.

$$\textbf{Social preference formula} = \frac{\text{Time spent in Stranger 1 zone}}{\text{Time spent in Stranger 1 zone+Time spent in Object zone}}$$

In the social memory phase, the object was replaced by a novel mouse, again being a mouse that the experimental mouse had never interacted with before, called stranger 2. See social memory formula below.

$$\textbf{Social memory formula} = \frac{\text{Time spent in Stranger 2 zone}}{\text{Time spent in Stranger 2 zone+Time spent in Stranger 1 zone}}$$

2.2.4.4. *Prepulse inhibition of acoustic startle (PPI)*

PPI is a measure of sensory-motor gating (Geyer and Swerdlow 2001). As described previously, mice were placed into a cylindrical Plexiglass enclosure, mounted on a Plexiglass base. Each session consisted of 50 trials, some of which only had a startle tone, and some trials had a prepulse played before the startle tone. A piezoelectric accelerometer fixed to the Plexiglass base was used to detect and transduce motion resulting from the animal's startle response. Percent PPI was calculated as follows.

$$\textbf{\% prepulse inhibition} = 100 - \left(\frac{\text{Startle amplitude on prepulse trials}}{\text{Startle amplitude on pulse alone trials}} \right) \text{x } 100$$

2.2.5. Microglial characterization

2.2.5.1. Microglia visualization and analysis

After the last behavioral test, poly IC- and saline-exposed male offspring were placed back into LD or LL for 4 weeks before their brains were harvested for immunohistochemistry. Further details are in Supplementary Methods.

Microglia from the dorsal hippocampus (both the dentate gyrus and CA1) and medial PFC were imaged using Z-stacks at a 20X magnification. Each stack contained ~30 slices (1 μm each). The images were analyzed using Fiji ImageJ software, while being blinded to the experimental conditions. Microglia density and morphology were characterized essentially as described by us previously (Hui, St-Pierre et al. 2018). A more reactive microglial profile would include a higher morphological index, a less circular cell body, greater density, and reduced spacing index. Further details are in Supplementary Methods.

2.2.6. Statistics

Data were analyzed and graphed using Prism version 9 (GraphPad). Differences were considered significant if $p < 0.05$. Details on the statistical analyses are available in the Supplementary Methods.

2.3. Results

2.3.1. Comparison and validation of poly IC lots

The behavioral data were collected from two different cohorts of mice, each treated with a different lot of poly IC. The results from the first cohort (poly IC lot 1) are presented in the main manuscript, and the results from the second cohort (poly IC lot 2) are presented in the Supplementary Information. We previously confirmed that both lots of poly IC used in the study induced significant inflammatory responses in pregnant dams (Delorme, Srivastava et al. 2021), although cytokine and chemokine levels induced by poly IC lot 1 were ~2 times higher compared

to poly IC lot 2. Additionally, using poly IC lot 1 but not lot 2, there was a significant treatment x age interaction, where poly IC exposure led to decreased weight in adulthood for both males and females (males: $F_{(1, 84)} = 7.946$, $p = 0.0060$; *post hoc* with adulthood poly IC versus saline, $p = 0.0122$; females: $F_{(1, 28)} = 7.385$, $p = 0.0112$; *post hoc* with adulthood poly IC versus saline, $p = 0.0440$) (**Supplementary Fig. 2.1C-F**).

2.3.2. Behavior

2.3.2.1. Hyperactive phenotype following prenatal poly IC exposure in males.

Spontaneous locomotion was assessed using the open field test. Using poly IC lot 1, poly IC-exposed males showed a hyperactive phenotype compared to saline-exposed males. This was seen in an increased amount of horizontal activity (main effect of treatment, $F_{(1, 42)} = 6.233$, $p = 0.0165$; *post hoc* saline versus poly IC under LD1, $p = 0.0456$) (**Fig. 2.2A**) and increased total distance traveled (main effect of treatment, $F_{(1, 42)} = 7.398$, $p = 0.0095$) (**Fig. 2.2C**). The complete statistical parameters from the behavioral studies are listed in **Supplementary Tables 2.1-2.4**. No significant treatment x lighting interactions were observed, and no significant differences were seen in thigmotaxis ratio (**Fig. 2.2B**). Interestingly, no significant differences were observed in locomotion using poly IC lot 2 in males (**Supplementary Fig. 2.2A-C**), and no differences were observed in females using either lot (**Fig. 2.2D-F, Supplementary Fig. 2.2D-F**). Taken together, no significant interactions were observed in spontaneous locomotion, but poly IC exposure induced a hyperlocomotive phenotype in males while using poly IC lot 1.

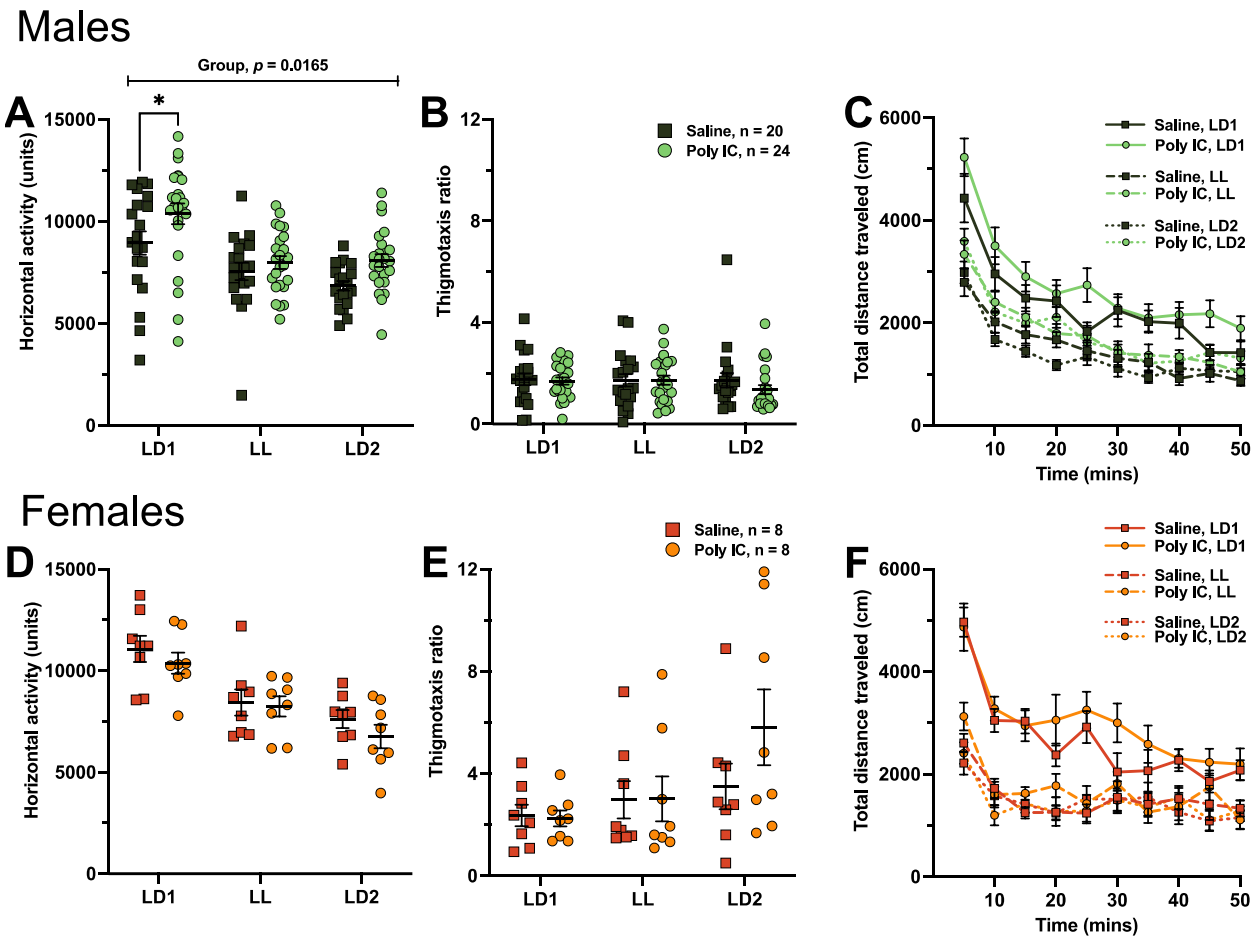


Fig. 2.2. Hyperactive phenotype in poly IC-exposed males. Spontaneous locomotor activity was measured in the open field test. Horizontal activity **(A, D)**, thigmotaxis **(B, E)** and total distance traveled (**C, F**) were assessed in males (**A-C**) and females (**D-F**). For panels **A, B, D and E** data points represent individual mice and are presented as mean ± SEM. Two-way ANOVAs (factors treatment x lighting with Tukey's post-hoc comparisons) were conducted. For panels **C** and **F,** group averages ± SEM are shown over each 10-minute bin of the test. Three-way ANOVAs (factors treatment x lighting x time) were conducted. See **Supplementary Table 2.1 and 2.2** for full statistics. *$p < 0.05$ (post hoc).

2.3.2.2. Limited effects of prenatal poly IC exposure on anxiety-like behavior

Anxiety-like behavior was measured using the EPM test. In males, no significant differences were observed in the time spent in the closed arms, and percent time spent and percent entries in open arms with either lot of poly IC (**Fig. 2.3A-C, Supplementary Fig. 2.3A-C**). Thus, neither poly IC exposure, LL exposure, or their interaction had a significant effect in anxiety-like behavior in males. This is consistent with the lack of differences in thigmotaxis in the open field test (**Fig. 2.2B**, **Supplementary Fig. 2.2B).**

In females, although poly IC-exposed mice showed no significant treatment x lighting interactions in the tested parameters, they exhibited a significant decrease in time spent in closed arms using lot 1 but not lot 2 (main effect of treatment; $F_{(1, 13)} = 12.47$, $p = 0.0036$) (**Fig. 2.3D, Supplementary Fig. 2.3D**). However, there was no difference in percent time spent and percent entries in open arms while using either lot (**Fig. 2.3E-F, Supplementary Fig. 2.3E-F).** Thus, some evidence supports that poly IC exposure in females led to less anxiety-like behavior, but this is not supported by percent time spent or percent entries in open arms, nor thigmotaxis, and was not replicated using poly IC lot 2.

2.3.2.3. Reduced sociability after LL exposure in poly IC-exposed males

Social interaction was measured using the three-chamber social interaction test. Due to practical reasons and time restraints, only a subset of males (and no females) was used in this test compared to the other behavioral tests. For the habituation phase, there were no significant differences in the amount of time spent in each chamber using either of the poly IC lots (**Fig. 2.3G, Supplementary Fig. 2.3G**). During the sociability phase, poly IC exposure led to reduced

sociability in poly IC lot 1 (main effect of treatment, $F_{(1, 90)} = 6.172$, $p = 0.0276$) but not lot 2 (**Fig. 2.3H, Supplementary Fig. 2.3H**). The effect reported in lot 1 was expected and has been reported for prenatal poly IC exposure (Malkova, Yu et al. 2012). Interestingly, *post hoc* analyses revealed that the overall reduction in sociability caused by poly IC was driven by a decreased sociability exhibited after LL exposure ($p = 0.0081$) (**Fig. 2.3H**), and after conducting *post hoc* comparisons on the lot 2 data, we saw a similar trend when we compared poly IC versus saline under LL ($p = 0.0985$) (**Supplementary Fig. 2.3H**). During the social memory phase, there were no significant differences in social memory (**Fig. 2.3I, Supplementary Fig. 2.3I**). In sum, LL exposure acted to uncover a reduced sociability phenotype in the MIA mice.

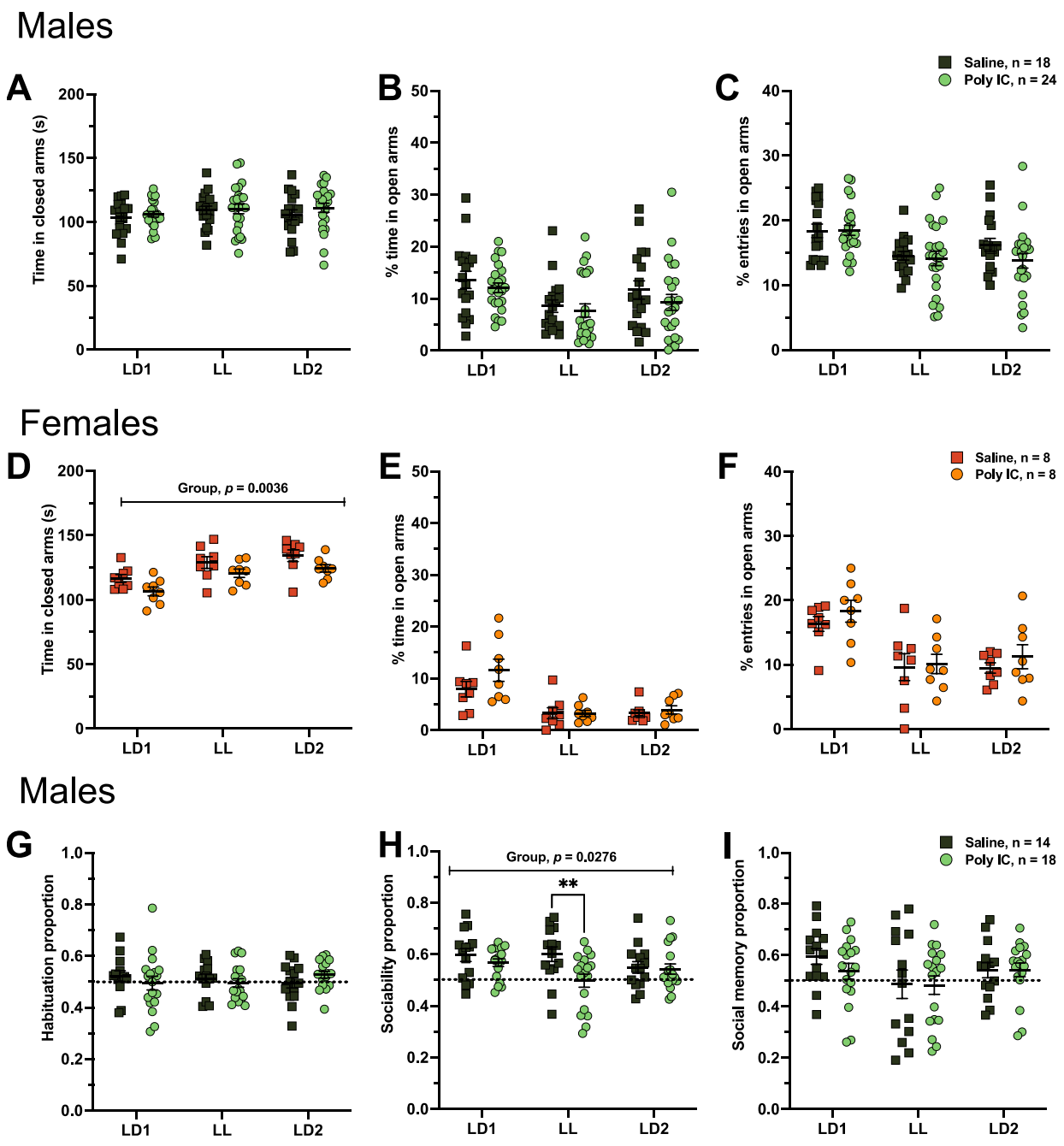


Fig. 2.3. Prenatal poly IC led to limited effects in EPM and reduced sociability after LL. The elevated plus maze was used to assess anxiety-like behavior. Time in closed arms (**A, D**), percent time in open arms (**B, E**) and percent entries in open arms (**C, F**) were assessed in males (**A-C**) and females (**D-F**). The three-chamber social interaction test was used to assess sociability and social memory. Preference proportions were assessed for the habituation phase **(G)**, sociability

phase (**H**) and social memory phase (**I**) in males. Data points represent individual mice, and are presented as mean ± SEM. Two-way ANOVAs (factors treatment x lighting with Tukey's post-hoc comparisons) were conducted. See **Supplementary Table 2.1 and 2.2** for full statistics. ** $p < 0.01$ (post hoc).

2.3.2.4. Deficits in PPI following prenatal poly IC exposure.

Sensory-motor gating was measured using the prepulse inhibition of acoustic startle (PPI) test. The baseline startle response was not significantly different between groups for either lots (**Fig. 2.4A, Supplementary Fig. 2.4A**). We found a significant three-way interaction for both lots of poly IC (prepulse x lighting x treatment: lot 1: $F_{(1, 40)} = 3.637$, $p = 0.008$; lot 2: $F_{(1, 40)} = 4.823$, $p = 0.047$) (**Fig. 2.4C, Supplementary Fig. 2.4C**) and we decomposed this three-way interaction by performing two-way ANOVAs between lighting x treatment. Consistent with the literature on SCZ (Geyer, Krebs-Thomson et al. 2001) and ASD (Perry, Minassian et al. 2007), poly IC exposure induced a decrease in percent PPI (main effect of treatment: lot 1: $F_{(1, 40)} = 4.498$, $p = 0.04$; lot 2: $F_{(1, 40)} = 3.827$, $p = 0.02$), without significant treatment x lighting interactions (**Fig. 2.4B, Supplementary Fig. 2.4B**). Thus, poly IC exposure led to a reduction in PPI, indicative of deficits in sensory-motor gating. The lack of treatment x lighting interactions or main effects of lighting suggests that the addition of LL exposure did not influence sensory-motor gating in males.

In females, no differences in baseline startle response were reported (**Fig. 2.4D, Supplementary Fig. 2.4D**). The three-way ANOVAs (prepulse x lighting x treatment) were not significant (**Fig. 2.4F, Supplementary Fig. 2.4F**), and since we also did not find a significant effect of prepulse, we averaged the prepulses together for each mouse and perform a two-way ANOVA on treatment x lighting. We found that poly IC exposure led to significantly decreased percent PPI compared to saline-exposed females using both lots of poly IC (main effect of treatment: lot 1: $F_{(1, 12)} = 6.082$, $p = 0.0061$; lot 2: $F_{(1, 14)} = 1.358$, $p = 0.0485$) (**Fig. 2.4E, Supplementary Fig. 2.4E**).

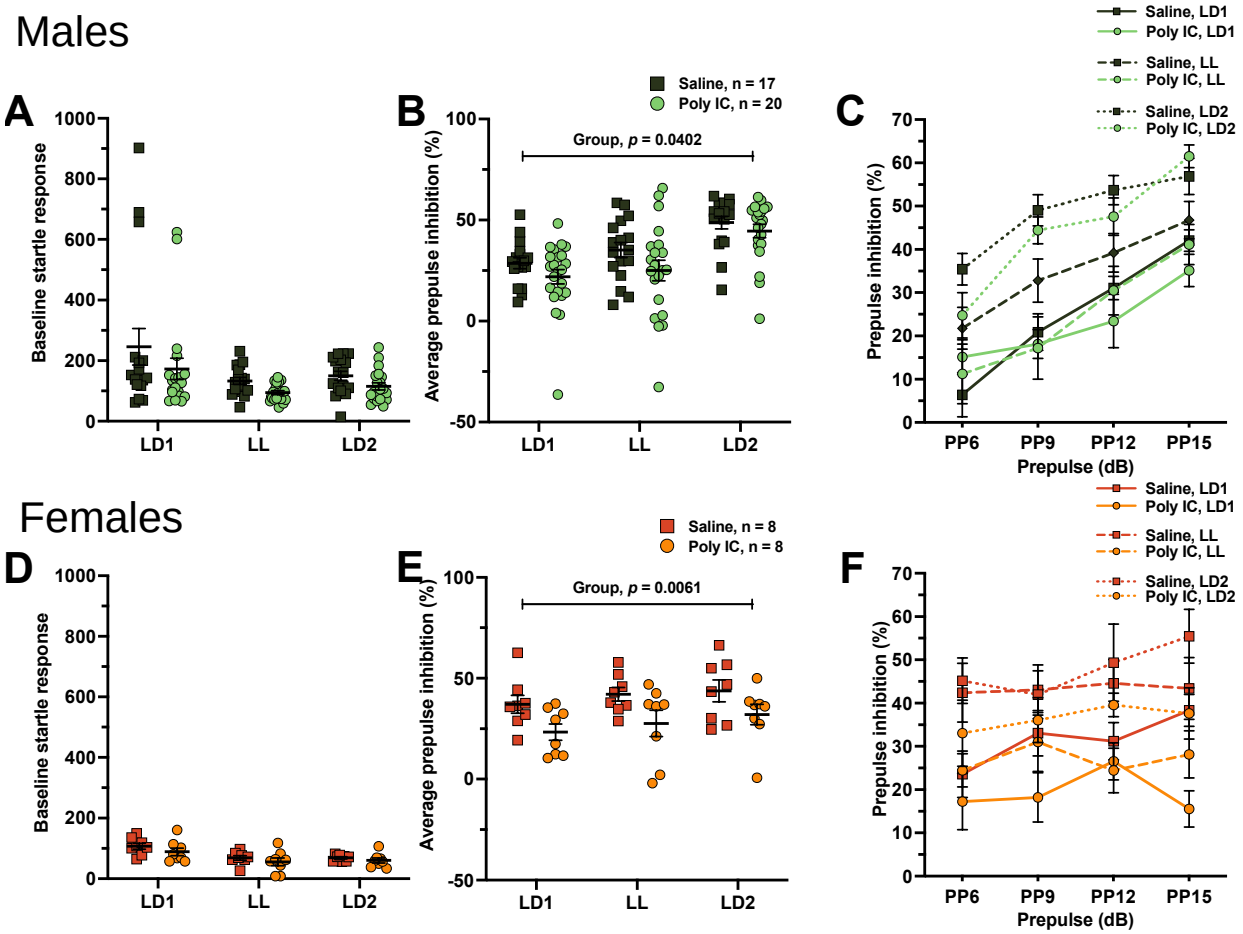


Fig. 2.4. Deficits in PPI following prenatal poly IC exposure. Prepulse inhibition of acoustic startle (PPI) was used to assess sensory motor gating. Baseline startle response (**A, D)**, average PPI (%) (**B, E**) and PPI (%) across each prepulse levels (**C, F**) were assessed in males (**A-C**) and females (**D-F**). For panels **A, B, D and E** data points represent individual mice and are presented as mean ± SEM. Two-way ANOVAs (factors treatment x lighting with Tukey's post-hoc comparisons) were conducted. For panels **C** and **F,** group averages ± SEM are shown over prepulse level. Three-way ANOVAs (factors treatment x lighting x time) were conducted. See **Supplementary Table 2.1 and 2.2** for full statistics.

2.3.3. Sex differences

To directly address sex differences, we performed three-way ANOVAs for treatment x lighting x sex (**Supplementary Table 2.5)**.

In the open field test, a significant three-way interaction was found when assessing thigmotaxis ($F_{(2, 112)} = 3.699$, $p = 0.0217$), where females had significantly higher thigmotaxis than males using both lots (main effect of sex: lot 1: $F_{(1, 56)} = 15.37$, $p = 0.0002$; lot 2: $F_{(1, 489)} = 19.64$, $p = 0.0001$). No sex differences were observed in horizontal activity or total distance traveled. In the EPM test, a significant treatment x sex interaction was found in time in closed arms ($F_{(1, 53)} = 4.334$, $p = 0.0422$), and females spent significantly more time in the closed arms (main effect of sex: $F_{(1, 53)} = 22.89$, $p = 0.0001$), less percent time in the open arms (main effect of sex: $F_{(1, 53)} = 15.31$, $p = 0.0003$) and less percent entries in the open arms (main effect of sex: $F_{(1, 55)} = 17.44$, $p = 0.0001$) compared to males using lot 1 but not lot 2. Finally, in PPI, no sex differences were observed, except females exhibited lower baseline startle response scores than males using lot 1 (main effect of sex: $F_{(1, 50)} = 12.41$, $p = 0.0009$) and lot 2 (main effect of sex: $F_{(1, 48)} = 14.88$, $p = 0.0003$).

2.3.4. Increased microglial morphology values and density by poly IC- exposure were attenuated by LL exposure in the dentate gyrus

Representative images of microglia from the DG for each group under each lighting condition are shown (**Fig. 2.5A-D**). As reported previously (Hui, St-Pierre et al. 2018), we confirmed that poly IC exposure had long term effects on microglia in resulting adult offspring. This is exhibited as a significantly increased morphological index in the poly IC-exposed mice compared to saline-exposed mice (main effect of treatment: $F_{(1, 18)} = 5.727$, $p = 0.0278$), with no

significant treatment x lighting interaction (**Fig. 2.5E**). Using *post hoc* tests, we found that under LD, poly IC induced a trending increase in morphological index (saline/LD versus poly IC/LD: $p = 0.0832$), which was not observed after LL exposure (saline/LL versus poly IC/LL) (**Fig. 2.5E**). When assessing cell body area, the *post hoc* test between saline/LD versus poly IC/LD was not significant, but due to a similar pattern being observed on morphological index, a t-test was performed and revealed a significant finding (t-test between saline/LD versus poly IC/LD, $p = 0.0340$) (**Fig. 2.5F**). No significant differences were observed in cell body circularity (**Fig. 2.5G**). Overall, we observed a diminished response of poly IC exposure on adult microglial morphology after LL exposure, compared to LD, and no significant differences in cell body area or cell body circularity.

We next explored microglial density. Density was significantly increased due to poly IC exposure (main effect of treatment: $F_{(1, 18)} = 4.766$, $p = 0.0425$), with no significant treatment x lighting interaction and no main effect of LL (**Fig. 2.5H**). A similar trend was observed when we conducted the nearest neighbor distance (NND) algorithm, which calculates the average distance of each cell to its nearest neighboring cell. Specifically, poly IC exposure led to a trending decrease in NND (main effect of treatment: $F_{(1, 18)} = 3.552$, $p = 0.0757$), with no treatment x lighting interaction and no main effect of LL (**Fig. 2.5I**). Lastly, we saw no significant differences in spacing index, which describes how the cells are distributed in the region of interest (**Fig. 2.5J**). Poly IC overall led to an increased density and perhaps a decreased NDD in the DG of males.

Representative images of microglia from CA1 and PFC for each treatment under each lighting condition are shown (**Supplementary Fig. 2.5A-D; Supplementary Fig. 2.6A-D**). No differences were observed in the morphological index, cell body area, cell body circularity, density, NND or spacing index (**Supplementary Fig. 2.5E-J; Supplementary Fig. 2.6E-J**).

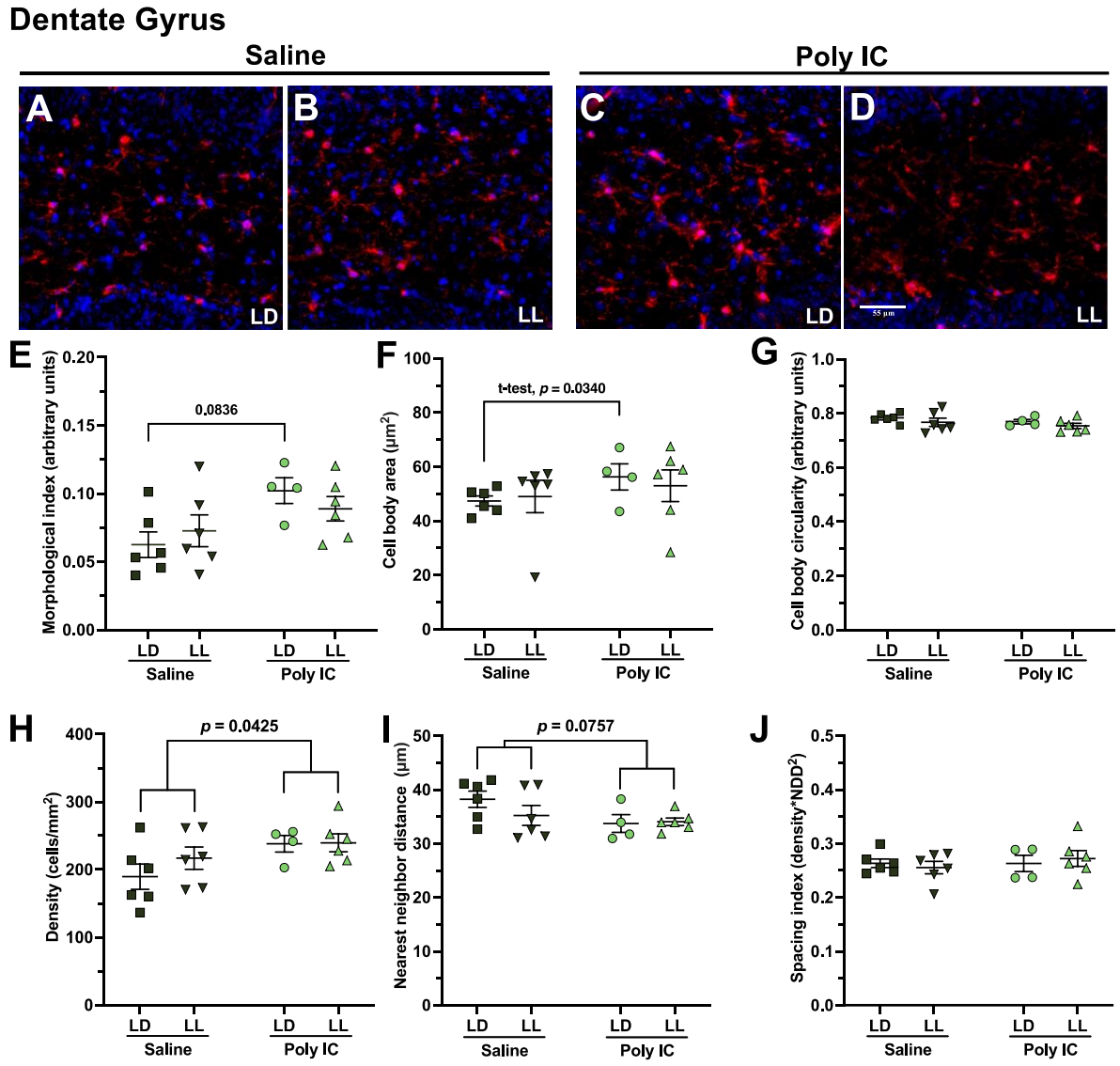


Fig. 2.5. In the dentate gyrus, LL exposure diminished effects on microglia caused by prenatal poly IC. Representative images of microglia from the DG for each group under each lighting condition are shown **(A-D)**. Morphological index (**E**), cell body area (**F**) and cell body circularity (**G**), microglial density (**H**), nearest neighbor distance (**I**), and spacing index (**J**) were assessed in males. Data points represent individual mice and are presented as mean ± SEM. Two-way ANOVAs (factors treatment x lighting with Tukey's post-hoc comparisons) were conducted.

2.4. Discussion

Our study showed a significant interaction between MIA and environmental circadian disruption, both at the behavioral and cellular levels. In particular, we found that MIA male offspring exhibited decreased sociability specifically after LL. Given that reduced sociability is a common symptom of SCZ (Goldberg and Schmidt 2001) and ASD (Orsmond, Shattuck et al. 2013), this finding aligned with our hypothesis that adulthood LL exposure interacts with pre-existing risk factors for NDDs, such as prenatal infection, to exacerbate behaviors related to NDDs. We also found that poly IC exposure alone led to various group differences, including deficits in PPI across both sexes, which is consistent with the literature on MIA (Meyer, Nyffeler et al. 2008), and is exhibited by individuals with NDDs (Geyer, Krebs-Thomson et al. 2001, Perry, Minassian et al. 2007). Females had a much milder phenotype than males, which is also consistent with literature on MIA in rodents (Haida, Al Sagheer et al. 2019). With respect to microglia, we found that poly IC exposure led to increased microglial morphology index and density in the DG, which would indicate a more reactive phenotype, while LL exposure seemed to attenuate these effects.

The behavioral effects observed seem dependent on the lot of poly IC used. Despite using the same dose of poly IC, the cytokine response in maternal serum between poly IC lots differed (Delorme, Srivastava et al. 2021), which may have influenced the severity of the behavioral deficits in adult offspring. This is unsurprising given that the effects of poly IC are dose-dependent (Shi, Fatemi et al. 2003, Meyer, Feldon et al. 2005) and, in humans, there is a positive correlation between the severity of maternal inflammation and NDDs outcomes (Hornig, Bresnahan et al. 2018). Additionally, the poly IC lot that triggered the stronger cytokine response in dams (lot 1) also led to significant weight loss in adult poly IC-exposed male and female offspring compared to their respective controls.

Microglia are immune cells in the CNS, contribute to CNS development, and rapidly respond to homeostasis disruptions and immune challenges (Salter and Stevens 2017). Microglia continually surveying their microenvironment by extending and contracting processes into nearby synapses and are responsible for sculpting synapses during development through processes such as synaptic pruning (Tremblay, Lowery et al. 2010, Tremblay, Stevens et al. 2011, Wang, Zhang et al. 2019). Studies have visualized and quantified characteristics of microglial activation using PET neuroimaging in vivo and by analyzing post-mortem brain tissue in ASD (Koyama and Ikegaya 2015) and SCZ (De Picker, Morrens et al. 2017). Despite mixed results, some patients exhibited characteristics of microglial activation in morphological state and increased density in cortical regions (e.g., PFC, visual cortex), hippocampus and cerebellum (Vargas, Nascimbene et al. 2005, Morgan, Chana et al. 2012, Tetreault, Hakeem et al. 2012, Najjar and Pearlman 2015, Bloomfield, Selvaraj et al. 2016). In animal studies, poly IC-exposed offspring exhibited increased microglial clustering, reduced arborization and increased "dark" microglia, indicative of a pro-inflammatory state (Hui, St-Pierre et al. 2018). It is worth noting that "dark" microglia are thought to be a sub-class of microglia that exhibit signs of increased oxidative stress giving them a dark appearance, and are reported to be abundant under chronic stress (Bisht, Sharma et al. 2016). Microglia have circadian clocks; they display 24-hour mRNA rhythms of several inflammatory factors and circadian clock genes (Fonken, Frank et al. 2015). Additionally, exposure to abnormal lighting conditions altered microglia cytokine expression following an immune challenge (Fonken, Weil et al. 2013, Griffin, Dimitry et al. 2019) and mice lacking the circadian clock protein, REV-ERBα, showed activated hippocampal microglia and increased proinflammatory gene transcription (Griffin, Dimitry et al. 2019). Given these results, and that microglia colonize the fetal brain around E8-9 (i.e., when we injected the poly IC) (Mendes and Majewska 2021) and

have the capacity to become and remain chronically "primed" (Town, Nikolic et al. 2005), we expected that environmental circadian disruption would heighten the activation-related characteristics of MIA-primed microglia. Surprisingly, we observed the reverse in our microglia data. However, it is possible that protective mechanisms are controlling and mitigating the response of microglia, rendering them tolerant to LL exposure. Future studies would benefit from exploring beyond morphology and density and assess whether microglial functions are similarly affected (Paolicelli, Sierra et al. 2022). Additionally, studying the state and function of microglia across 24 hours would also be informative, as well as whether these effects are observed in females.

Protocols of environmental circadian disruption vary between studies. Given that artificial and irregular light schedules are common, we chose to study the effects of LL exposure, meant to mimic light at night. The treatment x lighting interaction that we observed in social behavior may result from a direct effect on the suprachiasmatic nucleus (SCN), a brain region that plays a key role in coordinating circadian oscillations. Namely, LL desynchronizes rhythms of cells of the SCN (Ohta, Yamazaki et al. 2005), and this long term desynchrony may disrupt SCN-driven rhythms in behavior and physiology (Dibner, Schibler et al. 2010). Alternatively, LL may exert its effects on behavior by targeting brain regions other than the SCN. For example, light information is primarily transmitted to the brain via intrinsically photosensitive retinal ganglion cells (ipRGCs) in the retina (Guler, Ecker et al. 2008, LeGates, Fernandez et al. 2014). Although ipRGCs innervate the SCN, they also innervate various cortical and limbic areas, including the amygdala (Schmidt, Do et al. 2011), which play a role in social behavior (Folkes, Baldi et al. 2020). Lastly, it is possible that LL exerts its effects indirectly by targeting peripheral tissues. Exposure to light at night has many peripheral effects (Fonken and Nelson 2014), such as on metabolism (Coomans, Berg et al. 2013), and is known to affect peripheral clock gene expression and circadian hormones (Sudo,

Sasahara et al. 2003, Wideman and Murphy 2009). Since LL does not affect corticosterone secretion levels (Bhardwaj, Stojkovic et al. 2015), the behavioral effects induced by LL are not likely due to stress. In summary, it is unclear if LL exerts its effects on behavior through circuits involving the SCN, circuits in other brain areas directly impinged upon by ipRGCs, or peripheral effects. An additional consideration is the timing of the circadian disruption; perhaps an early exposure to environmental circadian disruption, such as during adolescence, which is a critical time for brain development, would lead to more pronounced differences.

The multifactorial aspect of NDDs, such as SCZ and ASD, prompts the study of the interaction between multiple risk factors, instead of studying risk factors in isolation. Here, we found interactions between MIA and circadian disruption at the behavioral and microglial level. Our data supports a role for circadian disruption as a risk factor for NDDs, which has already been shown in a genetic mouse model for schizophrenia (Bhardwaj, Stojkovic et al. 2015, Cloutier, Srivastava et al. 2022). Future research should address the mechanisms that underlie this interaction to inform the development of circadian-based therapies that aim to prevent or mitigate these serious diseases.

2.5. Acknowledgments

We thank the members of the Cermakian and Srivastava laboratories for helpful discussions, Shashank Srikanta, Margaret Sayeh, Katherine Lord, Nicola Ludin and Joseph Rochford for help with behavioral tests or analysis, the Douglas animal care staff for tending to the animals, Melina Jaramillo Garcia and Bita Khadivjam at the Molecular and Cellular

Microscopy Platform at the Douglas, and Elke Küster-Schöck at the Platform for Imaging by Microscopy at CHUSJ for help with cell analysis.

2.6. Funding

This work was supported by grants from the Canadian Institutes of Health Research (PJT-153299 to NC and LKS) and Velux Stiftung (Project 927 to NC). TCD was supported by graduate scholarships from the Schizophrenia Society of Canada Foundation, the Canadian College of Neuropsychopharmacology, and the Fonds de Recherche du Québec – Santé. WOL was supported by an NSERC-CREATE undergraduate student research award.

2.7. Author contributions

Author contributions included conception and design of the experiments (TCD, LKS, NC), data acquisition (TCD, WOL), data analysis (TCD, WOL) and interpretation of results (TCD, LKS, NC, WOL). Drafting manuscript and revising manuscript for publication (TCD, LKS, NC).

2.8. References

Abel, K. M., R. Drake and J. M. Goldstein (2010). "Sex differences in schizophrenia." International Review of Psychiatry **22**(5): 417-428.

Bhardwaj, S. K., K. Stojkovic, S. Kiessling, L. K. Srivastava and N. Cermakian (2015). "Constant light uncovers behavioral effects of a mutation in the schizophrenia risk gene Dtnbp1 in mice." Behav Brain Res **284**: 58–68.

Bisht, K., K. P. Sharma, C. Lecours, M. Gabriela Sánchez, H. El Hajj, G. Milior, A. Olmos-Alonso, D. Gómez-Nicola, G. Luheshi, L. Vallières, I. Branchi, L. Maggi, C. Limatola, O. Butovsky and M. È. Tremblay (2016). "Dark microglia: A new phenotype predominantly associated with pathological states." Glia **64**(5): 826-839.

Bloomfield, P. S., S. Selvaraj, M. Veronese, G. Rizzo, A. Bertoldo, D. R. Owen, M. A. Bloomfield, I. Bonoldi, N. Kalk, F. Turkheimer, P. McGuire, V. de Paola and O. D. Howes (2016). "Microglial Activity in People at Ultra High Risk of Psychosis and in Schizophrenia: An [(11)C]PBR28 PET Brain Imaging Study." Am J Psychiatry **173**(1): 44-52.

Boivin, D. B. (2000). "Influence of sleep-wake and circadian rhythm disturbances in psychiatric disorders." J Psychiatry Neurosci **25**(5): 446-458.

Boivin, D. B., P. Boudreau and A. Kosmadopoulos (2022). "Disturbance of the Circadian System in Shift Work and Its Health Impact." J Biol Rhythms **37**(1): 3-28.

Brown, A. S., M. D. Begg, S. Gravenstein, C. A. Schaefer, R. J. Wyatt, M. Bresnahan, V. P. Babulas and E. S. Susser (2004). "Serologic evidence of prenatal influenza in the etiology of schizophrenia." Arch Gen Psychiatry **61**(8): 774-780.

Brown, J. P., D. Martin, Z. Nagaria, A. C. Verceles, S. L. Jobe and E. M. Wickwire (2020). "Mental Health Consequences of Shift Work: An Updated Review." Curr Psychiatry Rep **22**(2): 7.

Cheroni, C., N. Caporale and G. Testa (2020). "Autism spectrum disorder at the crossroad between genes and environment: contributions, convergences, and interactions in ASD developmental pathophysiology." Mol Autism **11**(1): 69.

Cloutier, M. E., L. K. Srivastava and N. Cermakian (2022). "Exposure to Circadian Disruption During Adolescence Interacts With a Genetic Risk Factor to Modify Schizophrenia-relevant Behaviors in a Sex-dependent Manner." J Biol Rhythms **37**(6): 655-672.

Coomans, C. P., S. A. A. Berg, T. Houben, J. B. Klinken, R. Berg, A. C. M. Pronk, L. M. Havekes, J. A. Romijn, K. W. Dijk, N. R. Biermasz and J. H. Meijer (2013). "Detrimental effects of constant light exposure and high-fat diet on circadian energy metabolism and insulin sensitivity." The FASEB Journal **27**(4): 1721-1732.

Costa, G., E. Haus and R. Stevens (2010). "Shift work and cancer - considerations on rationale, mechanisms, and epidemiology." Scand J Work Environ Health **36**(2): 163-179.

Davis, J., H. Eyre, F. N. Jacka, S. Dodd, O. Dean, S. McEwen, M. Debnath, J. McGrath, M. Maes, P. Amminger, P. D. McGorry, C. Pantelis and M. Berk (2016). "A review of vulnerability and risks for schizophrenia: Beyond the two hit hypothesis." Neurosci Biobehav Rev **65**: 185-194.

De Picker, L. J., M. Morrens, S. A. Chance and D. Boche (2017). "Microglia and Brain Plasticity in Acute Psychosis and Schizophrenia Illness Course: A Meta-Review." Front Psychiatry **8**: 238.

Delorme, T. C., L. K. Srivastava and N. Cermakian (2020). "Are Circadian Disturbances a Core Pathophysiological Component of Schizophrenia?" J Biol Rhythms **35**(4): 325-339.

Delorme, T. C., L. K. Srivastava and N. Cermakian (2021). "Altered circadian rhythms in a mouse model of neurodevelopmental disorders based on prenatal maternal immune activation." Brain Behav Immun **93**: 119-131.

Demily, C., A. Poisson, E. Peyroux, V. Gatellier, A. Nicolas, C. Rigard, C. Schluth-Bolard, D. Sanlaville and M. Rossi (2017). "Autism spectrum disorder associated with 49,XYYYY: case report and review of the literature." BMC Med Genet **18**(1): 9.

Dibner, C., U. Schibler and U. Albrecht (2010). "The mammalian circadian timing system: organization and coordination of central and peripheral clocks." Annu Rev Physiol **72**: 517–549.

Drake, C. L., T. Roehrs, G. Richardson, J. K. Walsh and T. Roth (2004). "Shift work sleep disorder: prevalence and consequences beyond that of symptomatic day workers." Sleep **27**(8): 1453-1462.

Fatemi, S. H. and T. D. Folsom (2009). "The neurodevelopmental hypothesis of schizophrenia, revisited." Schizophr Bull **35**(3): 528-548.

Ferri, S. L., T. Abel and E. S. Brodkin (2018). "Sex Differences in Autism Spectrum Disorder: a Review." Curr Psychiatry Rep **20**(2): 9.

Folkes, O. M., R. Baldi, V. Kondev, D. J. Marcus, N. D. Hartley, B. D. Turner, J. K. Ayers, J. J. Baechle, M. P. Misra, M. Altemus, C. A. Grueter, B. A. Grueter and S. Patel (2020). "An endocannabinoid-regulated basolateral amygdala-nucleus accumbens circuit modulates sociability." J Clin Invest **130**(4): 1728-1742.

Fonken, L. K., M. G. Frank, M. M. Kitt, R. M. Barrientos, L. R. Watkins and S. F. Maier (2015). "Microglia inflammatory responses are controlled by an intrinsic circadian clock." Brain Behav Immun **45**: 171-179.

Fonken, L. K. and R. J. Nelson (2014). "The effects of light at night on circadian clocks and metabolism." Endocr Rev **35**(4): 648-670.

Fonken, L. K., Z. M. Weil and R. J. Nelson (2013). "Mice exposed to dim light at night exaggerate inflammatory responses to lipopolysaccharide." Brain Behav Immun **34**: 159-163.

Geyer, M. A., K. Krebs-Thomson, D. L. Braff and N. R. Swerdlow (2001). "Pharmacological studies of prepulse inhibition models of sensorimotor gating deficits in schizophrenia: a decade in review." Psychopharmacology (Berl) **156**(2-3): 117-154.

Geyer, M. A. and N. R. Swerdlow (2001). "Measurement of startle response, prepulse inhibition, and habituation." Curr Protoc Neurosci **Chapter 8**: Unit 8 7.

Goldberg, J. O. and L. A. Schmidt (2001). "Shyness, sociability, and social dysfunction in schizophrenia." Schizophr Res **48**(2-3): 343-349.

Griffin, P., J. M. Dimitry, P. W. Sheehan, B. V. Lananna, C. Guo, M. L. Robinette, M. E. Hayes, M. R. Cedeno, C. J. Nadarajah, L. A. Ezerskiy, M. Colonna, J. Zhang, A. Q. Bauer, T. P. Burris and E. S. Musiek (2019). "Circadian clock protein Rev-erbalpha regulates neuroinflammation." Proc Natl Acad Sci U S A **116**(11): 5102-5107.

Guler, A. D., J. L. Ecker, G. S. Lall, S. Haq, C. M. Altimus, H. W. Liao, A. R. Barnard, H. Cahill, T. C. Badea, H. Zhao, M. W. Hankins, D. M. Berson, R. J. Lucas, K. W. Yau and S. Hattar (2008). "Melanopsin cells are the principal conduits for rod-cone input to non-image-forming vision." Nature **453**(7191): 102-105.

Haddad, F. L., S. V. Patel and S. Schmid (2020). "Maternal Immune Activation by Poly I:C as a preclinical Model for Neurodevelopmental Disorders: A focus on Autism and Schizophrenia." Neurosci Biobehav Rev **113**: 546-567.

Haida, O., T. Al Sagheer, A. Balbous, M. Francheteau, E. Matas, F. Soria, P. O. Fernagut and M. Jaber (2019). "Sex-dependent behavioral deficits and neuropathology in a maternal immune activation model of autism." Transl Psychiatry **9**(1): 124.

Hastings, M. H., E. S. Maywood and M. Brancaccio (2018). "Generation of circadian rhythms in the suprachiasmatic nucleus." Nat Rev Neurosci **19**(8): 453-469.

Hornig, M., M. A. Bresnahan, X. Che, A. F. Schultz, J. E. Ukaigwe, M. L. Eddy, D. Hirtz, N. Gunnes, K. K. Lie, P. Magnus, S. Mjaaland, T. Reichborn-Kjennerud, S. Schjolberg, A. S. Oyen, B. Levin, E. S. Susser, C. Stoltenberg and W. I. Lipkin (2018). "Prenatal fever and autism risk." Mol Psychiatry **23**(3): 759-766.

Hui, C. W., A. St-Pierre, H. El Hajj, Y. Remy, S. S. Hebert, G. N. Luheshi, L. K. Srivastava and M. E. Tremblay (2018). "Prenatal Immune Challenge in Mice Leads to Partly Sex-Dependent Behavioral, Microglial, and Molecular Abnormalities Associated with Schizophrenia." Front Mol Neurosci **11**: 13.

Johansson, A. S., B. Owe-Larsson, J. Hetta and G. B. Lundkvist (2016). "Altered circadian clock gene expression in patients with schizophrenia." Schizophr Res **174**(1-3): 17-23.

Kaidanovich-Beilin, O., T. Lipina, I. Vukobradovic, J. Roder and J. R. Woodgett (2011). "Assessment of Social Interaction Behaviors." Journal of Visualized Experiments(48).

Karatsoreos, I. N., S. Bhagat, E. B. Bloss, J. H. Morrison and B. S. McEwen (2011). "Disruption of circadian clocks has ramifications for metabolism, brain, and behavior." Proc Natl Acad Sci U S A **108**(4): 1657-1662.

Koyama, R. and Y. Ikegaya (2015). "Microglia in the pathogenesis of autism spectrum disorders." Neurosci Res **100**: 1-5.

Krakowiak, P., B. Goodlin-Jones, I. Hertz-Picciotto, L. A. Croen and R. L. Hansen (2008). "Sleep problems in children with autism spectrum disorders, developmental delays, and typical development: a population-based study." J Sleep Res **17**(2): 197-206.

Kwon, H. K., G. B. Choi and J. R. Huh (2022). "Maternal inflammation and its ramifications on fetal neurodevelopment." Trends Immunol **43**(3): 230-244.

Lee, B. K., C. Magnusson, R. M. Gardner, A. Blomstrom, C. J. Newschaffer, I. Burstyn, H. Karlsson and C. Dalman (2015). "Maternal hospitalization with infection during pregnancy and risk of autism spectrum disorders." Brain Behav Immun **44**: 100-105.

LeGates, T. A., D. C. Fernandez and S. Hattar (2014). "Light as a central modulator of circadian rhythms, sleep and affect." Nat Rev Neurosci **15**(7): 443-454.

Loomes, R., L. Hull and W. P. L. Mandy (2017). "What Is the Male-to-Female Ratio in Autism Spectrum Disorder? A Systematic Review and Meta-Analysis." J Am Acad Child Adolesc Psychiatry **56**(6): 466-474.

Madore, C., Z. Yin, J. Leibowitz and O. Butovsky (2020). "Microglia, Lifestyle Stress, and Neurodegeneration." Immunity **52**(2): 222-240.

Malkova, N. V., C. Z. Yu, E. Y. Hsiao, M. J. Moore and P. H. Patterson (2012). "Maternal immune activation yields offspring displaying mouse versions of the three core symptoms of autism." Brain Behav Immun **26**(4): 607-616.

Massrali, A., D. Adhya, D. P. Srivastava, S. Baron-Cohen and M. R. Kotter (2022). "Virus-Induced Maternal Immune Activation as an Environmental Factor in the Etiology of Autism and Schizophrenia." Front Neurosci **16**: 834058.

Mattei, D., A. Ivanov, C. Ferrai, P. Jordan, D. Guneykaya, A. Buonfiglioli, W. Schaafsma, P. Przanowski, W. Deuther-Conrad, P. Brust, S. Hesse, M. Patt, O. Sabri, T. L. Ross, B. J. L.

Eggen, E. Boddeke, B. Kaminska, D. Beule, A. Pombo, H. Kettenmann and S. A. Wolf (2017). "Maternal immune activation results in complex microglial transcriptome signature in the adult offspring that is reversed by minocycline treatment." Transl Psychiatry **7**(5): e1120.

McGrath, J., S. Saha, J. Welham, O. El Saadi, C. MacCauley and D. Chant (2004). "A systematic review of the incidence of schizophrenia: the distribution of rates and the influence of sex, urbanicity, migrant status and methodology." BMC Med **2**: 13.

Mendes, M. S. and A. K. Majewska (2021). "An overview of microglia ontogeny and maturation in the homeostatic and pathological brain." Eur J Neurosci **53**(11): 3525-3547.

Meyer, U. (2014). "Prenatal poly(i:C) exposure and other developmental immune activation models in rodent systems." Biol Psychiatry **75**(4): 307-315.

Meyer, U., J. Feldon, M. Schedlowski and B. K. Yee (2005). "Towards an immuno-precipitated neurodevelopmental animal model of schizophrenia." Neurosci Biobehav Rev **29**(6): 913-947.

Meyer, U., M. Nyffeler, B. K. Yee, I. Knuesel and J. Feldon (2008). "Adult brain and behavioral pathological markers of prenatal immune challenge during early/middle and late fetal development in mice." Brain Behav Immun **22**(4): 469-486.

Mondelli, V., A. C. Vernon, F. Turkheimer, P. Dazzan and C. M. Pariante (2017). "Brain microglia in psychiatric disorders." Lancet Psychiatry **4**(7): 563-572.

Morgan, J. T., G. Chana, I. Abramson, K. Semendeferi, E. Courchesne and I. P. Everall (2012). "Abnormal microglial-neuronal spatial organization in the dorsolateral prefrontal cortex in autism." Brain Res **1456**: 72-81.

Murray, B. G., D. A. Davies, J. J. Molder and J. G. Howland (2017). "Maternal immune activation during pregnancy in rats impairs working memory capacity of the offspring." Neurobiol Learn Mem **141**: 150-156.

Najjar, S. and D. M. Pearlman (2015). "Neuroinflammation and white matter pathology in schizophrenia: systematic review." Schizophr Res **161**(1): 102-112.

Ohta, H., S. Yamazaki and D. G. McMahon (2005). "Constant light desynchronizes mammalian clock neurons." Nat Neurosci **8**(3): 267-269.

Orsmond, G. I., P. T. Shattuck, B. P. Cooper, P. R. Sterzing and K. A. Anderson (2013). "Social participation among young adults with an autism spectrum disorder." J Autism Dev Disord **43**(11): 2710-2719.

Paolicelli, R. C., A. Sierra, B. Stevens, M. E. Tremblay, A. Aguzzi, B. Ajami, I. Amit, E. Audinat, I. Bechmann, M. Bennett, F. Bennett, A. Bessis, K. Biber, S. Bilbo, M. Blurton-Jones, E. Boddeke, D. Brites, B. Brone, G. C. Brown, O. Butovsky, M. J. Carson, B. Castellano, M. Colonna, S. A. Cowley, C. Cunningham, D. Davalos, P. L. De Jager, B. de Strooper, A. Denes, B. J. L. Eggen, U. Eyo, E. Galea, S. Garel, F. Ginhoux, C. K. Glass, O. Gokce, D. Gomez-Nicola, B. Gonzalez, S. Gordon, M. B. Graeber, A. D. Greenhalgh, P. Gressens, M. Greter, D. H. Gutmann, C. Haass, M. T. Heneka, F. L. Heppner, S. Hong, D. A. Hume, S. Jung, H. Kettenmann, J. Kipnis, R. Koyama, G. Lemke, M. Lynch, A. Majewska, M. Malcangio, T. Malm, R. Mancuso, T. Masuda, M. Matteoli, B. W. McColl, V. E. Miron, A. V. Molofsky, M. Monje, E. Mracsko, A. Nadjar, J. J. Neher, U. Neniskyte, H. Neumann, M. Noda, B. Peng, F. Peri, V. H. Perry, P. G. Popovich, C. Pridans, J. Priller, M. Prinz, D. Ragozzino, R. M. Ransohoff, M. W. Salter, A. Schaefer, D. P. Schafer, M. Schwartz, M. Simons, C. J. Smith, W. J. Streit, T. L. Tay, L. H. Tsai, A. Verkhratsky, R. von Bernhardi,

H. Wake, V. Wittamer, S. A. Wolf, L. J. Wu and T. Wyss-Coray (2022). "Microglia states and nomenclature: A field at its crossroads." Neuron **110**(21): 3458-3483.

Patke, A., M. W. Young and S. Axelrod (2020). "Molecular mechanisms and physiological importance of circadian rhythms." Nat Rev Mol Cell Biol **21**(2): 67-84.

Perry, W., A. Minassian, B. Lopez, L. Maron and A. Lincoln (2007). "Sensorimotor gating deficits in adults with autism." Biol Psychiatry **61**(4): 482-486.

Presser, H. B. (2003). "Race-Ethnic and Gender Differences in Nonstandard Work Shifts." Work and Occupations **30**(4): 412-439.

Puttonen, S., M. Harma and C. Hublin (2010). "Shift work and cardiovascular disease - pathways from circadian stress to morbidity." Scand J Work Environ Health **36**(2): 96-108.

Rapoport, J. L., J. N. Giedd and N. Gogtay (2012). "Neurodevelopmental model of schizophrenia: update 2012." Mol Psychiatry **17**(12): 1228-1238.

Salter, M. W. and B. Stevens (2017). "Microglia emerge as central players in brain disease." Nat Med **23**(9): 1018-1027.

Schmidt, T. M., M. T. Do, D. Dacey, R. Lucas, S. Hattar and A. Matynia (2011). "Melanopsin-positive intrinsically photosensitive retinal ganglion cells: from form to function." J Neurosci **31**(45): 16094-16101.

Schmitt, A., P. Falkai and S. Papiol (2022). "Neurodevelopmental disturbances in schizophrenia: evidence from genetic and environmental factors." J Neural Transm (Vienna): 1-11.

Seibenhener, M. L. and M. C. Wooten (2015). "Use of the Open Field Maze to Measure Locomotor and Anxiety-like Behavior in Mice." Journal of Visualized Experiments(96).

Shi, L., S. H. Fatemi, R. W. Sidwell and P. H. Patterson (2003). "Maternal influenza infection causes marked behavioral and pharmacological changes in the offspring." J Neurosci **23**(1): 297-302.

Sudo, M., K. Sasahara, T. Moriya, M. Akiyama, T. Hamada and S. Shibata (2003). "Constant light housing attenuates circadian rhythms of mPer2 mRNA and mPER2 protein expression in the suprachiasmatic nucleus of mice." Neuroscience **121**(2): 493-499.

Tandon, R., J. E. Shipley, S. Taylor, J. F. Greden, A. Eiser, J. DeQuardo and J. Goodson (1992). "Electroencephalographic sleep abnormalities in schizophrenia. Relationship to positive/negative symptoms and prior neuroleptic treatment." Arch Gen Psychiatry **49**(3): 185-194.

Tandon, R., J. E. Shipley, S. Taylor, J. F. Greden, A. Eiser, J. DeQuardo and J. Goodson (1992). "Electroencephalographic sleep abnormalities in schizophrenia. Relationship to positive/negative symptoms and prior neuroleptic treatment." Arch Gen Psychiatry **49**(3): 185–194.

Tetreault, N. A., A. Y. Hakeem, S. Jiang, B. A. Williams, E. Allman, B. J. Wold and J. M. Allman (2012). "Microglia in the cerebral cortex in autism." J Autism Dev Disord **42**(12): 2569-2584.

Town, T., V. Nikolic and J. Tan (2005). "The microglial "activation" continuum: from innate to adaptive responses." J Neuroinflammation **2**: 24.

Tremblay, M. E., R. L. Lowery and A. K. Majewska (2010). "Microglial interactions with synapses are modulated by visual experience." PLoS Biol **8**(11): e1000527.

Tremblay, M. E., B. Stevens, A. Sierra, H. Wake, A. Bessis and A. Nimmerjahn (2011). "The role of microglia in the healthy brain." J Neurosci **31**(45): 16064-16069.

Valle, F. P. (1970). "Effects of strain, sex, and illumination on open-field behavior of rats." Am J Psychol **83**(1): 103-111.

Vargas, D. L., C. Nascimbene, C. Krishnan, A. W. Zimmerman and C. A. Pardo (2005). "Neuroglial activation and neuroinflammation in the brain of patients with autism." Ann Neurol **57**(1): 67-81.

Walf, A. A. and C. A. Frye (2007). "The use of the elevated plus maze as an assay of anxiety-related behavior in rodents." Nat Protoc **2**(2): 322-328.

Walker, W. H., 2nd, J. C. Walton, A. C. DeVries and R. J. Nelson (2020). "Circadian rhythm disruption and mental health." Transl Psychiatry **10**(1): 28.

Wang, M., L. Zhang and F. H. Gage (2019). "Microglia, complement and schizophrenia." Nat Neurosci **22**(3): 333-334.

Wideman, C. H. and H. M. Murphy (2009). "Constant light induces alterations in melatonin levels, food intake, feed efficiency, visceral adiposity, and circadian rhythms in rats." Nutritional Neuroscience **12**(5): 233-240.

Williams, C. (2008). "Work-life balance of shift workers."

Wintler, T., H. Schoch, M. G. Frank and L. Peixoto (2020). "Sleep, brain development, and autism spectrum disorders: Insights from animal models." J Neurosci Res **00**: 1-13.

Wulff, K., D. J. Dijk, B. Middleton, R. G. Foster and E. M. Joyce (2012). "Sleep and circadian rhythm disruption in schizophrenia." Br J Psychiatry **200**(4): 308-316

BEHAVIORAL AND CELLULAR RESPONSES TO CIRCADIAN DISRUPTION AND PRENATAL IMMUNE ACTIVATION IN MICE

Tara C. Delorme, William Ozell-Landry, Nicolas Cermakian, and Lalit K. Srivastava

Supplementary Information

Supplementary Methods

Animals and housing

Male and female C57BL/6J mice for the maternal immune activation (MIA) mating protocol were ordered from Jackson Laboratory (product number: 000664) at 8 weeks old. Male C57BL/6 mice used as strangers for the three-chamber social interaction test were ordered from Charles River Laboratories (Saint-Constant, QC, Canada) and were age and sex matched to the test mice. Offspring resulting from the MIA protocol and controls were placed in light-proof ventilated cabinets (Actimetrics, Wilmette, IL, USA). Cabinet light was controlled via an external timer, with a light intensity of 150-200 lux (cool white LED lighting).

Timed mating protocol for MIA

Male and female mice were placed under standard laboratory lighting (LD; 12 h of light, 12 h of dark) for 2 weeks to acclimate to their surroundings. Mice were mated overnight, and the presence of a vaginal plug was assessed in females within the first hour following lights on. The day of vaginal plug was considered embryonic day 0.5 (E0.5).

Litter details

In the first cohort, 20 female breeders were mated with 10 male breeders: 6 out of 12 poly IC-injected dams gave birth (average litter size: 6.7, total male offspring used = 24, total female offspring used = 8) and 6 out of 8 saline injected dams gave birth (average litter size: 7.8, total male offspring used = 20, total female offspring used = 8). For the second cohort, 19 female breeders were mated with 9 male breeders: 6 out of 11 poly IC-injected dams gave birth (average

litter size: 6.8, total male offspring used = 24, total female offspring used = 8) and 4 out of 8 saline-injected dams gave birth (average litter size: 6.5, total male offspring used = 13, total female offspring used = 8).

Experimental timeline and details

At 3 weeks old, poly IC- and saline-exposed male and female pups were weaned, tagged, and weighed. Two to three sex-matched littermates were group housed, offspring were aged to adulthood (8 weeks old) and were weighed before being placed in light-proof ventilated cabinets, where they were left to acclimatize to their environment for 2 weeks before behavioral tests began. All tests were performed under dim light conditions, where the lighting intensity at the center of the testing chambers was 10 lux. To minimize the effect of litter, mice from 4-6 litters were used in each experimental condition. Additionally, we visually confirmed that the significant effects that we reported were not driven by a cluster of mice from the same litter and ensured effects were still present after averaging individual mouse scores by litter.

Behavioral outcomes

The behavioral tests conducted under LD1 and LD2 were performed in the first 3 hours after the lights turned on (between Zeitgeber Time (ZT) 1-3). When we conducted the behavioral tests under LL, mice were "free running", meaning they were not entrained to an external stimulus (lights on/lights off). Thus, we used passive infrared sensors that measure general home cage locomotion, to assess when the mice were active versus inactive. The time the mice became active was considered Circadian Time (CT) 12, and CT 0 was defined as half a period (~12 hours) later, calculated using a chi-square periodogram (ClockLab software, version 6, Actimetrics, Wilmette,

IL, USA). Thus, behavioral tests under LL were conducted between CT 1-3 for each cage of mice. Behavioral tests were performed in order of least stressful to the most (i.e., open field followed by EPM, three-chamber social interaction, and PPI). Two rest days were scheduled between each test. In the EPM and three-chamber social interaction test, we used Swann cameras (DVR4-4575, 4 Channel digital video recorder & 2x PRO-T853 Cameras) to record behavior and the TopScan 2.0 software (Clever Sys, Restin, VA, USA) to track mouse movement.

Open field test

We used the VersaMax Legacy Open Field setup (AccuScan Instruments, Inc., Columbus, OH, USA) system, which consisted of acrylic chambers (Length × Width × Height = 17.5 cm × 10 cm x 26 cm) equipped with infrared sensors to record and score locomotion-related variables. Mice were free to explore the chamber for 50 minutes. Data were collected using the Versamax Software (version 4.0, 2004; AccuScan Instruments, Inc.).

Elevated plus maze (EPM) test

The apparatus was elevated 70 cm above the ground. The center area measured 5 cm × 5 cm, and each of the 4 arms measured Length = 50 cm × Width = 5 cm. Mice were placed in the intersection of the four arms, facing an open arm. Mice could freely explore the chamber for 5 minutes after which they were returned to their home cage. EPM is based on the conflict between the rodent's preference for enclosed areas (the closed arms) and their innate motivation to explore a novel environment (the open arms). A video camera positioned directly above the apparatus recorded the session, and time spent and entries in each arm was scored. Mice that jumped off the maze were excluded from the analysis. Higher scores on the EPM formula indicated less anxiety-

like behavior. Two male saline-exposed mice from lot 1 and 1 male saline-exposed mouse from lot 2 jumped off the maze and were excluded from the analysis.

Three-chamber social interaction test

The test is comprised of 3 consecutive phases, each lasting 10 minutes. In the *habituation phase*, mice could freely explore the testing apparatus, which consisted of a three-chamber plastic apparatus (Length = 26 cm x Width = 21.6 x Height = 21.6 cm) with an empty wire container (Diameter = 7.6 cm, Height = 9.5 cm) in each of the two extreme chambers. The habituation formula is shown below. A value of 0.5 indicates no preference for either chamber. A value between 0.5-1 indicates a preference for Zone 1 and a value between 0-0.5 indicates a preference for Zone 2. $$Habituation\ formula = \frac{\text{Time spent in Zone 1}}{\text{Time spent in Zone 1+Time spent in Zone 2}}$$

In the *social preference formula*, a value of 0.5 indicates no preference for either chamber. A value between 0.5-1 indicates a preference for stranger 1 (i.e., social preference) and a value between 0-0.5 indicates a preference for the object. The object used was a similar size to the stranger mice. Mice typically prefer to explore a mouse over an object.

In the *social memory formula*, a value of 0.5 indicates no preference for either chamber. A value between 0.5-1 indicates a preference for stranger 2 (i.e., social memory) and a value between 0-0.5 indicates a preference for the familiar stranger 1. Mice typically prefer to explore a novel mouse over a familiar one.

Stranger mice

The stranger mice were strain-, sex-, and aged-matched to the test mice and were habituated to the chamber and wire containers for 30 minutes the day before the test. The location of the

stranger mice and the object was counterbalanced. A video camera positioned directly above the apparatus recorded the 3 phases, and time in each chamber was scored.

Prepulse inhibition of acoustic startle (PPI)

PPI was measured using commercially available startle chambers (San Diego Instruments, San Diego, CA, USA), and a commercial software package by SR-LAB. Mice were placed into a cylindrical Plexiglass enclosure (Diameter = 8 cm, Length = 16 cm), mounted on a Plexiglass base, within a dimly lit (10 lux) and sound-attenuating chamber. The speaker that delivered the acoustic stimuli was in the ceiling of the chamber (24 cm above the animal) and delivered a 70-dB background noise. A piezoelectric accelerometer fixed to the Plexiglass base was used to detect and transduce motion resulting from the animal's startle response. The session began with a 5-minute acclimation period. Each session consisted of 50 trials. In the first 6 and final 6 trials, a 'startle tone' (120-dB for 50ms, broad-band burst) was played in the absence of a prepulse (called 'startle-only' trials). The "baseline startle response" was calculated by averaging the startle magnitude after the last 'startle-only' trials. For the middle 38 trials, 8 trials were 'startle-only' trials, 5 trials had no stimulus, and 25 trials had the startle tone preceded by a prepulse stimulus (30 ms, broad-band burst) that was either 6, 9, 12 or 15 dB above the background noise and presented 100 ms before the startle pulse. The order of the middle 38 trials varied randomly. The intertrial interval also varied randomly, with an average intertrial interval of 15 seconds. The higher the percent PPI, the greater the inhibition to the startle when preceded by a prepulse. In lot 1, 3 male saline-exposed and 4 male poly IC-exposed mice were excluded and in lot 2, 1 male saline-exposed mouse was excluded due to issues with the recording apparatus.

Microglia characterization

Tissue preparation

Mice were anesthetized with an intraperitoneal injection of ketamine/xylazine/acepromazine between ZT 1 and 3. Mice were transcardially perfused with ice-cold phosphate buffered saline (PBS), followed by 4% paraformaldehyde (# 31985-062, Invitrogen). Brains were post-fixed for 24 hours in 4% paraformaldehyde at 4°C, then transferred to a 30% sucrose solution for 48 hours at 4°C. Brains were placed in OCT compound and flash frozen in isopentane over dry ice before being stored at -80°C. Brains were sliced with a cryostat at 30 μm section. Sagittal sections containing Bregma 0.36 to 1.00 (which included the dorsal hippocampus and the medial prefrontal cortex), based on the stereotaxic atlas of Paxinos and Franklin (4th edition), were processed for immunofluorescence staining and imaging.

Immunohistochemistry

Slices were washed in PBS and Triton 0.2% and blocked in donkey serum (1:10, including 0.2% Triton X-100 and PBS) at room temperature for 1 hour to prevent non-specific antibody binding. Slices were incubated with primary antibody [1:1000, Rabbit anti-Iba1 (ionized calcium binding adaptor molecule 1, a marker for microglia), # 019-19741, Cedarlane] overnight at 4°C. Slices were rinsed in PBS and Tween 0.1% before they were incubated in secondary antibody (1:1000, Alexa fluor 594 Donkey anti-rabbit IgG, # 711-585-152, Cedarlane) at room temperature for 2 hours. All primary and secondary antibodies were diluted in the blocking buffer mentioned above. Sections were washed in PBS and Tween 0.1%, counter-stained with DAPI (1:20000, # D9542, Sigma), and mounted with antifade mounting medium (H-1000, lot # T0606, Vector Laboratories) under a glass coverslip.

Microglia visualization and analysis

Brain sections were visualized using an Olympus BX63 automated fluorescence microscope. We characterized the microglia based on morphology, density, and spacing.

Morphology

Measures of morphology included soma area and perimeter, arborization area and perimeter and soma circularity. Arborization area and process perimeter were measured using the 'polygon' tool to trace around the edge of all the processes of a single microglia. To determine soma area, soma perimeter, and soma circularity, the 'freehand' tool was used to trace around the cell body seen on the Iba1 stain. Soma circularity was calculated using the 'shape descriptors' tool. A morphological index was calculated by dividing the soma area by the arborization area for each cell.

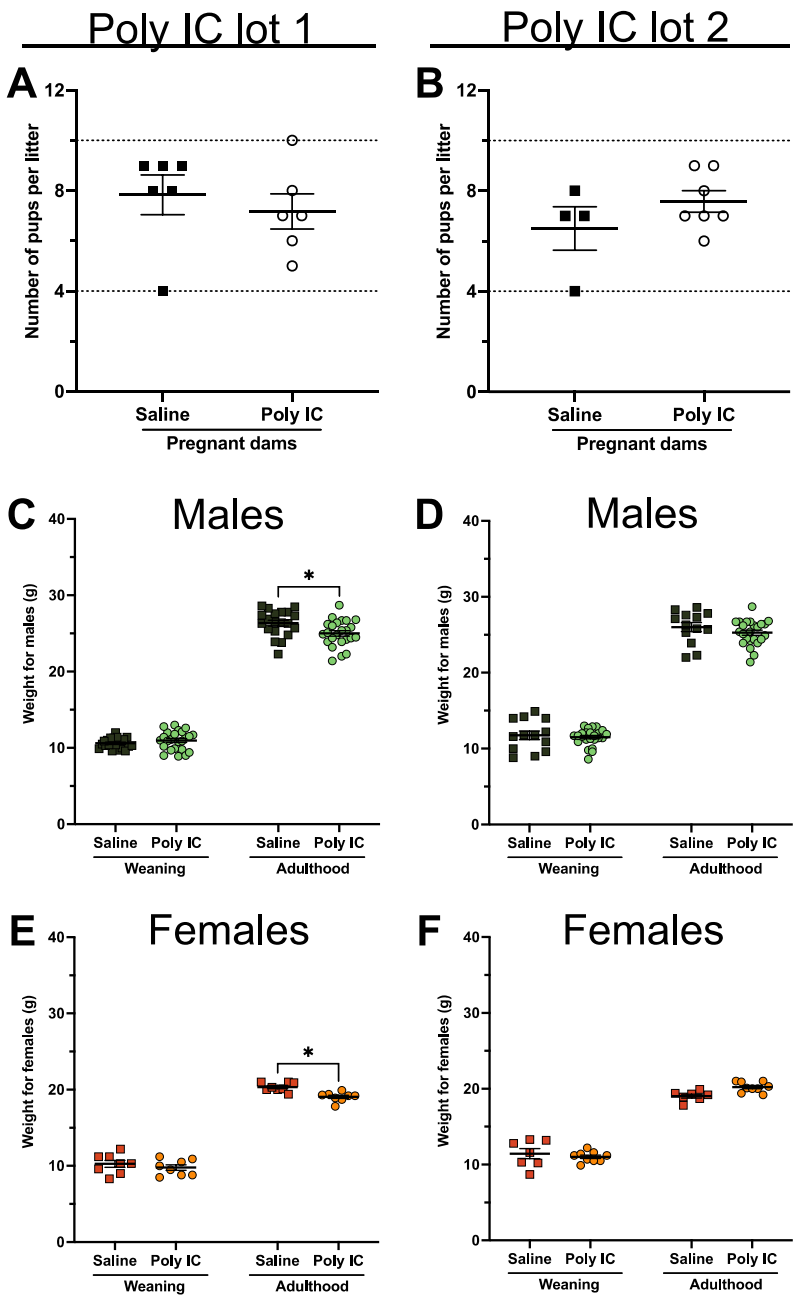
Density

To determine the density of microglia per image, we first used the 'freehand' tool to trace a region of interest. The 'threshold' tool was used to identify each microglia, and the 'analyze particles' function was used to automatically record the number of identified microglia, as well as each of their spatial coordinates. The 'nearest neighbor distance' plugin was then applied to determine the distance between each cell and its closest neighbor. The density of microglia for each image was calculated by dividing the number of cells in that image by the region of interest identified. A spacing index was calculated by multiplying the average density for each animal obtained above by the square of the average nearest neighbor distance.

Statistics

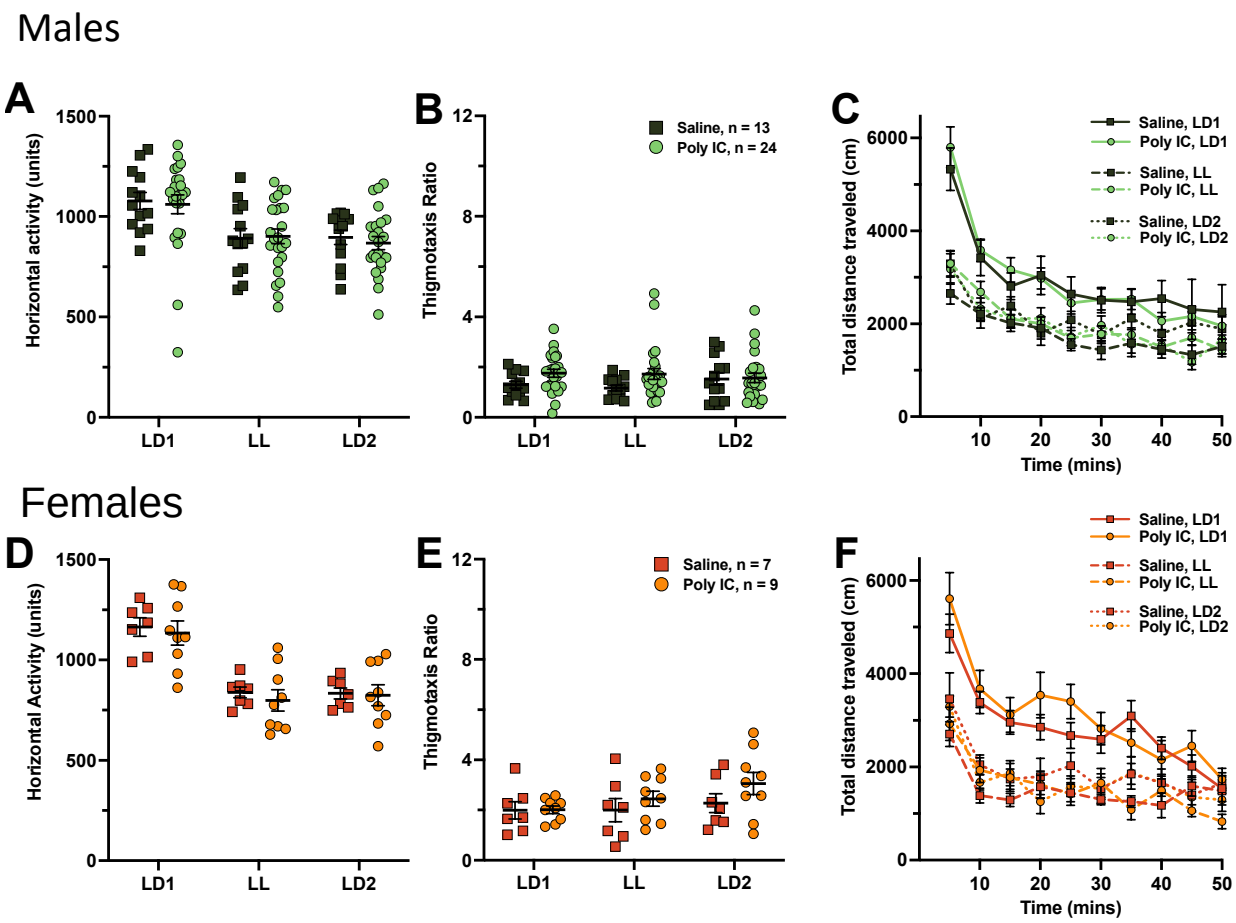
For the behavioral data (**Fig. 2.2-2.4, Supplementary Fig. 2.2-2.4**), 2-way mixed effect ANOVAs were conducted using Tukey's post hoc comparisons, with treatment (poly IC or saline) as a between-subjects variable and lighting (LD1, LL, and LD2) as a within-subjects variable. Main effects were explored if no significant treatment x lighting interaction was observed. Including lighting as a within-subjects variable allows for the same mice to be tested throughout the experiment, but it does introduce the likelihood that mice habituate to the test, which is a limitation of this design. Three-way ANOVAs were also conducted (using SPSS statistical software version 29) for prepulse inhibition (3^{rd} factor: prepulse) and locomotion (3^{rd} factor: time). For the microglia data (**Fig. 2.5, Supplementary Fig. 2.5-2.6**), 2-way mixed effect ANOVAs were conducted using Tukey's post hoc comparisons, with treatment (poly IC or saline) and lighting condition (LD or LL) both as between-subjects variables. If no interaction was found, main effects were explored. The data describing number of pups per litter (**Supplementary Fig. 2.1A-B)**, passed the Shapiro-Wilk normality test and had equal variances (assessed by an F-test); thus, independent samples t-test was used between poly IC- and saline-injected pregnant dams. For offspring weight data (**Supplementary Fig. 2.1C-F)**, we conducted 2-way mixed effect ANOVAs, with treatment (poly IC or saline) as a between-subjects variable and age (weaning and adulthood) as a within-subjects variable. We explored treatment x age interactions and performed Tukey's post hoc comparisons when applicable. The data describing sex differences (**Supplementary Table 2.5)** were assessed using three-way ANOVAs (treatment x lighting x sex) and decomposed by assessing the 2-way interaction (treatment x sex) and observing the main effect of sex. No outliers were identified (ROUT's test, Q = 1%).

Supplementary Figures

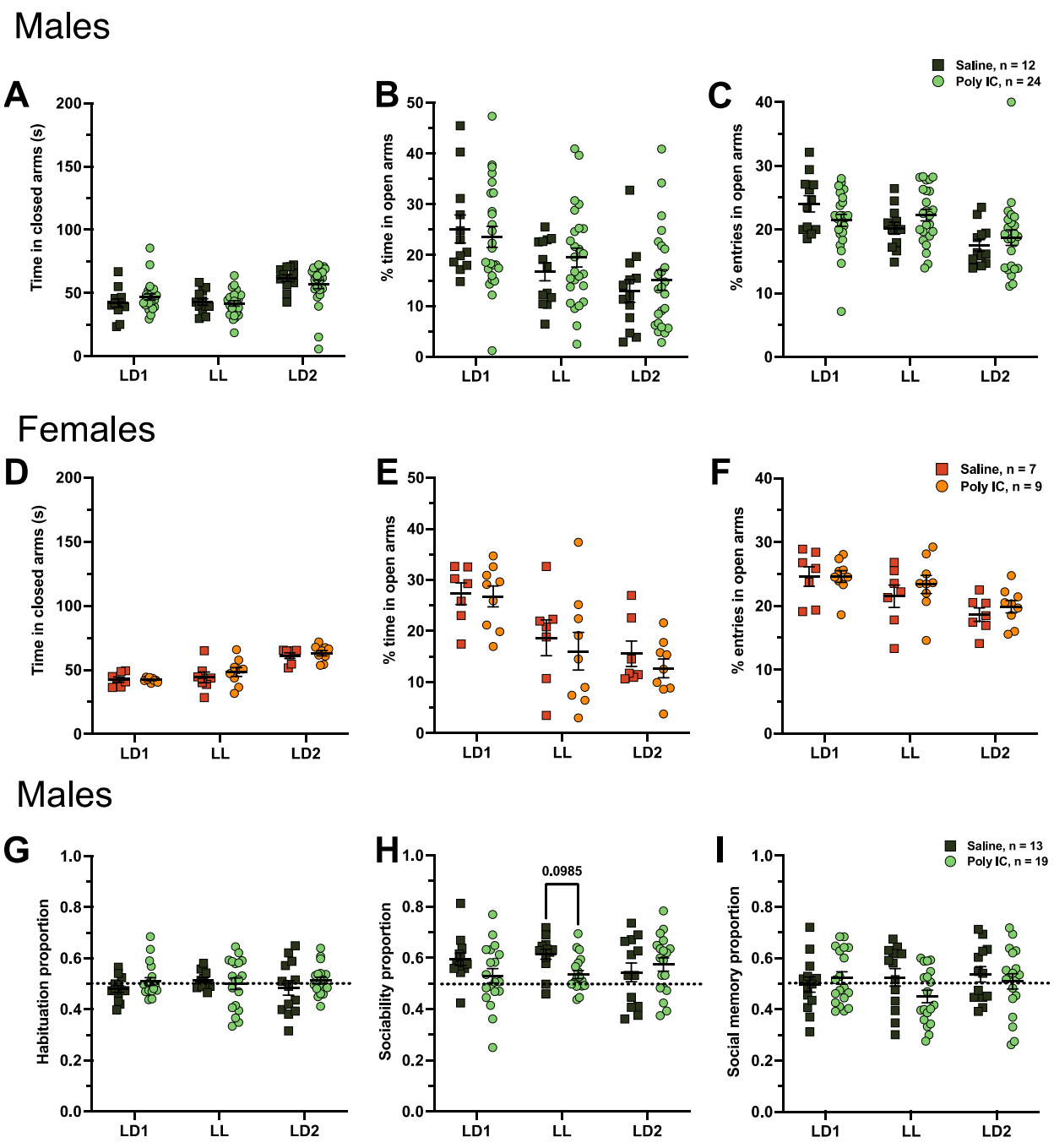


Supplementary Fig. 2.1. Litter details and poly IC- and saline-exposed offspring body weights at weaning and adulthood. Number of pups from each poly IC and saline litter (**A**). Litters had a minimum of 4 pups/litters and a maximum of 10 pups/litter. Individual points represent a litter and data are represented as mean ± SEM. Independent samples t-tests were

conducted. Body weight between groups at weaning (3 weeks old) (**B**) and adulthood (8 weeks old) (**C**) are presented. Data points represent independent offspring and data are represented as mean ± SEM. Two-way ANOVAs (factors treatment x lighting with Tukey's post hoc comparisons) were conducted. *$p < 0.05$ (post hoc).

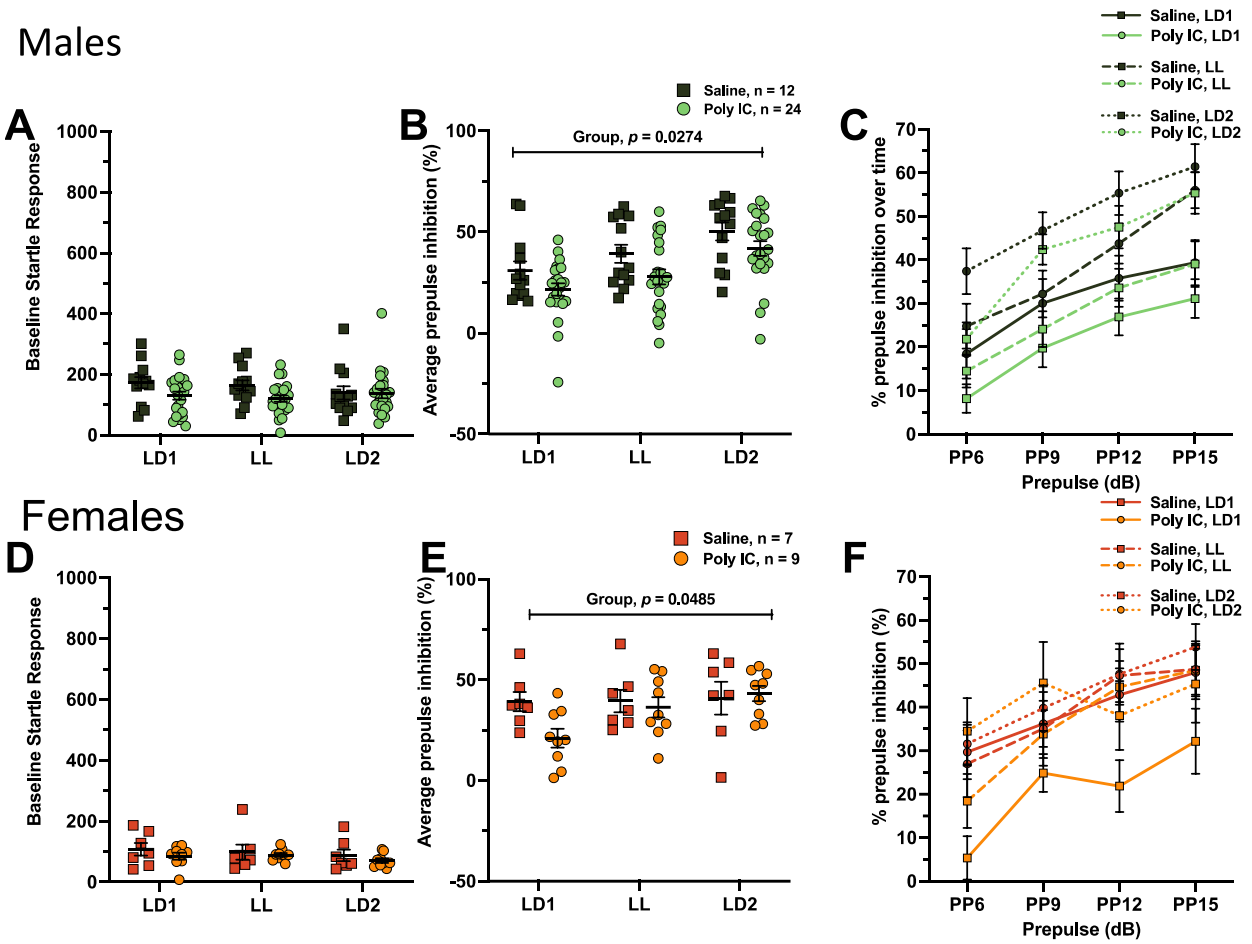


Supplementary Fig. 2.2. No significant effects in spontaneous locomotion while using poly IC lot 2. Spontaneous locomotor activity was measured in the open field test. Horizontal activity **(A, D)**, thigmotaxis **(B, E)** and total distance traveled (**C, F**) were assessed in males (**A-C**) and females (**D-F**). For panels **A, B, D and E** data points represent individual mice, and are presented as mean ± SEM. Two-way ANOVAs (factors treatment x lighting with Tukey's post hoc comparisons) were conducted. For panels **C** and **F,** group averages ± SEM are shown over each 10-minute bin of the test. Three-way ANOVAs (factors treatment x lighting x time) were conducted. See **Supplementary Table 2.3 and 2.4** for full statistics.

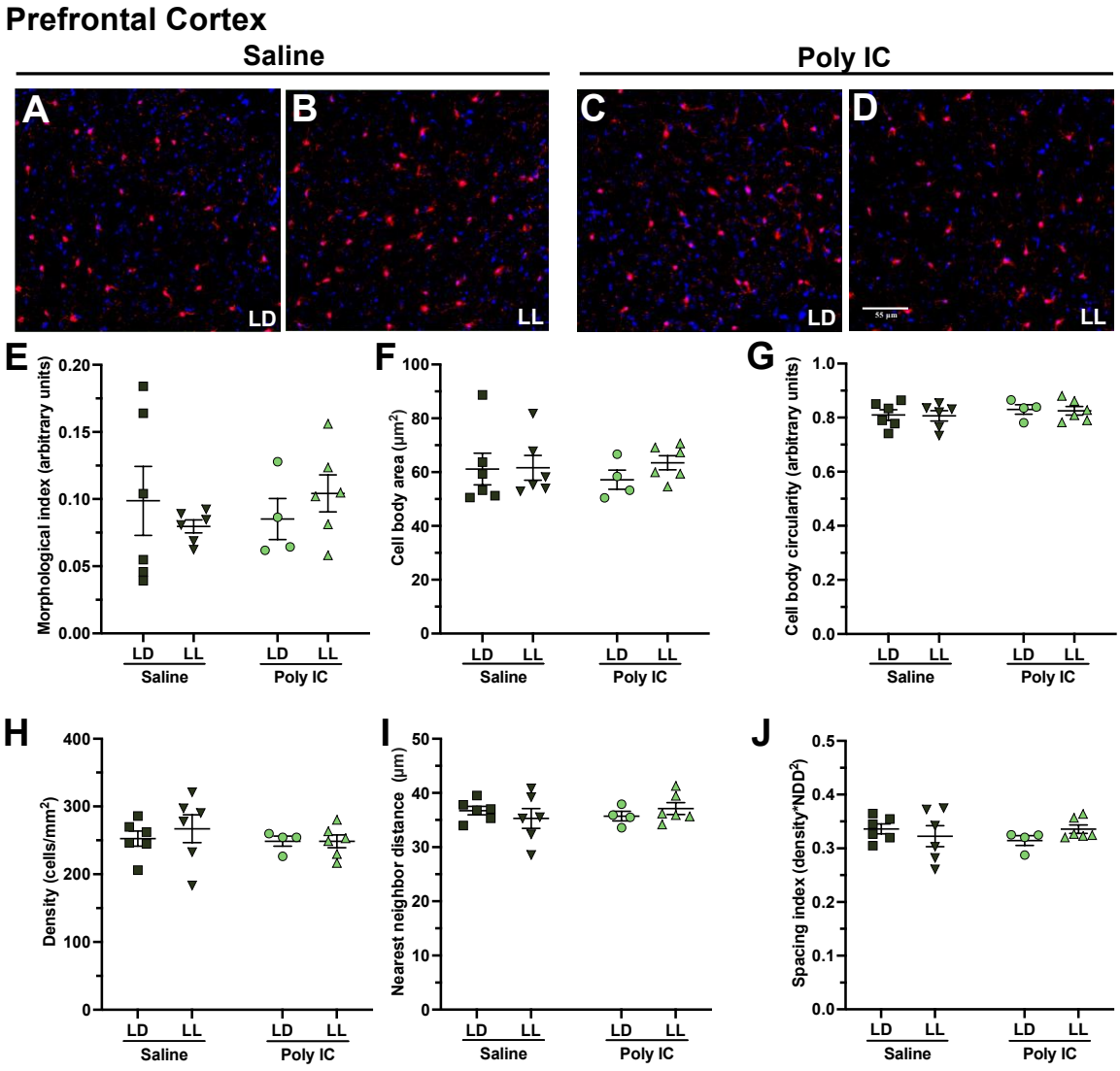


Supplementary Fig. 2.3. No differences in anxiety-like behavior, and a trending effect for poly IC-exposed males to exhibit reduced sociability after LL. In the elevated plus maze, time in closed arms (**A, D**), percent time in open arms (**B, E**) and percent entries in open arms (**C, F**) were assessed in males (**A-C**) and females (**D-F**). In the three-chamber social interaction test,

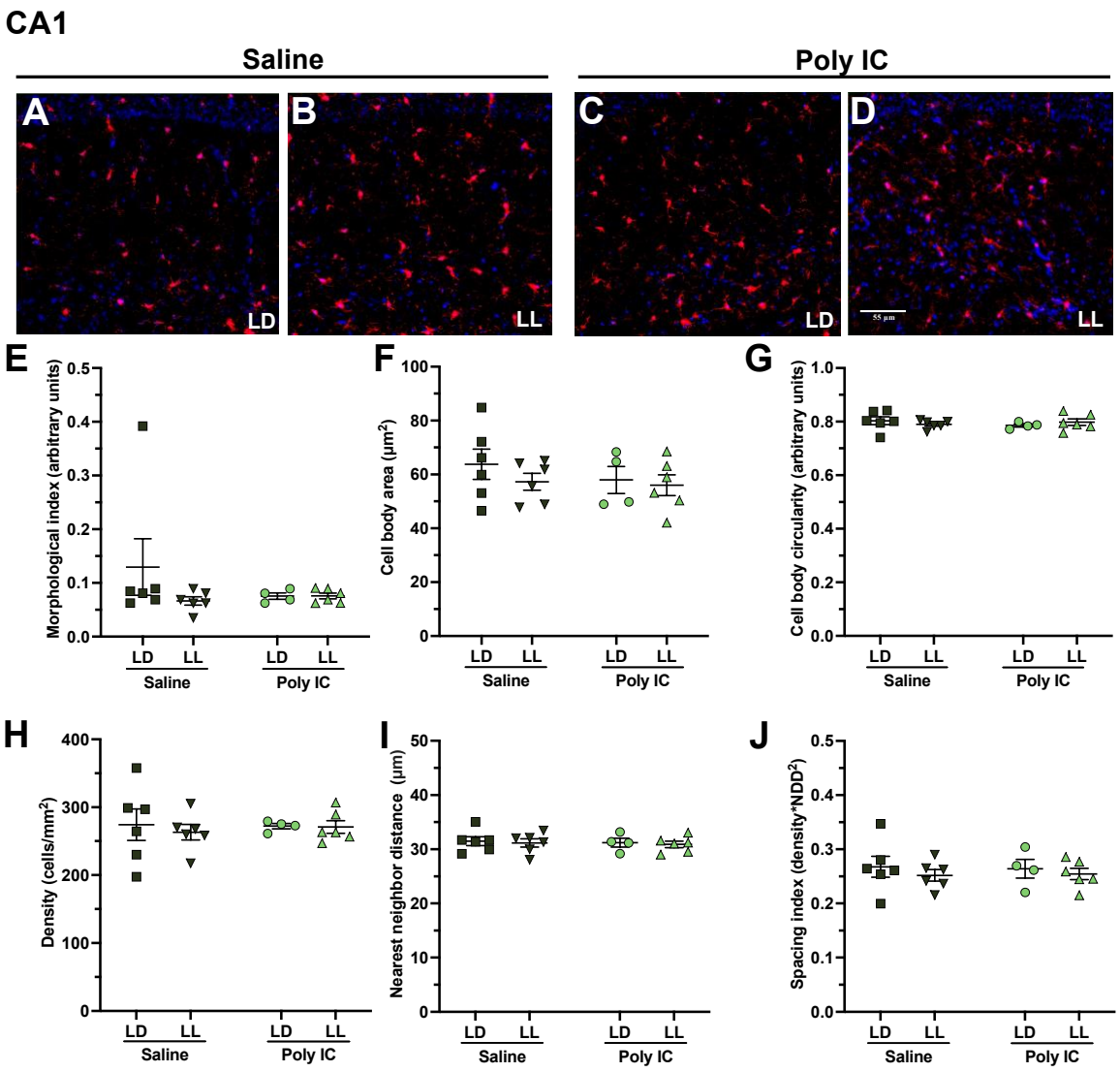
preference proportions were assessed for the habituation phase **(G)**, sociability phase (**H**) and social memory phase (**I**) in males. Data points represent individual mice, and are presented as mean ± SEM. Two-way ANOVAs (factors treatment x lighting with Tukey's post hoc comparisons) were conducted. See **Supplementary Table 2.3 and 2.4** for full statistics.



Supplementary Fig. 2.4. Deficits in PPI following prenatal poly IC exposure. Prepulse inhibition of acoustic startle (PPI) was used to assess sensory motor gating. Baseline startle response (**A, D)**, average PPI (%) (**B, E**) and PPI (%) across each prepulse levels (**C, F**) were assessed in males (**A-C**) and females (**D-F**). For panels **A, B, D and E** data points represent individual mice, and are presented as mean ± SEM. Two-way ANOVAs (factors treatment x lighting with Tukey's post hoc comparisons) were conducted. For panels **C** and **F,** group averages ± SEM are shown over prepulse level. Three-way ANOVAs (factors treatment x lighting x time) were conducted. See **Supplementary Table 2.3 and 2.4** for full statistics.



Supplementary Fig. 2.5. In the PFC, no morphological or density-related differences in microglia were observed. Representative images of microglia from the PFC for each group under each lighting condition are shown **(A-D)**. Morphological index (**E**), cell body area (**F**) and cell body circularity (**G**), microglial density (**H**), nearest neighbor distance (**I**), and spacing index (**J**) were assessed in males. Data points represent individual mice, and are presented as mean ± SEM. Two-way ANOVAs (factors treatment x lighting with Tukey's post hoc comparisons) were conducted.



Supplementary Fig. 2.6. In area CA1, no differences were observed in microglia after poly IC alone, LL alone or the combined effects of both poly IC and LL. Representative images of microglia from the CA1 for each treatment under each lighting condition are shown (A-D). Morphological index (E), cell body area (F) and cell body circularity (G), microglial density (H), nearest neighbor distance (I), and spacing index (J) were assessed in males. Data points represent individual mice, and are presented as mean ± SEM. Two-way ANOVAs (factors treatment x lighting with Tukey's post hoc comparisons) were conducted.

Supplementary Table 2.1. Poly IC and saline-exposed males (cohort 1).

	Treatment x lighting		Treatment		Multiple comparisons *p*-values		
	F	*p* value	F	*p* value	LD1	LL	LD2
Open field							
Horizontal activity	1.121	0.3308	6.233	**0.0165**	**0.0456**	0.9999	0.1055
Thigmotaxis ratio	0.5049	0.6054	0.6147	0.4374			
Total distance traveled	0.3186	0.7281	7.398	**0.0095**	*0.0741*	0.5920	0.1417
Elevated plus maze							
Time in closed arms	0.2992	0.7422	0.7579	0.3892			
Percent in open arms	0.2075	0.8131	1.241	0.2719			
Entries in open arms	1.081	0.3441	1.013	0.3201			
Three-chamber social interaction							
Habituation ratio	1.284	0.2818	0.0760	0.7834			
Sociability ratio	2.575	***0.0846***	5.365	**0.0276**	0.9999	**0.0064**	0.9999
Social memory ratio	0.4207	0.6579	0.4752	0.4924			
Prepulse inhibition of acoustic startle							
Baseline startle response	0.2655	0.7689	1.889	0.1925			
Null response	1.228	0.3094	3.414	0.0875			
Average PPI	0.5246	0.5979	8.158	**0.0135**	0.2015	0.1891	0.6034

Notes: Results from two-way ANOVAs (treatment x lighting) for behavioral parameters from poly IC and saline-exposed males (cohort 1). ANOVA factors include treatment (poly IC, saline) and lighting (LD1, LL, LD2). P-values in **bold** represent significance, and p-values in ***bold and italics*** represent trending significance.

Supplementary Table 2.2. Poly IC and saline-exposed females (cohort 1).

	Treatment x lighting		Treatment		Multiple comparisons *p*-values		
	F	*p* value	F	*p* value	LD1	LL	LD2
Open field							
Horizontal activity	0.2626	0.7710	1.070	0.3185			
Thigmotaxis ratio	1.957	0.1602	0.6185	0.4447			
Total distance traveled	0.3498	0.7078	0.0761	0.7886			
Elevated plus maze							
Time in closed arms	0.0336	0.9670	14.63	**0.0019**	0.1709	0.3618	0.1988
Percent time open arms	1.083	0.3523	2.729	0.1208			
Percent entries open arms	0.1307	0.8778	1.188	0.2820			
Prepulse inhibition of acoustic startle							
Baseline startle response	0.1514	0.8602	2.463	0.1389			
Null response	1.228	0.3094	3.414	0.0875			
Average PPI	0.0445	0.9565	10.40	**0.0061**	0.1107	0.2148	0.3625

Notes: Results from two-way ANOVAs (treatment x lighting) for behavioral parameters from poly IC and saline-exposed females (cohort 1). ANOVA factors include treatment (poly IC, saline) and lighting (LD1, LL, LD2). P-values in **bold** represent significance.

Supplementary Table 2.3. Poly IC and saline-exposed males (cohort 2).

	Treatment x lighting		Treatment		Multiple comparisons *p*-values		
	F	*p* value	F	*p* value	LD1	LL	LD2
Open field							
Horizontal activity	0.1392	0.8703	0.0721	0.7899			
Thigmotaxis ratio	1.225	0.2999	2.944	0.0951			
Total distance traveled	0.4777	0.6222	0.0009	0.9754			
Elevated plus maze							
Time in closed arms	1.711	0.1882	0.0011	0.9742			
Percent in open arms	0.5300	0.5909	0.3528	0.5561			
Entries in open arms	2.602	***0.0813***	0.0067	0.9352	0.9999	0.9999	0.2475
Three-chamber social interaction							
Habituation ratio	0.8884	0.4167	0.9791	0.3303			
Sociability ratio	2.903	*0.0626*	2.665	0.1130	0.2553	*0.0985*	0.9999
Social memory ratio	2.814	0.0679	0.5923	0.4475			
Prepulse inhibition of acoustic startle							
Baseline startle response	1.670	0.1959	2.882	0.0987			
Null response	0.2325	0.7932	1.921	0.1748			
Average PPI	0.9913	0.9058	5.314	**0.0274**			

Notes: Results from two-way ANOVAs (treatment x lighting) for behavioral parameters from poly IC and saline-exposed males (cohort 2). ANOVA factors include treatment (poly IC, saline) and lighting (LD1, LL, LD2). P-values in **bold** represent significance, and p-values in ***bold and italics*** represent trending significance.

Supplementary Table 2.4. Poly IC and saline-exposed females (cohort 2).

	Treatment x lighting		Treatment		Multiple comparisons *p*-values		
	F	*p* value	F	*p* value	LD1	LL	LD2
Open field							
Horizontal activity	0.1265	0.8817	0.2458	0.6277			
Thigmotaxis ratio	0.4366	0.6505	1.473	0.2450			
Total distance traveled	3.161	***0.0579***	0.9729	3.407	***0.0983***	0.9999	0.9999
Elevated plus maze							
Time in closed arms	0.2639	0.7700	0.7849	0.3888			
Percent in open arms	0.1771	0.8386	0.8595	0.3677			
Entries in open arms	0.5131	0.6043	0.4869	0.4953			
Prepulse inhibition of acoustic startle							
Baseline startle response	0.1876	0.8299	0.9038	0.3579			
Null response	0.3736	0.6916	2.316	0.1503			
Percent PPI	2.657	***0.0878***	1.358	0.2633	***0.0642***	0.9999	0.9999

Notes: Results from two-way ANOVAs (treatment x lighting) for behavioral parameters from poly IC and saline-exposed females (cohort 2). ANOVA factors include treatment (poly IC, saline) and lighting (LD1, LL, LD2). P-values in ***bold and italics*** represent trending significance.

Supplementary Table 2.5. Sex differences statistics from poly IC and saline-exposed mice.

	Cohort 1						Cohort 2					
	Treatment x Sex x Lighting		Treatment x Sex		Main Effect Sex		Treatment x Sex x Lighting		Treatment x Sex		Main Effect of Sex	
	F	*p-value*	F	*p-value*	F	*p-value*	F	*p-value*	F	p-value	F	p-value
Open field												
Horizontal activity	0.9857	0.3764	2.497	0.1197	2.070	0.1558	0.0233	0.9770	0.0182	0.8932	0.2762	0.6016
Thigmotaxis ratio	3.966	**0.0217**	0.9519	0.3334	15.37	**0.0002**	0.6026	0.5494	3.987	***0.0514***	19.64	**0.0001**
Total distance traveled	0.3353	0.7158	0.8703	0.3549	0.050	0.8226	0.7899	0.4567	0.4038	0.5281	0.6017	0.4417
Elevated plus maze												
Time in closed arms	0.2433	0.7844	4.334	**0.0422**	22.89	**0.0001**	0.9537	0.3890	0.2298	0.6338	0.3918	0.5343
Percent in open arms	0.4060	0.6673	1.222	0.2739	15.31	**0.0003**	0.4148	0.6617	1.128	0.2934	0.0550	0.8155
Entries in open arms	0.5478	0.5799	2.194	0.1443	17.44	**0.0001**	0.6839	0.3814	0.1210	0.7295	2.627	0.1116
Prepulse inhibition												
Baseline startle response	0.0193	0.9809	0.1920	0.6631	12.41	**0.0009**	0.7600	0.4705	0.2227	0.6391	14.88	**0.0003**
Average PPI	0.0403	0.9605	1.084	0.3024	0.010	0.9207	1.651	0.1972	0.2288	0.6346	0.1630	0.6882

Notes: Results from three-way ANOVAs (treatment x lighting x sex) for behavioral parameters from poly IC and saline-exposed mice, while also highlighting the treatment x sex interactions and main effects of sex analysis. ANOVA factors include sex (male, female),

treatment (poly IC, saline exposure) and lighting (LD1, LL, LD2). P-values in **bold** represent significance, and p-values in ***bold and italics*** represent trending significance.

CHAPTER 3: EFFECTS OF PRENATAL IMMUNE ACTIVATION AND EXPOSURE TO CIRCADIAN DISRUPTION DURING ADOLESCENCE: EXPLORING THE TWO-HIT MODEL OF NEURODEVELOPMENTAL DISORDERS

Preface:

Chapter 2 presented behavioral and microglial findings after adulthood subjected to LL in mice exposed to prenatal poly IC. **Chapter 3** builds upon these findings, by investigating the behavioral, microglial, and transcriptomic effects in male and female MIA offspring after exposure to LL in adolescence. Adolescence was chosen as the time point for the exposure to LL because it is a time when the brain undergoes significant structural and functional changes, there is also evidence for adolescence being a time of heightened sensitivity to stressors compared to adulthood, and finally, adolescence is when drastic changes in sleep and circadian rhythms occur.

CHAPTER 3: EFFECTS OF PRENATAL IMMUNE ACTIVATION AND EXPOSURE TO CIRCADIAN DISRUPTION DURING ADOLESCENCE: EXPLORING THE TWO-HIT MODEL OF NEURODEVELOPMENTAL DISORDERS

Tara C. Delorme[1,2], Nikki Huebener[1], Danusa M. Arcego[1,3], Nick O'Toole[1], Patrícia P. Silveira[1,3], Lalit K. Srivastava[1,3,*] and Nicolas Cermakian[1,3,*]

[1] Douglas Mental Health University Institute, Montréal, Québec, H4H 1R3.

[2] Integrated Program in Neuroscience, McGill University, Montréal, Québec, H3A 2B4.

[3] Department of Psychiatry, McGill University, Montréal, Québec, H3A 1A1.

*Co-corresponding authors

3.0. Abstract

Around 80% of individuals with neurodevelopmental disorders such as schizophrenia and autism spectrum disorders experience disruptions in their sleep/circadian rhythms. Given that neurodevelopmental disorders are caused by exposure to multiple risk factors, we explored whether prenatal infection, an established risk factor for neurodevelopmental disorders, and circadian disruption could synergistically induce sex-specific deficits in offspring. The maternal immune activation (MIA) protocol was implemented by injecting pregnant mice with a viral mimic poly IC (or saline) at embryonic day 9.5. Offspring were subjected to either standard lighting (12:12LD) or constant light (LL) between 3-7 weeks of age (i.e. juvenile and adolescent ages). At the end of 7 weeks, separate groups of LD and LL mice with or without MIA were used for each of the three experiments: the first cohort underwent behavioral testing and differences in cognitive, anxiety and sociability-related behaviors were observed. In the second cohort, brains were harvested for immunohistochemistry to analyze microglia in the prefrontal cortex and hippocampus. MIA in males, but not females, caused a more activated microglial profile, while LL exposure induced a less reactive profile and less dense distribution of microglia in the dentate gyrus but not the prefrontal cortex, and the CA1 region. In the third cohort, gene expression profile using RNA sequencing was performed in the dorsal hippocampus. MIA led to several differentially expressed genes in males, which was diminished by LL. The rank-rank hypergeometric overlap algorithm identified transcriptional synchrony between groups within sexes, and discordant patterns between sexes. The weighted correlation network analysis identified significant clusters of co-expressed genes that were associated with either MIA or LL. Given that circadian disruption is ubiquitously experienced in the 24-hour society and the high transmission of viral infections that affects many pregnant individuals, it is critical to understand the interaction between circadian

disruption and MIA. Our findings provide insights into the cellular and molecular mechanisms on how circadian disruption affects development of neurodevelopmental disorders.

3.1. Introduction

Epidemiological evidence suggests that prenatal infection, which induces maternal immune activation (MIA), is a risk factor for schizophrenia and autism spectrum disorders (ASD) (Brown, Begg et al. 2004, Lee, Magnusson et al. 2015). MIA has been described as a disease primer, increasing an individual's susceptibility to develop different disorders (Estes and McAllister 2016). Genetic mutations and environmental adversities (especially in early life) can further increase risk, and how different risk factors interact with each other is an active area of research (Davis, Eyre et al. 2016, Schaafsma, Gagnidze et al. 2017).

Psychiatric symptoms in individuals with schizophrenia typically begin in a prodromal stage (Hafner and Maurer 2006), followed by the full display of symptoms in adolescence to early adulthood. Specifically, in males this occurs around 15 to 25 years old and in females around 15 to 30 years old, with a second flatter peak at menopausal age (44 to 49 years old) (Häfner 2019). Adolescence is also a period of significant changes in the brain, especially in limbic and cortical regions, and in the maturation of connectivity between brains regions. In addition, there are increases in axons and synapses, followed by their rapid pruning later in adolescence, as well as maturation of many neurotransmitter systems (Spear 2000). Adolescence is also a period of increased vulnerability to stressors. For example, exposure to drugs of abuse (e.g., cannabis, ethanol, and nicotine) or stress during adolescence induces stronger and more persistent adverse effects later in life compared with exposure in adulthood (Slawecki, Thorsell et al. 2004, Meier, Caspi et al. 2012, McCormick and Green 2013).

Circadian rhythms are ~24 hour cycles in behavior and physiology that are generated through clock mechanisms present in most mammalian cells (Patke, Young et al. 2020). These endogenously generated rhythms need rhythmic environmental cue, such as light exposure, to maintain synchrony with our environment (Hastings, Maywood et al. 2018). Disruptions to the circadian system can be detrimental to mental and physical health, increasing the risk for cardiovascular disease (Puttonen, Harma et al. 2010), cancer (Costa, Haus et al. 2010), sleep disorders (Drake, Roehrs et al. 2004) and negative mental health outcomes (Brown, Martin et al. 2020). Many individuals with schizophrenia and ASD experience disruptions in sleep, rest/activity rhythms, daily hormone rhythms and circadian clock gene expression (Krakowiak, Goodlin-Jones et al. 2008, Wulff, Dijk et al. 2012, Johansson, Owe-Larsson et al. 2016). Circadian disruption is also prevalent during adolescence. Firstly, the timing of sleep onset and waking and other biological rhythms becomes later in adolescence (reviewed here: Logan and McClung 2019). This usually results in sleep deprivation during the school week and needing to oversleep on weekend (Perkinson-Gloor, Lemola et al. 2013). Exposure to light at night is also prominent in adolescents from light emitting devices for reading, socializing and entertainment (Fonken and Nelson 2016). Thus, we were particularly interested in exploring the risk of exposure to circadian rhythm disruption during adolescence to the development of psychiatric disorders such as schizophrenia.

In this study, we investigated if MIA (first risk factor in-utero) combined with exposure to circadian disruption (second risk factor during adolescence) in mice would synergistically induce behavioral, microglial and transcriptomics changes associated with neurodevelopmental disorders in males and females. Prenatal infection was modelled in mice by injecting pregnant mice with a viral mimic, called polyinosinic:polycytidylic acid (poly IC) at embryonic day 9.5 (Haddad, Patel et al. 2020). We modeled circadian disruption by exposing mice to constant light (LL) exposure in

early development (3-7 weeks of age).

3.2. Materials and Methods

3.2.1. Animals and housing

As previously described (Delorme, Srivastava et al. 2021), 8-week-old male and female C57BL/6J mice (Jackson Laboratory; product number: 000664) were used in a mating protocol. For the three-chamber social interaction test, male and female C57BL/6 mice were obtained from Charles River Laboratories (Saint-Constant, QC, Canada) and matched for age and sex with the test mice. Offspring from the MIA protocol and controls were housed in light-proof, ventilated cabinets with a light intensity of 150-200 lux (cool white LED lighting) controlled by an external timer (Actimetrics, Wilmette, IL, USA). Animal use adhered to the Canadian Council of Animal Care guidelines and was approved by the Douglas/McGill University Animal Care Committee.

3.2.2. MIA protocol and litter details

MIA was performed as previously described (Delorme, Srivastava et al. 2021). After a 2-week habituation period, male and female mice were mated overnight, and females were checked within the first hour of lights on for the presence of a vaginal plug. On embryonic day 9.5 (9 days after the plug was found), pregnant dams were intraperitoneally injected with poly IC dissolved in double-distilled water (5 mg/kg; lot 1: 059M4167V; Sigma-Aldrich, St. Louis, MO, USA) or sterile saline, and dams were left undisturbed to deliver their litters.

In the first experiment (behavior) (**Supplementary Fig. 3.1A-C**), 29 female breeders were mated with 15 male breeders: 3 dams were not pregnant, 11 out of 14 poly IC-injected dams gave birth (average litter size: 6.44, total male offspring used= 35, total female offspring used = 22) and

10 out of 12 saline injected dams gave birth (average litter size: 6.55, total male offspring used = 33, total female offspring used = 25). For the second experiment (microglia) (**Supplementary Fig. 3.1D-F**), 18 female breeders were mated with 9 male breeders: 2 dams were not pregnant, 6 out of 9 poly IC-injected dams gave birth (average litter size: 7.25, total male offspring used = 29, total female offspring used = 28) and 6 out of 7 saline-injected dams gave birth (average litter size: 6.4, total male offspring used = 13, total female offspring used = 29). For the third experiment (transcriptomics) (**Supplementary Fig. 3.1G-I**), 20 female breeders were mated with 10 male breeders: 5 dams were not pregnant, 4 out of 8 poly IC-injected dams gave birth (average litter size: 7.5, total male offspring used = 14, total female offspring used = 16) and 7 out of 7 saline-injected dams gave birth (average litter size: 6.9, total male offspring used = 27, total female offspring used = 21).

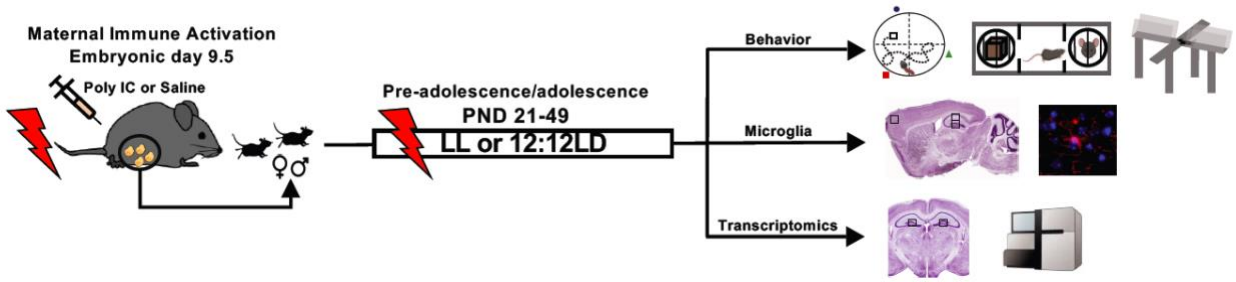


Fig. 3.1. Experimental timeline. On E9.5 pregnant dams were intraperitoneally injected with poly IC or saline. Poly IC- and saline-exposed male and female offspring were aged to pre-adolescence/adolescence and were subjected to 4 weeks of either: standard lighting (12:12LD) or constant light (LL). Three separate cohorts of mice were used: the first underwent behavioral testing and tests included the open field test, elevated plus maze, three-chamber social interaction test, Barnes maze and prepulse inhibition of acoustic startle. The second cohort had their brains harvested for immunohistochemistry to analyze microglia in the prefrontal cortex, dentate gyrus, and area CA1. In the third cohort, the hippocampus was microdissected and RNA sequencing was performed.

3.2.3. Verification of inflammatory response in pregnant dams after poly IC infection

As previously described (Delorme, Srivastava et al. 2021), trunk blood was collected from pregnant dams 3 hours after poly IC or saline injections. The Mouse Cytokine Array Proinflammatory Focused 10-plex (MDF10, Eve Technologies, Calgary, AB, Canada) was used for the following pro/anti-inflammatory biomarkers: IL-6, IL-2, IL-1β, IL-10, TNFα, MCP-1, IL-4, IL-12p70 and GM-CSF (**Supplementary Fig. 3.2**). Samples that were below the detectable range were designated as 0 pg/mL, and IFNγ was not reported because all samples were below the minimum detectable concentration.

3.2.4. Experimental timeline

The experimental protocol is depicted in **Fig. 3.1.** Poly IC- and saline-exposed male and female offspring were aged to 3 weeks old, weaned and placed into light-proof, ventilated cabinets and subjected to 4 weeks of either standard lighting (12:12LD) or constant light (LL) (i.e., from 3 weeks to 7 weeks, equivalent to juvenile/adolescence). At 7 weeks, separate groups of mice were used for each of the three experiments: the first underwent behavioral testing under dim light conditions, where the lighting intensity at the center of the testing chambers was 10 lux (except for Barnes maze). In the second cohort, brains were harvested for immunohistochemistry to analyze microglia in the prefrontal cortex, dentate gyrus, and the CA1 region. In the third cohort, the hippocampus was microdissected, and RNA sequencing was performed.

3.2.5. Behavioral outcomes

For the behavioral tests, 16-18 males and 10-14 females were used per group (MIA/saline), lighting condition (LD/LL) for males and females. Due to practical constraints, a subset of mice

(n=8 and n=9-11) were randomly chosen per group for the social interaction test and the Barnes Maze respectively. All behavioral tests were conducted as described previously (Delorme, Ozell-Landry et al. 2023). The behavioral tests were performed between 1 hour and 3 hours after lights on [Zeitgeber Time (ZT) 1-3]. In the LL condition, mice were free running, i.e., they were not entrained to any external stimulus (lights on/lights off). Thus, passive infrared sensors were used to measure general home cage locomotion to assess when the mice were active versus inactive. The time at which the mice became active was considered Circadian Time (CT) 12, and CT 0 was defined as half a period (~12 hours) later. Thus, behavioral tests under LL were conducted between CT 1-3 for each cage of mice. Behavioral tests were performed in order of least to most stressful (i.e., open field, EPM, three-chamber social interaction, PPI, Barnes maze). Two days of rest were scheduled between each test. The testing room was dimly lit for each test (10 lux) except for the Barnes Maze.

3.2.5.1. Open field test

We utilized the VersaMax Legacy Open Field setup (AccuScan Instruments, Inc., Columbus, OH, USA) system, and collected data using the Versamax Software (version 4.0, 2004; AccuScan Instruments, Inc.). Measures such as total distance traveled and thigmotaxis (time spent in the outer edges of the apparatus divided by time spent in the center) were analyzed (Valle 1970).

3.2.5.2. Elevated plus maze (EPM) test

The elevated apparatus consisted of a plus-shaped maze where 2 opposing arms (closed arms) were enclosed by high walls, while the other two opposing arms (open arms) did not have walls. Mice were placed in the intersection of the four arms, facing an open arm. Mice were

allowed to freely explore the chamber for 5 minutes, after which they were returned to their home cage. Time spent and entries in each arm was scored.

Formula for anxiety-like behavior =

$$\frac{\text{Time spent in open arms (seconds)}}{\text{Time spent in open arms (seconds)}+\text{Time spent in closed arms (seconds)}} \times 100$$

3.2.5.3. Three-chamber social interaction test

The test is comprised of 3 consecutive phases. In the habituation phase, mice were allowed to freely explore the three-chamber testing apparatus.

Formula for Habituation =

$$\frac{\text{Time spent in chamber 1 (seconds)}}{\text{Time spent in chamber 1 (seconds)}+\text{Time spent in chamber 2 (seconds)}}$$

In the social preference phase, the test mouse was free to interact with stranger 1, a mouse located in one of the chambers, or an object, located in the other chamber.

Formula to calculate social preference =

$$\frac{\text{Time spent in stranger 1 chamber (seconds)}}{\text{Time spent in stranger 1 chamber (seconds)}+\text{Time spent in object chamber (seconds)}}$$

In the social memory phase, the test mouse was free to interact with stranger 2, a novel mouse located in the chamber that previously had an object, or stranger 1, the same mouse as in the previous trial.

Formula to calculate social memory =

$$\frac{\text{Time spent in stranger 2 chamber (seconds)}}{\text{Time spent in stranger 2 chamber (seconds)}+\text{Time spent in stranger 1 chamber (seconds)}}$$

3.2.5.4. Barnes maze

The Barnes maze was a circular platform elevated 150 cm off the ground, with 20 holes around the perimeter and a diameter of 120 cm. A camera and bright light were placed above the platform, and a white noise sound was played (application called "white noise" on android, sound: blue noise, system volume: 10, 50 decibels) to motivate the test mice to seek shelter in an escape box, which is placed under one of the holes of the apparatus. Mice were trained to locate this escape box using a configuration of visual cues located around the testing area (an image of a black square, circle and triangle located equidistant around the apparatus). There were two training days: the first training day consisted of three trials, and the second training day consisted of two trials, where the test mice had three minutes to locate the escape box. If the mice did not locate the escape box, they were gently guided to the escape box and a lid was placed on the escape box for 1 minute. The latency to the escape box was assessed for each training trial. On the test day, the escape box was removed, and the time spent in the target location was assessed for 90 seconds. The latency to the target quadrant, entries and duration in target quadrant, duration in each quadrant and number of errors was assessed.

3.2.5.5. Prepulse inhibition of acoustic startle (PPI)

PPI was measured using SR-LAB startle chambers (San Diego Instruments, San Diego, CA, USA), and a software package by SR-LAB. Mice were placed into a cylindrical Plexiglass enclosure. Each session consisted of 50 trials, some of which only had a startle tone, and some trials had a prepulse played before the startle tone. A piezoelectric accelerometer fixed to the Plexiglass base was used to detect and transduce motion resulting from the animal's startle

response. Each trial began with a 5-minute acclimation period. In the first 6 and final 6 trials, a 'startle tone' (120 dB for 50 ms, broad-band noise burst) was played in the absence of a prepulse (called 'startle-only' trials). In the next 38 trials, 8 trials were 'startle-only' trials, 5 trials had no stimulus, and 25 trials had the startle tone preceded by a prepulse stimulus (30 ms, broad-band burst) that was either 6, 9, 12 or 15 dB above the background noise and presented 100 ms before the startle pulse.

$$\% \text{ prepulse inhibition } = 100 - \left(\frac{\text{Startle amplitude on prepulse trials}}{\text{Startle amplitude on pulse alone trials}}\right) \times 100$$

3.2.6. Microglia characterization

The microglia characterization was conducted in a separate cohort of male and female mice as described previously (Delorme, Ozell-Landry et al. 2023).

3.2.6.1. Tissue preparation and immunohistochemistry

Mice [40 mice: 5 mice per group (MIA/saline), lighting condition (LD/LL) for males and females] were transcardially perfused using 4% paraformaldehyde (# 31985-062, Invitrogen). 30 μm thick sagittal sections containing the medial prefrontal cortex and dorsal hippocampus regions (lateral 1.08 mm until 1.80 mm; medial prefrontal cortex bregma +2.46 mm until +1.94 mm; dorsal hippocampus -1.46 mm until -2.06 mm) based on the stereotaxic atlas of Paxinos and Franklin (3rd edition) (Paxinos and Franklin 2019), were processed for immunofluorescence staining for a microglial marker Iba1 (ionized calcium binding adaptor molecule 1) and imaging. The primary antibody used was Rabbit anti-Iba1 (1:1000, # 019-19741, Cedarlane) and the secondary antibody was Alexa fluor 594 Donkey anti-rabbit IgG (1:1000, # 711-585-152, Cedarlane). The sections were counter stained with DAPI (1:20000, # D9542, Sigma).

3.2.6.2. Microglia visualization and analysis

Iba-1 immunostained microglia from the dorsal hippocampus (dentate gyrus and CA1) and medial PFC were imaged using Z-stacks at a 20X magnification. Brain sections were visualized using an Olympus BX63 automated fluorescence microscope. The images were analyzed using Fiji ImageJ software, while being blinded to the experimental conditions. Measures of morphology included soma area and perimeter, arborization area and perimeter and soma circularity. A morphological index was calculated by dividing the soma area by the arborization area for each cell. The 'nearest neighbor distance' plugin was then applied to determine the distance between each cell and its closest neighbor. The density of microglia for each image was calculated by dividing the number of cells in that image by the region of interest identified. A spacing index was calculated by multiplying the average density for each animal obtained above by the square of the average nearest neighbor distance.

3.2.7. Transcriptomics

Transcriptomic profiles were assessed in a separate cohort of mice (**Fig. 3.1)** in the dorsal hippocampus (1 mm thick punch containing bregma –1.46 mm until -2.46 mm) using next generation RNA sequencing.

3.2.7.1. Tissue collection and pre-processing of data

Male and female mice were euthanized by decapitation, brains were rapidly extracted and sliced (1 mm sections). The dorsal hippocampus tissue was punched (0.5 mm diameter), and flash frozen on dry ice. RNA was extracted using trizol/chloroform, and RNA quantity and integrity

was analyzed using a Nanodrop (Thermo Fisher). Sequencing was performed at the IRIC (Institute for Research in Immunology and Cancer) genomics sequencing platform at the University of Montreal (Nextseq 1x75bp, around 20M reads/samples) for 32 samples [4 samples per group (MIA/saline), lighting condition (LD/LL) for males and females]. Pre-alignment quality control was performed at the sequencing platform using FastQC. Alignment was performed using the STAR aligner on the mouse genome build (Mouse_ENSEMBL_Gene_ID_Human_Orthologs). 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 4 samples were excluded. Only genes annotated as protein coding according to the ensembl's biomart Mus musculus package were used for analyses (https://bioconductor.org/packages/release/data/annotation/html/Mus.musculus.html). We assessed outliers in normalized data using unsupervised hierarchical clustering of mice by principal component analysis (PCA).

3.2.7.2. Differential expression (DE) analysis

DE analysis was performed using Deseq2 package (Love, Huber et al. 2014), which modelled the raw counts (using normalization factors to account for differences in library depth), then estimated the gene-wise dispersions and shrunk these estimates to generate more accurate estimates of dispersion to model the counts. Then finally, Deseq2 fit the negative binomial generalized linear model.

3.2.7.3. Rank rank hypergeometric overlap 2 algorithm (RRHO2)

The updated and threshold-free algorithm now called RRHO2 was used to directly compare separate gene sets to reveal overlapping trends. Differential expression lists were firstly ranked by

their -$\log_{10}$(p-value) and effect size direction, then lists were compared to identify significant overlap in genes across a continuous significance gradient. RRHO was used to identify concordant and discordant overlap, which described overlap in genes that were respectively up and downregulated in both gene expression signatures, as well as overlap of expression profiles moving in opposite directions in the same dataset (Cahill, Huo et al. 2018). We assessed concordant and discordant between sexes for MIA versus saline, and LD versus LL. RRHO maps are heatmaps which visualized the intensity of significant overlap between two ranked lists. They computed the normal approximation of difference in log odds ratios and standard error of overlap. The z-scores were then converted to a p-value and corrected for multiple comparisons across pixels.

3.2.7.4. Weighted Gene Co-expression Network analysis

RNA sequencing data were analyzed using the R package Weighted Gene Co-expression Network analysis (WGCNA) to identify gene co-expression networks related to the different interventions (Langfelder and Horvath 2008). WGCNA allowed for the identification of gene co-expression modules, within these network structures, related to either poly IC and LL exposure in males and females. A correlation coefficient (r) was reported for each identified module, with either a positive value to indicate a positive association or a negative value to indicate a negative association. A p-value was also reported for each identified module.

3.2.8. Statistics

Data were analyzed and graphed using Prism version 9 (GraphPad). Data describing number of pups per saline versus poly IC litter, and maternal cytokine and chemokine expression

levels were analyzed using an independent samples t-test. Data that did not pass the Shapiro-Wilk normality test were analyzed by Mann-Whitney U test, and data with unequal variances (assessed by an F-test) were analyzed by an independent samples t-test with Welsh's correction (**Supplementary Fig. 3.1A, D, G; Supplementary Fig. 3.2).** For offspring weight data, we conducted 2-way mixed effect ANOVAs, with group (poly IC or saline) as a between-subjects variable and age (post-natal week 3, 5, 8) as a within-subjects variable. We explored group x age interactions and main effects for each sex separately (**Supplementary Fig. 3.1B, C, E, F, H, I).** For the behavior and microglia data, 2-way mixed effect ANOVAs were conducted using Tukey's post hoc comparisons, with group (poly IC or saline) and lighting condition (LD, LL) as a between-subjects variables. Main effects were explored if no significant group x lighting interaction was observed (**Fig. 3.2-3.6, Supplementary Fig. 3.3-3.6**). Three-way ANOVAs were also conducted (using SPSS statistical software version 29) for prepulse inhibition (3^{rd} factor: prepulse) and locomotion (3^{rd} factor: time) (**Supplementary Fig. 3.3-3.4**). WGCNA (**Fig. 3.7-3.8)** and RRHO (**Supplementary Fig. 3.7)** analyses were assessed as previously described (Langfelder and Horvath 2008, Cahill, Huo et al. 2018). Sex differences (**Supplementary Table 3.5)** were assessed using three-way ANOVAs (group x lighting x sex) and decomposed by assessing the 2-way interaction (group x sex) and observing the main effect of sex. Differences were considered significant if $p < 0.05$.

3.3. Results

3.3.1. Litter details

Litters from poly IC- and saline-exposed dams did not differ in number of pups (behavior: $p = 0.9094$; microglia: $p = 0.5009$; transcriptomics: $p = 0.3527$). (**Supplementary Fig. 3.1A, D, G**). Only litters with 4 to 10 pups inclusively were used for testing, thus 1 saline and 2 poly IC from the first cohort (behavior) were excluded as these litters only had 3 pups. Mice in 4-6 litters were used in each experimental condition, and we visually confirmed that significant effects reported were not driven by mice from the same litter. Additionally, MIA offspring weighed significantly less than saline-exposed offspring only in the cohort used for behavior (**Supplementary Fig. 3.1B, C**) (effect of group male: $p = 0.0006$, female: $p < 0.0001$), but not the cohorts used for microglia analysis or transcriptomics (**Supplementary Fig. 3.1E, F, H, I**).

3.3.2. Verification of inflammatory response in pregnant dams after poly IC infection

We confirmed that the lot of poly IC induced an inflammatory response in pregnant dams (n=3 per group). Poly IC-injected dams had a significant elevation in blood plasma expression of the following pro- and anti-inflammatory cytokines: IL-6 ($p = 0.0427$), IL-10 ($p = 0.0014$), TNFα ($p = 0.0029$) and MCP-1 ($p = 0.0384$) compared to controls, with no significant differences in IL-12(p70) ($p = 0.4608$), IL-2 ($p = 0.8754$), IL-1β ($p = 0.6798$), IL-4 ($p = 0.4990$) and GM-CSF ($p = 0.2844$) (**Supplementary Fig. 3.2A-I**).

3.3.3. Behavior

3.3.3.1. Similar spontaneous locomotion in saline and poly IC-exposed mice.

Male and female poly IC- and saline-exposed offspring under LD and LL had similar scores on total distance traveled in the open field test, which is a measure of spontaneous locomotion (**Supplementary Fig. 3.3B, D**).

3.3.3.2. LL causes decreased anxiety-like behavior in saline- but not poly IC- exposed male mice, while no differences are observed in females.

In males, while no significant interactions were observed, a main effect of lighting condition was noted in time spent in open arms (p = 0.0078), percent time in open arms (p = 0.0045) and percent entries in open arms (p = 0.0091). We further explored this data by conducting post doc comparisons, which revealed that, LL exposure in saline-exposed mice caused a decrease in anxiety-like behavior compared to saline/LD (time spent in open arms, p = 0.0204, percent time in open arms, p = 0.0237 and percent entries in open arms, p = 0.0121). Interestingly, LL did not have this effect in poly IC exposed mice (**Fig. 3.2A-C).** In females, poly IC-exposed mice showed no significant differences in the tested parameters (**Fig. 3.2D-F**). Additionally, no differences were observed in thigmotaxis ratio in the open field test (**Supplementary Fig. 3.3A, C**).

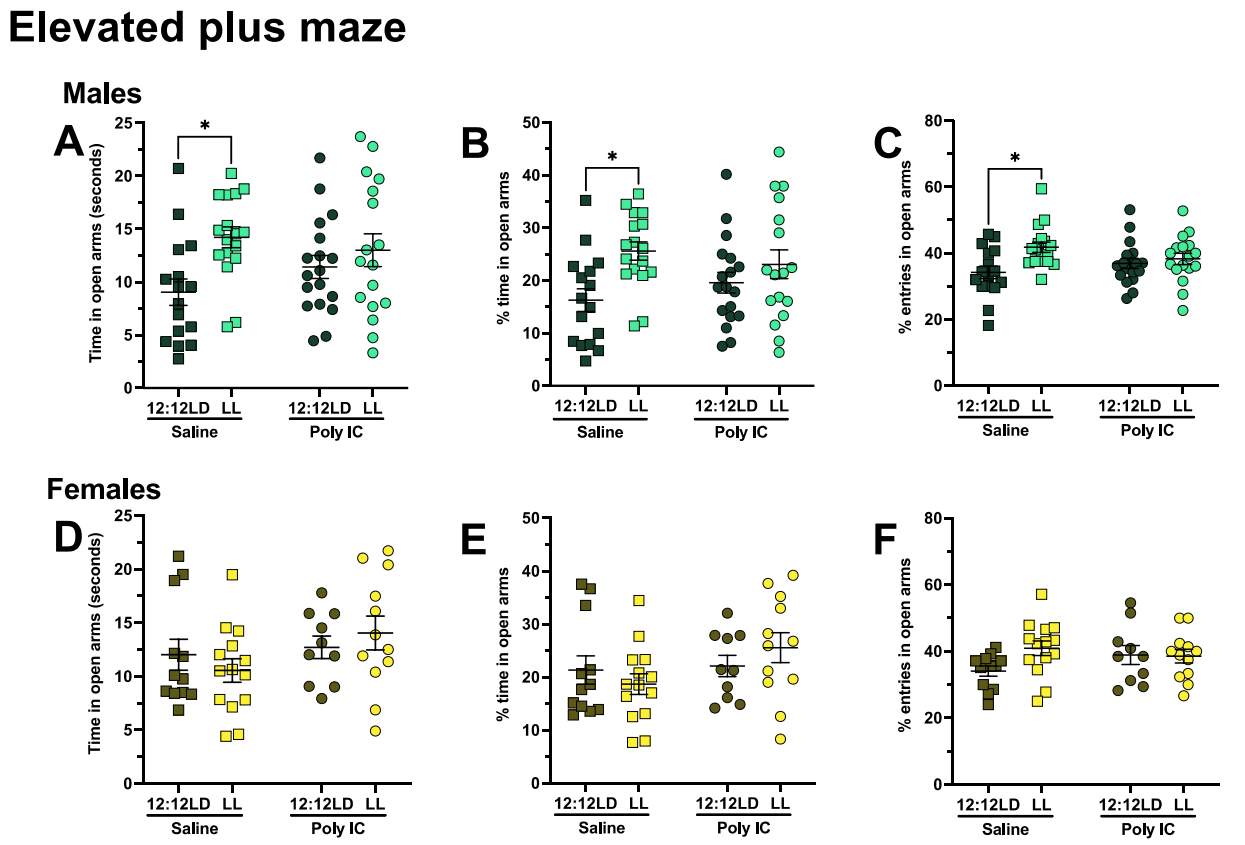


Fig. 3.2. LL causes decreased anxiety-like behavior in saline- but not poly IC- exposed males, while no differences are observed in females. The elevated plus maze was used to assess anxiety-like behavior. Time in open arms (**A, D**), percent time in open arms (**B, E**) and percent entries in open arms (**C, F**) were assessed in males (**A-C**) and females (**D-F**). Data points represent individual mice, and are presented as mean ± SEM. Two-way ANOVAs (factors group x lighting with Tukey's post-hoc comparisons) were conducted. *$p < 0.05$ (post hoc).

3.3.3.3. Reduced sociability in males following prenatal poly IC exposure after 12:12LD, but not LL.

In the habituation phase, male and female test mice spent a similar amount of time in each chamber (**Fig. 3.3A, D**). In the sociability phase, a trend for a group x lighting interaction was observed in male mice ($F_{(1, 28)} = 4.166$, $p = 0.0508$). Interestingly, further analysis revealed that prenatal poly IC exposure led to a trending decrease in sociability under 12:12LD ($p = 0.0642$), but not under LL (**Fig. 3.3B).** For females, although most showed a preference for the stranger 1 zone (over an object), no differences were seen between groups (**Fig. 3.3E).** During the social memory phase, there were no significant differences in social memory in males (**Fig. 3.3C**) or females (**Fig. 3.3F**). In sum, poly IC led to a reduced sociability phenotype in males, which was not apparent under LL exposure; these effects were not seen in females.

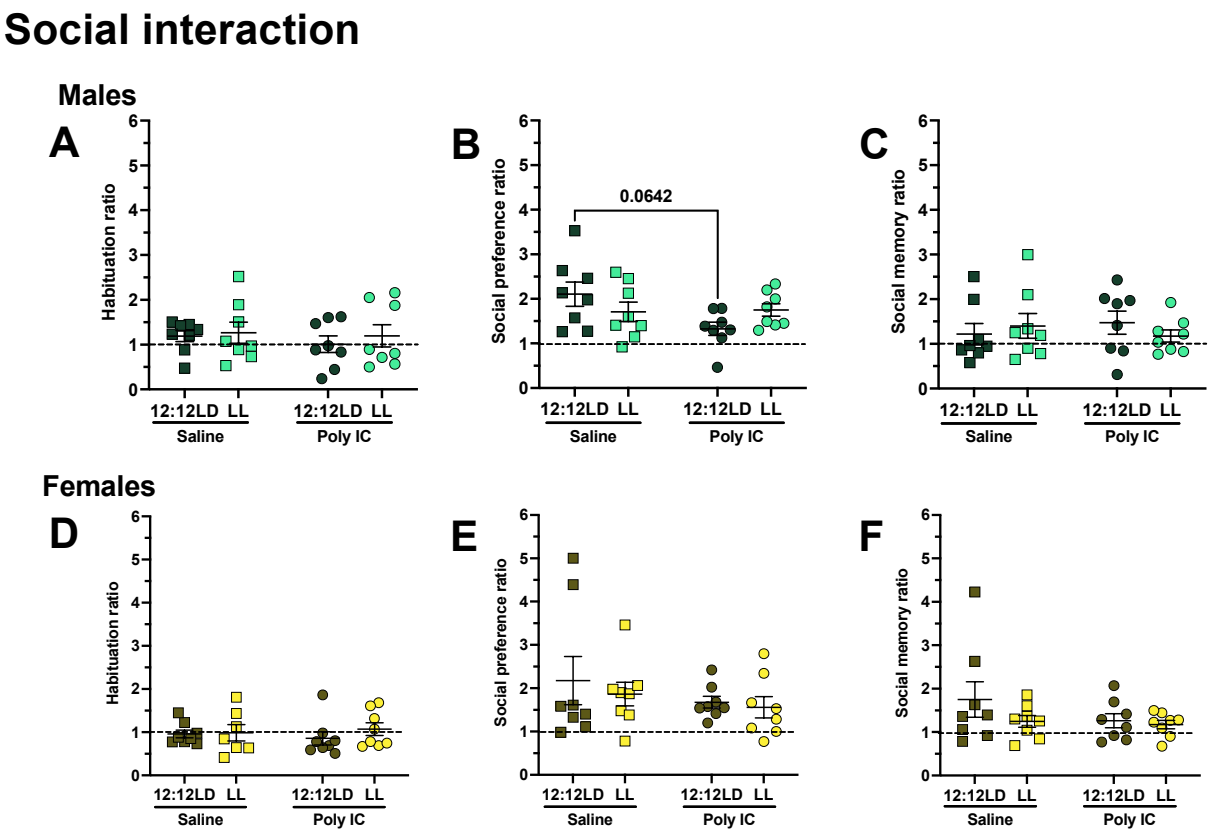


Fig. 3.3. Reduced sociability following prenatal poly IC exposure under 12:12LD, but not LL, and no differences observed in females. The three-chamber social interaction test was used to assess sociability and social memory. Preference ratios were assessed for the habituation phase **(A, D)**, sociability phase (**B, E**) and social memory phase (**C, F**) were assessed in males (**A-C**) and females (**D-F**). Data points represent individual mice, and are presented as mean ± SEM. Two-way ANOVAs (factors group x lighting with Tukey's post-hoc comparisons) were conducted.

3.3.3.4. Improved performance on Barnes maze after prenatal poly IC exposure in males.

During the training phase, all groups spent a similar amount of time locating the escape box (**Fig. 3.4A**). On the test day, all groups spent significantly more time in the target quadrant location, than other quadrants (effect of quadrant, $F_{(1, 38)} = 2.283$, $p < 0.0001$) (**Fig. 3.4B**). Prenatal poly IC led to increased duration ($p = 0.0322$) and entries ($p = 0.0438$) in the target quadrant, without significant treatment x lighting interactions being observed (**Fig. 3.4C, D**). A trend for a group x lighting interaction was observed in the latency to reach the target quadrant ($F_{(1, 36)} = 3.357$, $p = 0.0752$), with post hoc tests revealing a trend for a difference between poly IC/LL and all other groups: saline/12:12LD ($p = 0.0807$), poly IC/12:12LD ($p = 0.0703$) and saline/LL ($p = 0.0534$) (**Fig. 3.4E**). Lastly, when considering the number of errors made before reaching the area the escape box used to be, poly IC-exposed mice showed a trending effect for less errors (effect of group, $F_{(1, 37)} = 3.538$, $p = 0.0679$) (**Fig. 3.4F**), with no significant treatment x lighting interaction. Post hoc analyses showed that poly IC/LL is likely driving these effects (saline/12:12LD vs. poly IC/LL*:* $p = 0.0422$) (**Fig. 3.4F**). Overall, this shows that poly IC-exposed mice performed better on the Barne's maze, especially the poly IC/LL group.

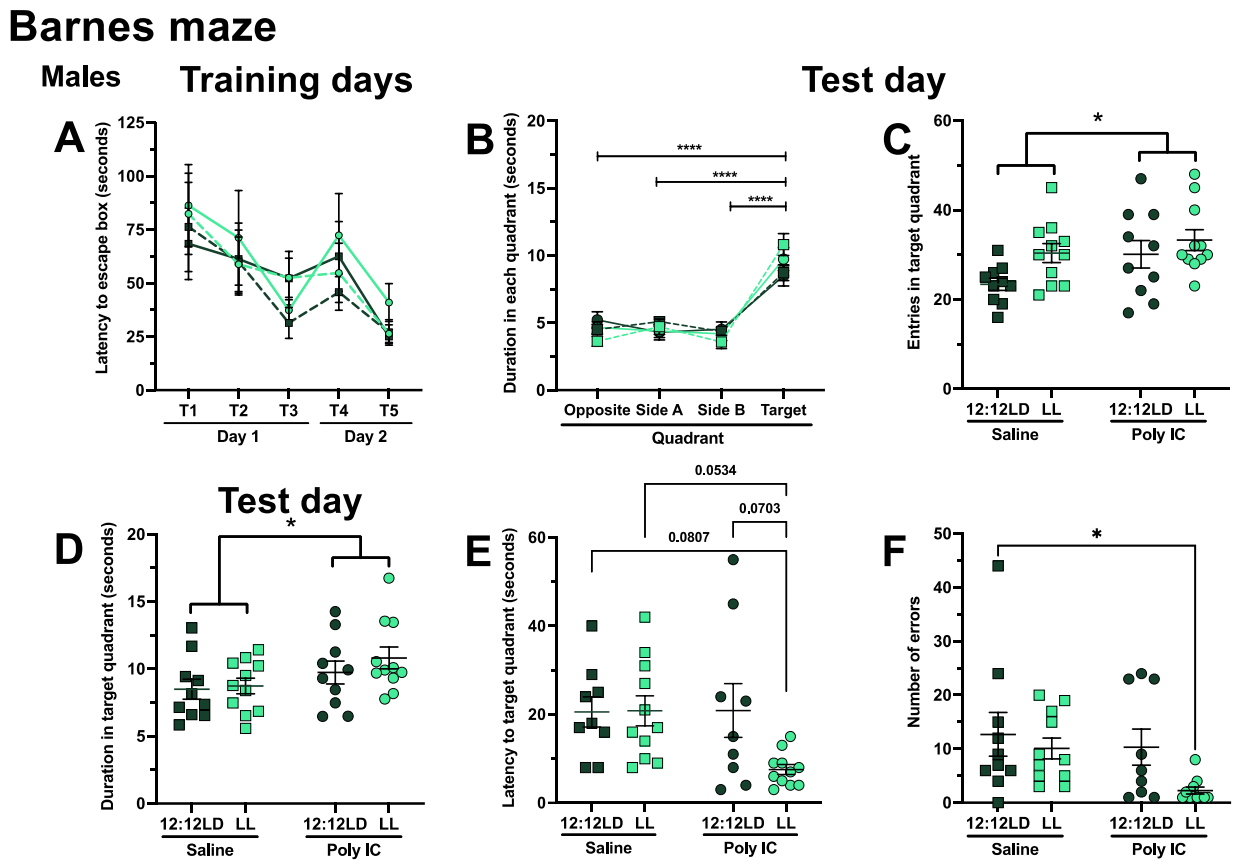


Fig. 3.4. Improved performance on Barnes maze after prenatal poly IC exposure in males. The Barnes maze was used to assess learning and memory. During the 2 training days, latency to target box in the 5 learning trials (**A)** was assessed. On the test day, latency to target quadrant (**A**), entries in target quadrant (**B**), duration in each quadrant (**D**), duration in target quadrant (**E**) and number of errors made (**F**) were assessed in males. For panels **B, C, E and E** data points represent individual mice and are presented as mean ± SEM. Two-way ANOVAs (factors group x lighting with Tukey's post-hoc comparisons) were conducted. For panels **A** and **D,** group averages ± SEM are shown. Three-way ANOVAs (factors group x lighting x training days (**A**) or quadrant (**D**)) were conducted.

3.3.3.5. No PPI deficits following prenatal poly IC exposure.

No significant group x lighting interactions or main effects were observed in baseline startle response, and average percent prepulse inhibition for males (**Supplementary Fig. 3.4A-B**) and females (**Supplementary Fig. 3.4D-E**). The 3-way interaction (prepulse x lighting x group) was also not significant for males (**Supplementary Fig. 3.4C**) or females (**Supplementary Fig. 3.4F**).

3.3.4. Microglia

3.3.4.1. Prenatal poly IC exposure led to increased morphological index in the dentate gyrus but not prefrontal cortex and CA1.

Poly IC exposure led to significantly increased morphological index compared to saline-exposed mice (main effect of group: $F_{(1, 16)} = 4.436$, $p = 0.0513$), with no significant group x lighting interaction (**Fig. 3.5A**). Using *post hoc* tests, we found a trend of increased morphological index between the saline/LL and poly IC/LD males ($p = 0.0696$) (**Fig. 3.5A**). No significant differences were observed in cell body area or circularity in males (**Fig. 3.5B-C**) or females (**Fig. 3.5G-I**). Finally, no differences were seen when assessing microglial density, NND or spacing index in the dentate gyrus in males or females (**Fig. 3.5D-F, J-L**). No differences were observed in the morphological index, cell body area, cell body circularity, density, NND or spacing index in the prefrontal cortex for males (**Supplementary Fig. 3.5A-C)** or females **(Supplementary Fig. 3.5D-F**), or in CA1 for males (**Supplementary Fig. 3.5A-F)** or females **(Supplementary Fig. 3.5G-L**). Overall, differences were only observed in the dentate gyrus, with poly IC-exposed males exhibiting a more inflammatory morphological state.

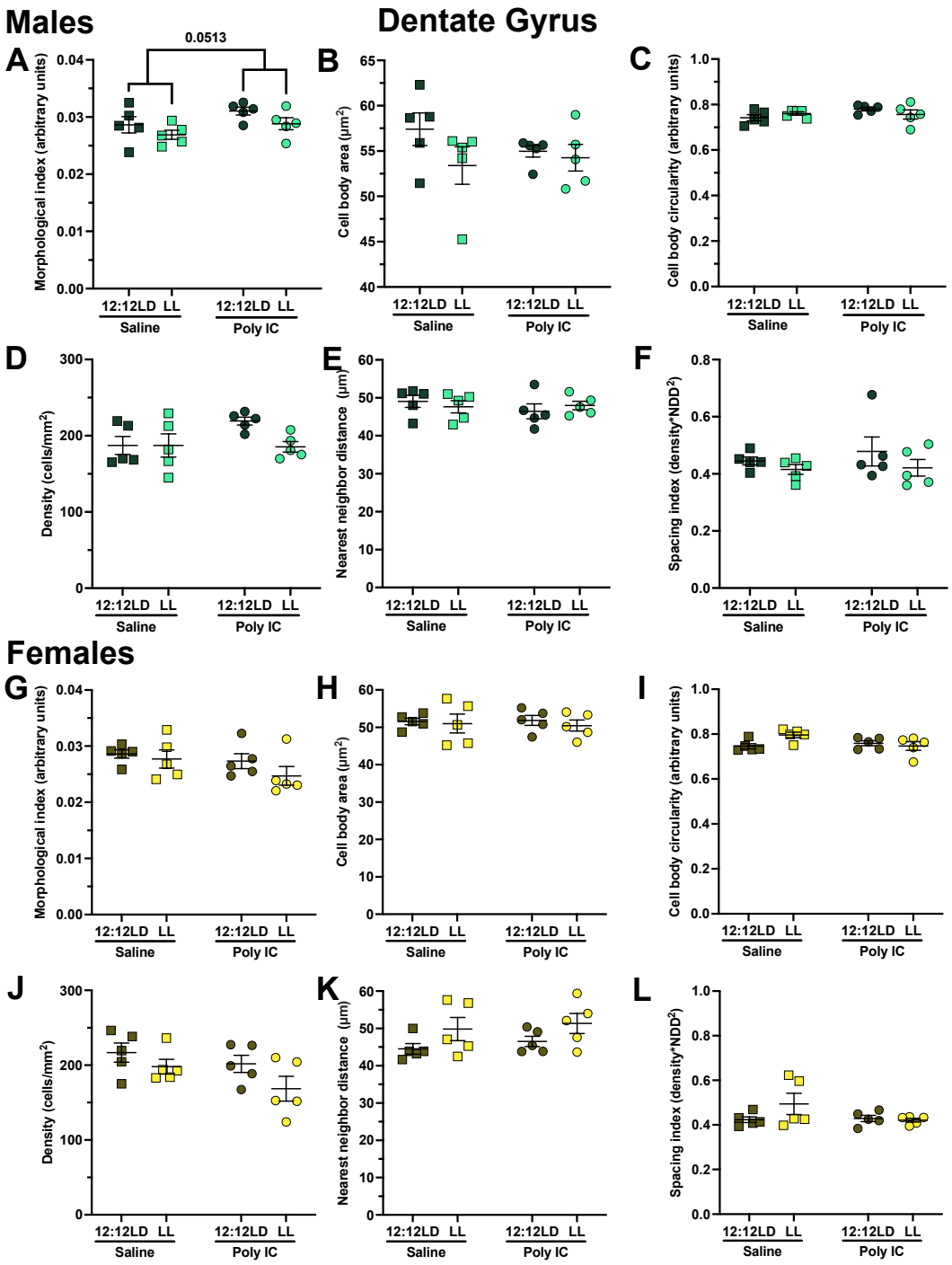


Fig. 3.5. Prenatal poly IC exposure led to increased morphological index. Morphological index (**A, G**), cell body area (**B, H**) and cell body circularity (**C, I**), microglial density (**D, J**), nearest neighbor distance (**E, K**), and spacing index (**F, L**) were assessed in males (**A-F**) and females (**G-**

L). Data points represent individual mice and are presented as mean ± SEM. Two-way ANOVAs (factors group x lighting with Tukey's post-hoc comparisons) were conducted.

3.3.5. Sex differences

Sex differences were assessed in the behavioral data, and no treatment x sex x lighting or treatment x sex interactions were found in any of the tested behavioral parameters. We report a main effect of sex in the thigmotaxis ratio in the open field test, where females had significant higher scores than males, indicative of more anxiety-like behavior ($p<0.001$). Additionally, we found a significant main effect of sex for baseline startle response in the PPI test where females had significantly lower scores than males ($p = 0.0485$) (**Supplementary Table. 3.2**).

Sex differences were also assessed in the microglia data and while no treatment x sex x lighting interactions were found, but we did report some treatment x sex interactions. Namely, in the dentate gyrus, we found a significant treatment x sex interaction for morphological index ($p = 0.0183$) and density ($p = 0.0320$). Lastly, we found several main effects of sex, including on morphological index ($p = 0.0496$) and cell body area ($p = 0.0024$) in the dentate gyrus, morphological index ($p = 0.0160$) in the prefrontal cortex and cell body area ($p = 0.0001$) in CA1 (**Supplementary Table. 3.4**).

3.3.6. RNA seq

3.3.6.1. Sex-specific effects of poly IC and LL on the hippocampus transcriptome.

We found several differentially expressed (DE) genes in the dorsal hippocampus between groups, which are presented in the heatmaps for males (**Fig. 3.6B**) and females (**Fig. 3.6B**). MIA led to the most DE genes in males, with a total of 1055. Of those 465 were significantly upregulated and 590 are significantly downregulated (**Fig. 3.6A**). Of those 1055 DE genes, 112 were similarly DE in the comparison between poly IC males under LL. In contrast, we did not see any DE genes due to prenatal poly IC in females (**Fig. 3.6A**). Regarding the effect of poly IC under LL, we found

no DE genes in males, but 83 DE genes in females, with 13 being significantly upregulated and 70 being significantly downregulated (**Fig. 3.6A**). Of these 83 DE genes, there was an overlap in only 2 genes that were also DE when exploring the effect of LL in saline-exposed females. When we assessed the effect of LL in saline-exposed mice, we did not find many DE genes in either sex (male = 1 significantly downregulated, females = 1 significantly upregulated and 1 significantly downregulated) (**Fig. 3.6A**). Lastly, when we assessed the effect of LL in poly IC-exposed mice, while there were no DE genes in females, we found 189 DE genes in males, with 110 being significantly upregulated and 79 being significantly downregulated (**Fig. 3.6A**). Overall, prenatal infection seems to affect the male hippocampal transcriptome more than females, and poly IC-exposed males seem sensitive to LL-exposure, while females do not.

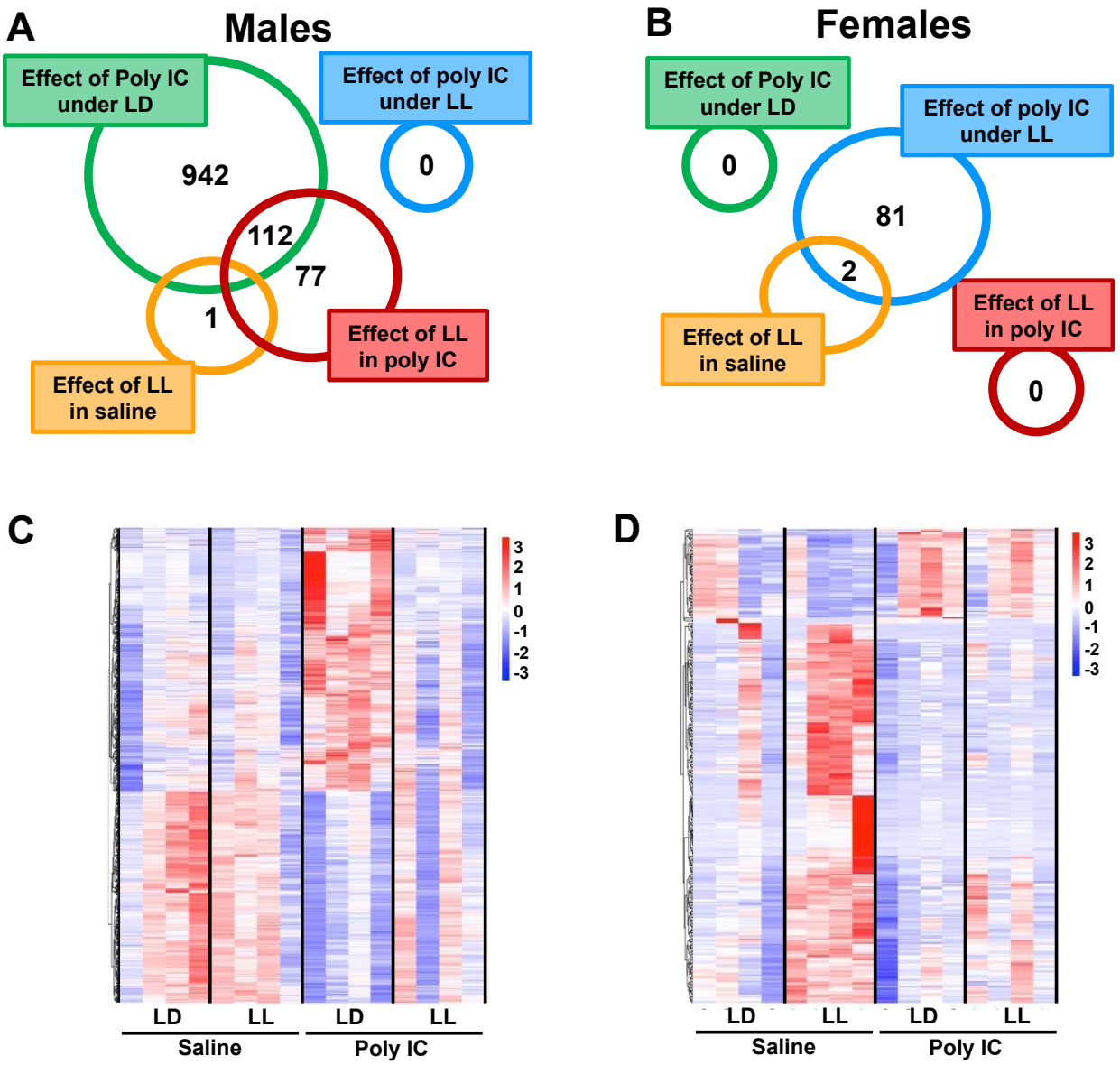


Fig. 3.6. Several differentially expressed genes associated with poly IC and LL exposure. Number of differentially expressed genes in between groups in males (**A**) and females (**B**). All differentially expressed genes were presented in a heatmap for males (**C**) and females (**D**).

3.3.6.2. Rank rank hypergeometric overlap (RRHO) analysis results in discordant patterns between sexes.

Using RRHO analysis, when we compared sex differences between all the groups of interest, we found discordant pattern of gene expression overlap between male and females when comparing the effect of poly IC under LD (saline LD versus poly IC LD) (**Fig. 3.7A**), the effect of LL in poly IC-exposed mice (poly IC LD versus poly IC LL) (**Fig. 3.7B**), the effect poly IC under LL (saline LL versus poly IC LL) (**Fig. 3.7C**) and the effect of LL in saline-exposed mice (saline LD versus saline LL) (**Fig. 3.7D**). Sex differences such as these are also apparent in the behavioral, microglial and DE analysis data.

In addition, we found concordant patterns of gene expression overlap between poly IC- and saline-exposed males (**Supplementary Fig. 3.7A**), poly IC- and saline-exposed females (**Supplementary Fig. 3.7B**), males after LD or LL exposure (**Supplementary Fig. 3.7C**) and females after LD or LL exposure (**Supplementary Fig. 3.7D**).

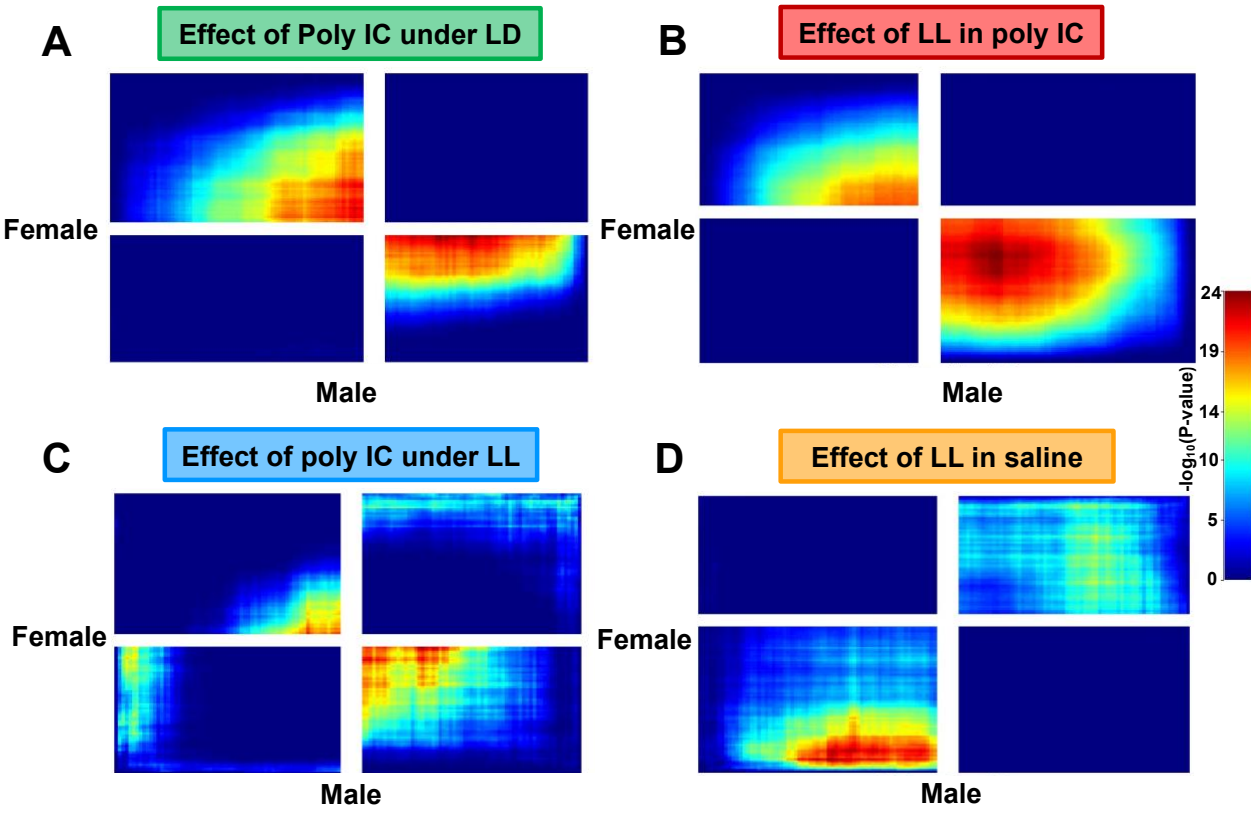


Fig. 3.7. Rank rank hypergeometric function analysis results in discordant patterns between sexes. The effect of poly IC under LD (**A**), the effect of LL in poly IC-exposed mice (**B**), the effect of poly IC under LL (**C**) and the effect of LL in saline-exposed mice (**D**) are compared between males (horizontal axis) and females (vertical axis).

3.3.6.3. Significant modules associated with poly IC and LL exposure in males and females

Using the WGCNA analysis, in males, a total of 6 modules (i.e. clusters of genes that are co-expressed and color coded in the figure) were significantly associated with the effect of prenatal poly IC exposure. One module was positively associated (green, r = +0.52, p = 0.04), while the rest were negatively associated (turquoise, r = -0.54, p = 0.03), saddle brown (r = -0.56, p = 0.02), purple (r = -0.57, p = 0.02), light green (r = -0.49, p = 0.05), brown (r = -47, p = 0.06). Eight modules were significantly associated with the effect of LL exposure. Of those, 5 were positively associated with LL exposure (purple, r = +0.61, p = 0.01; cyan, r = +0.57, p = 0.02; skyblue3, r = +0.69, p = 0.003; brown, r = +0.56, p = 0.02; white, r = +0.49, p = 0.05), and 3 were negatively associated (dark grey, r = -0.52, p = 0.04; magenta, r = -0.8, p = 0.00002; skyblue3, r = -0.61, p = 0.01). Two other modules exhibited a trend for an association with LL exposure (yellow, r = -0.47, p = 0.07; salmon, r = -0.48, p = 0.06) (**Fig. 3.8A**). Importantly, some modules were significantly associated with the effect of both prenatal poly IC and LL exposure in males. For example, the purple and brown modules were negatively associated with the effect of prenatal poly IC and positively associated with the effect of LL.

In females, 4 modules were significantly associated with the effect of prenatal poly IC exposure (light green; r = -0.64, p = 0.0007; magenta, r = -0.58, p = 0.02; cyan, r = -0.57, p = 0.02; royal blue, r = +0.63, p = 0.0009). Of those, the first 3 mentioned were negatively associated and the last module mentioned was positively associated. Two modules revealed a trend for an association with the effect of LL exposure, but none were significant (turquoise, r = +0.49, p = 0.06 and blue, r = -0.46, p = 0.07) (**Fig. 3.8B**). Overall, more modules were significantly associated with poly IC and LL exposure in males than females.

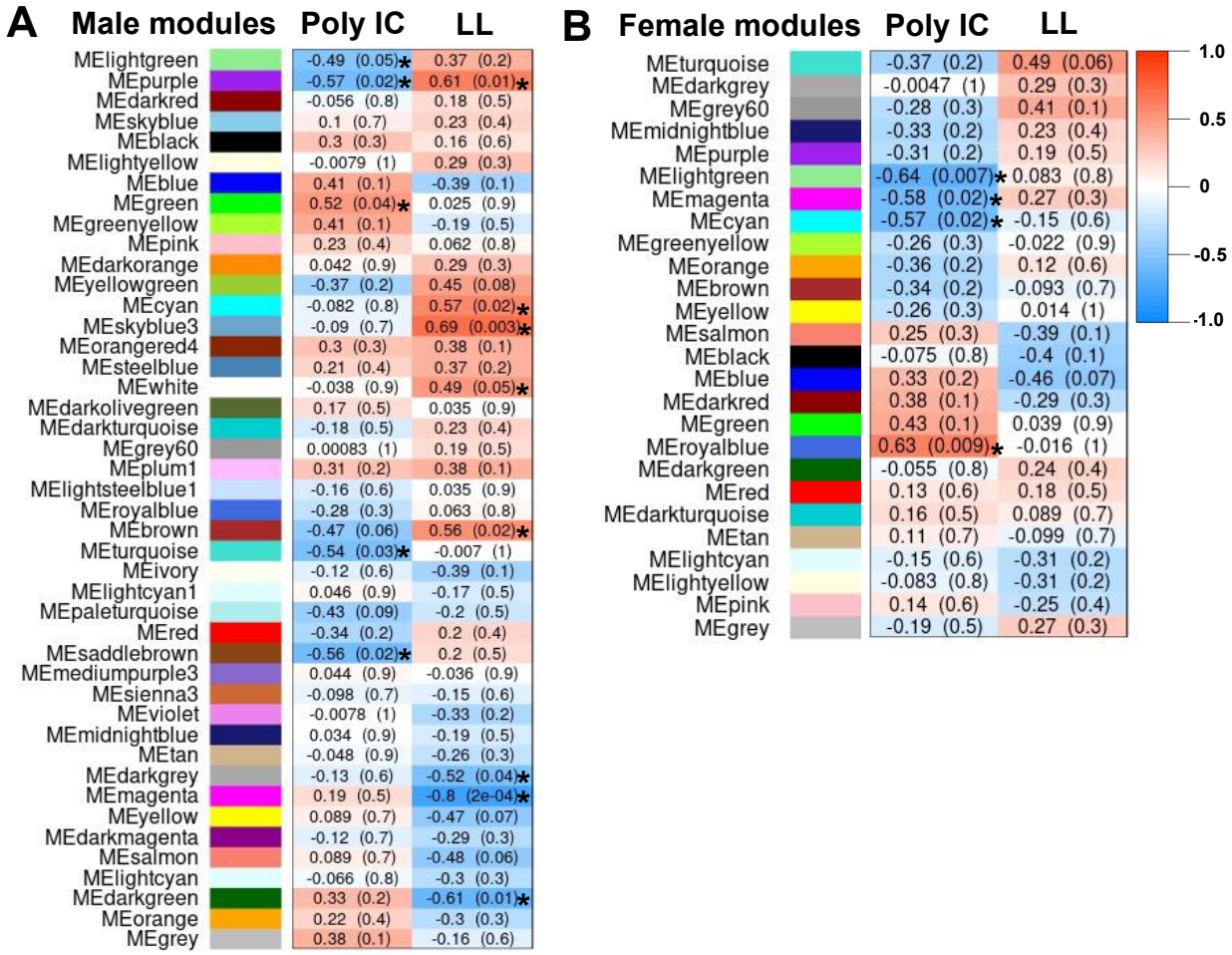


Fig. 3.8. WGCNA analysis identified several significant modules associated with poly IC and LL exposure. Modules associated with poly IC and LL exposure in males (**A**) and females (**B**). Each identified module shows the correlation coefficient (r), with either a positive value to indicate a positive association or a negative value to indicate a negative association. The p-value is presented beside the r value. The modules with significant correlations with the exposure are identified with a *.

3.4. Discussion

Our study demonstrated that maternal immune activation in mice in combination with circadian disruption during adolescence induced significant changes in behavior, microglia, and transcriptomics in a sex-dependent manner. Specifically, constant light (LL) exposure reduced anxiety-like behavior in saline-exposed males, but not between poly IC-exposed groups. Prenatal poly IC also led a 'hidden talent' phenotype in the Barnes maze and reduced sociability in males under 12:12LD, but not LL. Poly IC exposure increased microglial morphology index in the dentate gyrus, indicative of a more reactive phenotype, while LL exposure seemed to attenuate these effects. Lastly, hippocampal gene expression was significantly altered by poly IC exposure in males but not females, although poly IC exposure did have an effect in females under LL exposure. While LL caused a modest change in gene expression in saline-exposed mice of both sexes, it had more of an effect in males exposed to prenatal poly IC. Discordant patterns of gene expression overlap were found between the sexes, highlighting sex differences in our data. Additionally, concordant patterns were observed between poly IC- and saline-exposed mice and between LD and LL groups. Finally, many clusters of genes were significantly co-expressed due to prenatal poly IC and LL exposure in males and some in females.

Significant variability exists in the phenotype of prenatally challenged offspring (Meyer 2014), which could be explained by a variety of factors including individual differences in the maternal response (discussed below) and methodological differences between studies on the MIA and behavioral testing protocols. Studies exploring anxiety-like phenotypes following prenatal poly IC report conflicting results. Some studies report increased anxiety-like behavior in rats as assessed on the EPM test (Li, Chen et al. 2021), while others report decreased anxiety-like behavior in adult (but not adolescent) mice offspring as measured by the thigmotaxis ratio in the open field

test (Ozawa and et al. 2006), and lastly other studies report no differences as measured by both EPM and open field in mice (Tartaglione, Villani et al. 2022) using different MIA protocols. In contract, anxiety-like behavior does seem to be influenced by exposure to environmental circadian disruption. Studies that subjected adult rats, mice, and hamsters to LL or dim light at night found reduced anxiety-like behavior (Ma, Cao et al. 2007, Fonken, Finy et al. 2009, Bedrosian, Fonken et al. 2011) as well as reduced or no differences in corticosterone concentrations compared to 12:12LD (Claustrat, Valatx et al. 2008, Fonken, Finy et al. 2009, Bedrosian, Fonken et al. 2011, Coomans, Berg et al. 2013, Bhardwaj, Stojkovic et al. 2015). Notably, one study found increased anxiety-like behavior after pre-adolescent (3-week-old) and adolescent (5-week-old) exposure to dim light at night, suggesting that the effects of circadian disruption depend on age. However, a limitation of this study was that it did not include a group subjected to dim light at night during adulthood (8 weeks old), to show opposite effects or no effects compared to earlier disruption. Our results report no effect of MIA on anxiety-like behavior, which is consistent with some of the literature on prenatal infection. However, we observed an effect of LL in saline-exposed but not in poly IC-exposed males, suggests an interplay between these two factors on anxiety-like behavior.

Reduced sociability is a common symptom of SCZ (Goldberg and Schmidt 2001) and ASD (Orsmond, Shattuck et al. 2013) and has been reported in some studies exploring prenatal poly IC during early gestation (Hui, St-Pierre et al. 2018), but not all (Guma, Bordignon et al. 2021). Poly IC induced sociability deficits were shown to be rescued by environmental enrichment (compared to social isolation) (Zhao, Mohammed et al. 2021) and co-administration of anti-IL-6 (Smith, Li et al. 2007). Previous studies from our group exploring the effect of environmental circadian disruption on social interaction showed that LL (4 weeks, 200 lux) led to reduced sociability in

poly IC exposed mice, but had no effect on saline exposed males (Delorme, Ozell-Landry et al. 2023), whereas chronic jetlag exposure reduced sociability in both males and females (Cloutier, Srivastava et al. 2022). In the current study, while prenatal poly IC led to reduced sociability in males under 12:12LD, both the saline and poly IC groups exposed to LL did not exhibit these differences, suggesting an interplay between prenatal poly IC and LL exposure in males on sociability.

Cognitive impairments are a core feature of schizophrenia (Green, Horan et al. 2019, McCutcheon, Keefe et al. 2023) and ASD (Banker, Gu et al. 2021). Importantly, some individuals with ASD exhibit cognitive strengths, including attention and memory to details and a strong drive to detect patterns (Baron-Cohen 2017). Some MIA models using mice report decreased cognitive-like behavior when MIA was induced in early gestation (poly IC E9.5, at 10 mg/kg) (Li, Chen et al. 2021); however there is an emerging literature on improved cognitive-behavior in poly IC-exposed mice ('hidden talents'), whereby prenatal poly IC exposure conferred some functional advantages at relatively low doses (Zhao, Tran et al. 2021). It is possible that this phenotype is present in our Barne's maze findings, where prenatal poly IC leads to better performance, especially in the poly IC/LL group.

Lastly, PPI measures the ability to filter out sensory information. Deficits in PPI have been observed in individuals with neurodevelopmental disorders, including schizophrenia (Mena, Ruiz-Salas et al. 2016) and in ASD children but not adults (Cheng, Chan et al. 2018). While many studies report deficits in PPI in offspring after various doses of prenatal poly IC at E9.5 (Hui, St-Pierre et al. 2018, Chang, Li et al. 2020, Delorme, Ozell-Landry et al. 2023), other studies do not (Zhao, Erickson et al. 2022), and some studies only find PPI deficits in adolescent but not adult offspring (Guma, Bordignon et al. 2021). Our previous studies exploring the effects of

environmental circadian disruption on PPI reported that wild type mice showed no significant differences in PPI after LL or chronic jetlag exposure (Bhardwaj, Stojkovic et al. 2015, Cloutier, Srivastava et al. 2022). The current study also did not find differences in PPI after MIA or LL exposures or a combination of the two. Our previous studies and this study suggest that PPI scores do not seem to be influenced by exposure to environmental circadian disruption.

Some individuals with schizophrenia and ASD exhibit characteristics of microglial activation and increased density in cortical regions (e.g., PFC, visual cortex), hippocampus and cerebellum (Morgan, Chana et al. 2012, Bloomfield, Selvaraj et al. 2016). Prenatal poly IC-exposure led to a pro-inflammatory state in microglia, as measured by morphology (Hui, St-Pierre et al. 2018). While LL exposure was originally hypothesized to heighten the activation-related characteristics of MIA-primed microglia, there is some literature on microglia exhibited an exhaustive phenotype after exposure to two events that are known to activate microglia. For example, researchers looked at the combined effects of air pollution and maternal stress during pregnancy and found that the combined effects of these 2 stressors diminished microglia phagocytic function and inhibited (Block, Eroglu et al. 2022). In another study, mice were exposed to prenatal MIA and researchers found that the microglia from the offspring had a long-lived decrease in immune reactivity across different developmental trajectories. For example, single-cell RNA-sequencing analysis revealed that MIA decreased the amount of inflammatory microglia states in offspring, and interestingly, prenatal replacement of microglia improved the immune blunting effect caused by prenatal MIA (Hayes, An et al. 2022). In line with this hypothesis, in a previous study, we tested the combined effects of prenatal MIA and LL exposure during adulthood (Delorme, Ozell-Landry et al. 2023). Similar to the findings in the current paper, we found that poly IC exposure led to increased microglial morphology index in the dentate gyrus but not the

prefrontal cortex and CA1, and LL exposure attenuated these effects. Future studies would benefit from exploring beyond morphology and density and assess whether microglial functions are similarly affected (Paolicelli, Sierra et al. 2022).

With regards to the transcriptomics study, our findings that prenatal immune activation had a greater effect in male mice compared to females in the dorsal hippocampus was consistent with past literature (Guma, Bordignon et al. 2021). An interesting finding from our study was that LL exposure had a modest effect in saline-exposed mice. Perhaps the effects we are reporting in behavior were not dependent on LL indirectly affecting the dorsal hippocampus. Finally, LL exposure had an effect in males after LL exposure, which may highlight an important interplay between these 2 factors in males, but not females. The WGCNA analysis revealed many significantly associated modules; more so in males than in females which is consistent with prenatal poly IC exhibiting a greater effect in males. Future analyses will need to be done exploring the genes within these modules and exploring which pathways are enriched in our modules of interest to determine biological relevance. Sex differences observed in behavior, microglia and in the transcriptomic study are reflected by the RRHO, which showed discordant patterns of overlap between sexes.

There is some evidence for adolescence being a time of heightened sensitivity to stressors compared to adulthood. For example, our group tested LL exposure in adulthood in mice that underwent prenatal poly IC or saline exposure, and found less effects on behavioral and microglia than the present study, where the LL exposure was presented during adolescence (Delorme, Ozell-Landry et al. 2023). In addition, studies showed that social stress exposure leads to worse behavioral outcome when exposure is in adolescence and not adulthood. This is seen in a study by Morrissey and colleagues, where Long-Evans male and female rats were exposed to social stress

(daily 1 hour isolation and change of cage partners for 16 days) during adulthood (PND 62) or adolescence (PND 22) (Morrissey, Mathews et al. 2011, McCormick, Mongillo et al. 2013). As well, in another study where female Sprague-Dawley rats were exposed to repeated social defeat stress starting at PND 29-31 or at PND 69-71 (Ver Hoeve, Kelly et al. 2013). In the latter study, researchers also found that adolescent (but not adulthood) stress was associated with less neuronal activation in the prefrontal cortex, cingulate and thalamus. Interestingly, Buwalda and colleagues reported that the effects of social stress in adolescence can be buffered if rats were socially housed after exposure to stress (Buwalda, Stubbendorff et al. 2013). However, how exactly different types of stressors interact with the ongoing processes related to brain developmental during adolescence is largely unknown.

An unexpected finding in this study was that the lot of poly IC used led to decreased weight in both male and female offspring in the first cohort (used for behavioral tests) but not for the second and third cohorts (used for microglia and transcriptomic analyses respectively). The same dose and lot of poly IC was given to pregnant dams in all cohorts, and in a separate group of pregnant dams, we confirmed that the lot used in our study induced an immune response. Factors that could have contributed to the offspring weight difference are the first cohort having more power to detect such differences (more litters thus more pups used) and individual dam differences in response to the poly IC immune challenge.

The multifactorial aspect of neurodevelopmental disorders, such as schizophrenia and ASD, prompts the study of the interaction between multiple risk factors. Adolescence is a sensitive point in development. Adolescence is when psychiatric disorders typically emerge (Kessler, Amminger et al. 2007), and is a period of significant changes in the brain (Spear 2000), a period of increased vulnerability to stressors (Slawecki, Thorsell et al. 2004, Meier, Caspi et al. 2012,

McCormick and Green 2013) and a time when sleep onset, wake time and circadian rhythms shift to later in the day (Logan and McClung 2019). For these reasons, we decided to study the effects of environmental circadian disruption during adolescence. In this paper, we found interactions between MIA and circadian disruption during juvenile/adolescence at the behavioral, microglial level and transcriptomic level. Exposure to environmental circadian disruption has already been shown to exacerbate phenotypes in a genetic mouse model for schizophrenia (Bhardwaj, Stojkovic et al. 2015, Cloutier, Srivastava et al. 2022), but future research should address the mechanisms that underlie this interaction to inform the development of circadian-based therapies.

3.5. Acknowledgments

We thank the members of the Cermakian and Srivastava laboratories for helpful discussions, Dr. Elisa Guma, Shashank Srikanta and Margaret Sayeh for help with behavioral tests or analysis, the Douglas staff for animal care, and Melina Jaramillo Garcia at the Molecular and Cellular Microscopy Platform at the Douglas.

3.6. Funding

This work was supported by grants from the Canadian Institutes of Health Research (PJT-153299 to NC and LKS), Velux Stiftung (Project 927 to NC) and Ludmer – MI4 Collaborative Seed Fund Grant (to NC and PPS). TCD was supported by graduate scholarships from the Schizophrenia Society of Canada Foundation, the Canadian College of

Neuropsychopharmacology, and the Fonds de Recherche du Québec – Santé. NH was supported by an NSERC undergraduate student research award.

3.7. Author contributions

Author contributions included conception and design of the experiments (TCD, LKS, NC, PPS), data acquisition (TCD), data analysis (TCD, NH, DMA, NO) and interpretation of results (TCD, NH, DMA, NO, LKS, NC, PPS). Drafting manuscript and revising manuscript for publication (TCD, LKS, NC).

3.8. Competing interests

The authors have nothing to disclose.

3.9. References

Banker, S. M., X. Gu, D. Schiller and J. H. Foss-Feig (2021). "Hippocampal contributions to social and cognitive deficits in autism spectrum disorder." Trends Neurosci **44**(10): 793-807.

Baron-Cohen, S. (2017). "Editorial Perspective: Neurodiversity - a revolutionary concept for autism and psychiatry." J Child Psychol Psychiatry **58**(6): 744-747.

Bedrosian, T. A., L. K. Fonken, J. C. Walton, A. Haim and R. J. Nelson (2011). "Dim light at night provokes depression-like behaviors and reduces CA1 dendritic spine density in female hamsters." Psychoneuroendocrinology **36**(7): 1062–1069.

Bhardwaj, S. K., K. Stojkovic, S. Kiessling, L. K. Srivastava and N. Cermakian (2015). "Constant light uncovers behavioral effects of a mutation in the schizophrenia risk gene Dtnbp1 in mice." Behav Brain Res **284**: 58–68.

Block, C. L., O. Eroglu, S. D. Mague, C. J. Smith, A. M. Ceasrine, C. Sriworarat, C. Blount, K. A. Beben, K. E. Malacon, N. Ndubuizu, A. Talbot, N. M. Gallagher, Y. Chan Jo, T. Nyangacha, D. E. Carlson, K. Dzirasa, C. Eroglu and S. D. Bilbo (2022). "Prenatal environmental stressors impair postnatal microglia function and adult behavior in males." Cell Reports **40**(5): 111161.

Bloomfield, P. S., S. Selvaraj, M. Veronese, G. Rizzo, A. Bertoldo, D. R. Owen, M. A. Bloomfield, I. Bonoldi, N. Kalk, F. Turkheimer, P. McGuire, V. de Paola and O. D. Howes (2016). "Microglial Activity in People at Ultra High Risk of Psychosis and in Schizophrenia: An [(11)C]PBR28 PET Brain Imaging Study." Am J Psychiatry **173**(1): 44-52.

Brown, A. S., M. D. Begg, S. Gravenstein, C. A. Schaefer, R. J. Wyatt, M. Bresnahan, V. P. Babulas and E. S. Susser (2004). "Serologic evidence of prenatal influenza in the etiology of schizophrenia." Arch Gen Psychiatry **61**(8): 774–780.

Brown, J. P., D. Martin, Z. Nagaria, A. C. Verceles, S. L. Jobe and E. M. Wickwire (2020). "Mental Health Consequences of Shift Work: An Updated Review." Curr Psychiatry Rep **22**(2): 7.

Buwalda, B., C. Stubbendorff, N. Zickert and J. M. Koolhaas (2013). "Adolescent social stress does not necessarily lead to a compromised adaptive capacity during adulthood: A study on the consequences of social stress in rats." Neuroscience **249**: 258-270.

Cahill, K. M., Z. Huo, G. C. Tseng, R. W. Logan and M. L. Seney (2018). "Improved identification of concordant and discordant gene expression signatures using an updated rank-rank hypergeometric overlap approach." Scientific Reports **8**(1): 9588.

Chang, Y.-C., W.-Y. Li, L. J.-H. Lee and L.-J. Lee (2020). "Interplay of Prenatal and Postnatal Risk Factors in the Behavioral and Histological Features of a "Two-Hit" Non-Genetic Mouse Model of Schizophrenia." International Journal of Molecular Sciences **21**(22): 8518.

Cheng, C. H., P. S. Chan, S. C. Hsu and C. Y. Liu (2018). "Meta-analysis of sensorimotor gating in patients with autism spectrum disorders." Psychiatry Res **262**: 413-419.

Claustrat, B., J. L. Valatx, C. Harthé and J. Brun (2008). "Effect of Constant Light on Prolactin and Corticosterone Rhythms Evaluated Using a Noninvasive Urine Sampling Protocol in the Rat." Hormone and Metabolic Research **40**(6): 398-403.

Cloutier, M. E., L. K. Srivastava and N. Cermakian (2022). "Exposure to Circadian Disruption During Adolescence Interacts With a Genetic Risk Factor to Modify Schizophrenia-relevant Behaviors in a Sex-dependent Manner." J Biol Rhythms **37**(6): 655-672.

Coomans, C. P., S. A. A. Berg, T. Houben, J. B. Klinken, R. Berg, A. C. M. Pronk, L. M. Havekes, J. A. Romijn, K. W. Dijk, N. R. Biermasz and J. H. Meijer (2013). "Detrimental effects of

constant light exposure and high-fat diet on circadian energy metabolism and insulin sensitivity." The FASEB Journal **27**(4): 1721–1732.

Costa, G., E. Haus and R. Stevens (2010). "Shift work and cancer - considerations on rationale, mechanisms, and epidemiology." Scand J Work Environ Health **36**(2): 163-179.

Davis, J., H. Eyre, F. N. Jacka, S. Dodd, O. Dean, S. McEwen, M. Debnath, J. McGrath, M. Maes, P. Amminger, P. D. McGorry, C. Pantelis and M. Berk (2016). "A review of vulnerability and risks for schizophrenia: Beyond the two hit hypothesis." Neurosci Biobehav Rev **65**: 185–194.

Delorme, T. C., W. Ozell-Landry, N. Cermakian and L. K. Srivastava (2023). "Behavioral and cellular responses to circadian disruption and prenatal immune activation in mice." Scientific Reports.

Delorme, T. C., L. K. Srivastava and N. Cermakian (2021). "Altered circadian rhythms in a mouse model of neurodevelopmental disorders based on prenatal maternal immune activation." Brain Behav Immun **93**: 119–131.

Drake, C. L., T. Roehrs, G. Richardson, J. K. Walsh and T. Roth (2004). "Shift work sleep disorder: prevalence and consequences beyond that of symptomatic day workers." Sleep **27**(8): 1453-1462.

Estes, M. L. and A. K. McAllister (2016). "Maternal immune activation: Implications for neuropsychiatric disorders." Science **353**(6301): 772–777.

Fonken, L. K., M. S. Finy, J. C. Walton, Z. M. Weil, J. L. Workman, J. Ross and R. J. Nelson (2009). "Influence of light at night on murine anxiety- and depressive-like responses." Behav Brain Res **205**(2): 349-354.

Fonken, L. K. and R. J. Nelson (2016). "Effects of light exposure at night during development." Current Opinion in Behavioral Sciences **7**: 33-39.

Goldberg, J. O. and L. A. Schmidt (2001). "Shyness, sociability, and social dysfunction in schizophrenia." Schizophr Res **48**(2-3): 343-349.

Guma, E., P. D. C. Bordignon, G. A. Devenyi, D. Gallino, C. Anastassiadis, V. Cvetkovska, A. D. Barry, E. Snook, J. Germann, C. M. T. Greenwood, B. Misic, R. C. Bagot and M. M. Chakravarty (2021). "Early or Late Gestational Exposure to Maternal Immune Activation Alters Neurodevelopmental Trajectories in Mice: An Integrated Neuroimaging, Behavioral, and Transcriptional Study." Biological Psychiatry **90**(5): 328-341.

Haddad, F. L., S. V. Patel and S. Schmid (2020). "Maternal Immune Activation by Poly I:C as a preclinical Model for Neurodevelopmental Disorders: A focus on Autism and Schizophrenia." Neurosci Biobehav Rev **113**: 546-567.

Hafner, H. and K. Maurer (2006). "Early detection of schizophrenia: current evidence and future perspectives." World Psychiatry **5**(3): 130-138.

Hastings, M. H., E. S. Maywood and M. Brancaccio (2018). "Generation of circadian rhythms in the suprachiasmatic nucleus." Nat Rev Neurosci **19**(8): 453-469.

Hayes, L. N., K. An, E. Carloni, F. Li, E. Vincent, C. Trippaers, M. Paranjpe, G. Dölen, L. A. Goff, A. Ramos, S.-I. Kano and A. Sawa (2022). "Prenatal immune stress blunts microglia reactivity, impairing neurocircuitry." Nature **610**(7931): 327-334.

Hui, C. W., A. St-Pierre, H. El Hajj, Y. Remy, S. S. Hebert, G. N. Luheshi, L. K. Srivastava and M. E. Tremblay (2018). "Prenatal Immune Challenge in Mice Leads to Partly Sex-Dependent Behavioral, Microglial, and Molecular Abnormalities Associated with Schizophrenia." Front Mol Neurosci **11**: 13.

Häfner, H. (2019). "From Onset and Prodromal Stage to a Life-Long Course of Schizophrenia and Its Symptom Dimensions: How Sex, Age, and Other Risk Factors Influence Incidence and Course of Illness." Psychiatry Journal **2019**: 1-15.

Johansson, A. S., B. Owe-Larsson, J. Hetta and G. B. Lundkvist (2016). "Altered circadian clock gene expression in patients with schizophrenia." Schizophr Res **174**(1-3): 17-23.

Kessler, R. C., G. P. Amminger, S. Aguilar-Gaxiola, J. Alonso, S. Lee and T. B. Ustun (2007). "Age of onset of mental disorders: a review of recent literature." Current Opinion in Psychiatry **20**(4): 359-364.

Krakowiak, P., B. Goodlin-Jones, I. Hertz-Picciotto, L. A. Croen and R. L. Hansen (2008). "Sleep problems in children with autism spectrum disorders, developmental delays, and typical development: a population-based study." J Sleep Res **17**(2): 197-206.

Langfelder, P. and S. Horvath (2008). "WGCNA: an R package for weighted correlation network analysis." BMC bioinformatics **9**(1): 1-13.

Lee, B. K., C. Magnusson, R. M. Gardner, A. Blomstrom, C. J. Newschaffer, I. Burstyn, H. Karlsson and C. Dalman (2015). "Maternal hospitalization with infection during pregnancy and risk of autism spectrum disorders." Brain Behav Immun **44**: 100–105.

Li, W., M. Chen, X. Feng, M. Song, M. Shao, Y. Yang, L. Zhang, Q. Liu, L. Lv and X. Su (2021). "Maternal immune activation alters adult behavior, intestinal integrity, gut microbiota and the gut inflammation." Brain and Behavior **11**(5).

Logan, R. W. and C. A. McClung (2019). "Rhythms of life: circadian disruption and brain disorders across the lifespan." Nat Rev Neurosci **20**(1): 49–65.

Love, M., W. Huber and S. Anders (2014). "Moderated estimation of fold changes and dispersion for RNA-seq data with Deseq2. vol. 15." Genome Biol.

Ma, W.-P., J. Cao, M. Tian, M.-H. Cui, H.-L. Han, Y.-X. Yang and L. Xu (2007). "Exposure to chronic constant light impairs spatial memory and influences long-term depression in rats." Neuroscience Research **59**(2): 224-230.

McCormick, C. M. and M. R. Green (2013). "From the stressed adolescent to the anxious and depressed adult: investigations in rodent models." Neuroscience **249**: 242-257.

McCormick, C. M., D. L. Mongillo and J. J. Simone (2013). "Age and adolescent social stress effects on fear extinction in female rats." Stress **16**(6): 678-688.

Meier, M. H., A. Caspi, A. Ambler, H. Harrington, R. Houts, R. S. Keefe, K. McDonald, A. Ward, R. Poulton and T. E. Moffitt (2012). "Persistent cannabis users show neuropsychological decline from childhood to midlife." Proc Natl Acad Sci U S A **109**(40): E2657-2664.

Mena, A., J. C. Ruiz-Salas, A. Puentes, I. Dorado, M. Ruiz-Veguilla and L. G. De la Casa (2016). "Reduced Prepulse Inhibition as a Biomarker of Schizophrenia." Front Behav Neurosci **10**: 202.

Meyer, U. (2014). "Prenatal poly(i:C) exposure and other developmental immune activation models in rodent systems." Biol Psychiatry **75**(4): 307-315.

Morgan, J. T., G. Chana, I. Abramson, K. Semendeferi, E. Courchesne and I. P. Everall (2012). "Abnormal microglial-neuronal spatial organization in the dorsolateral prefrontal cortex in autism." Brain Res **1456**: 72-81.

Morrissey, M. D., I. Z. Mathews and C. M. McCormick (2011). "Enduring deficits in contextual and auditory fear conditioning after adolescent, not adult, social instability stress in male rats." Neurobiology of Learning and Memory **95**(1): 46-56.

Orsmond, G. I., P. T. Shattuck, B. P. Cooper, P. R. Sterzing and K. A. Anderson (2013). "Social participation among young adults with an autism spectrum disorder." J Autism Dev Disord **43**(11): 2710-2719.

Ozawa, K. and et al. (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 **59**(6): 546.

Paolicelli, R. C., A. Sierra, B. Stevens, M. E. Tremblay, A. Aguzzi, B. Ajami, I. Amit, E. Audinat, I. Bechmann, M. Bennett, F. Bennett, A. Bessis, K. Biber, S. Bilbo, M. Blurton-Jones, E. Boddeke, D. Brites, B. Brone, G. C. Brown, O. Butovsky, M. J. Carson, B. Castellano, M. Colonna, S. A. Cowley, C. Cunningham, D. Davalos, P. L. De Jager, B. de Strooper, A. Denes, B. J. L. Eggen, U. Eyo, E. Galea, S. Garel, F. Ginhoux, C. K. Glass, O. Gokce, D. Gomez-Nicola, B. Gonzalez, S. Gordon, M. B. Graeber, A. D. Greenhalgh, P. Gressens, M. Greter, D. H. Gutmann, C. Haass, M. T. Heneka, F. L. Heppner, S. Hong, D. A. Hume, S. Jung, H. Kettenmann, J. Kipnis, R. Koyama, G. Lemke, M. Lynch, A. Majewska, M. Malcangio, T. Malm, R. Mancuso, T. Masuda, M. Matteoli, B. W. McColl, V. E. Miron, A. V. Molofsky, M. Monje, E. Mracsko, A. Nadjar, J. J. Neher, U. Neniskyte, H. Neumann, M. Noda, B. Peng, F. Peri, V. H. Perry, P. G. Popovich, C. Pridans, J. Priller, M. Prinz, D. Ragozzino, R. M. Ransohoff, M. W. Salter, A. Schaefer, D. P. Schafer, M. Schwartz, M. Simons, C. J. Smith, W. J. Streit, T. L. Tay, L. H. Tsai, A. Verkhratsky, R. von Bernhardi, H. Wake, V. Wittamer, S. A. Wolf, L. J. Wu and T. Wyss-Coray (2022). "Microglia states and nomenclature: A field at its crossroads." Neuron **110**(21): 3458-3483.

Patke, A., M. W. Young and S. Axelrod (2020). "Molecular mechanisms and physiological importance of circadian rhythms." Nat Rev Mol Cell Biol **21**(2): 67-84.

Perkinson-Gloor, N., S. Lemola and A. Grob (2013). "Sleep duration, positive attitude toward life, and academic achievement: The role of daytime tiredness, behavioral persistence, and school start times." Journal of Adolescence **36**(2): 311-318.

Puttonen, S., M. Harma and C. Hublin (2010). "Shift work and cardiovascular disease - pathways from circadian stress to morbidity." Scand J Work Environ Health **36**(2): 96-108.

Schaafsma, S. M., K. Gagnidze, A. Reyes, N. Norstedt, K. Månsson, K. Francis and D. W. Pfaff (2017). "Sex-specific gene–environment interactions underlying ASD-like behaviors." Proceedings of the National Academy of Sciences **114**(6): 1383-1388.

Slawecki, C. J., A. Thorsell and C. L. Ehlers (2004). "Long-term neurobehavioral effects of alcohol or nicotine exposure in adolescent animal models." Ann N Y Acad Sci **1021**: 448-452.

Smith, S. E., J. Li, K. Garbett, K. Mirnics and P. H. Patterson (2007). "Maternal immune activation alters fetal brain development through interleukin-6." J Neurosci **27**(40): 10695–10702.

Spear, L. P. (2000). "The adolescent brain and age-related behavioral manifestations." Neuroscience & Biobehavioral Reviews **24**(4): 417-463.

Tartaglione, A. M., A. Villani, M. A. Ajmone-Cat, L. Minghetti, L. Ricceri, V. Pazienza, R. De Simone and G. Calamandrei (2022). "Maternal immune activation induces autism-like changes in behavior, neuroinflammatory profile and gut microbiota in mouse offspring of both sexes." Translational Psychiatry **12**(1).

Tripathi, A., S. K. Kar and R. Shukla (2018). "Cognitive Deficits in Schizophrenia: Understanding the Biological Correlates and Remediation Strategies." Clinical Psychopharmacology and Neuroscience **16**(1): 7-17.

Valle, F. P. (1970). "Effects of strain, sex, and illumination on open-field behavior of rats." Am J Psychol **83**(1): 103-111.

Ver Hoeve, E. S., G. Kelly, S. Luz, S. Ghanshani and S. Bhatnagar (2013). "Short-term and long-term effects of repeated social defeat during adolescence or adulthood in female rats." Neuroscience **249**: 63-73.

Wulff, K., D. J. Dijk, B. Middleton, R. G. Foster and E. M. Joyce (2012). "Sleep and circadian rhythm disruption in schizophrenia." Br J Psychiatry **200**(4): 308-316.

Zhao, X., M. Erickson, R. Mohammed and A. C. Kentner (2022). "Maternal immune activation accelerates puberty initiation and alters mechanical allodynia in male and female C57BL6/J mice." Developmental Psychobiology **64**(5).

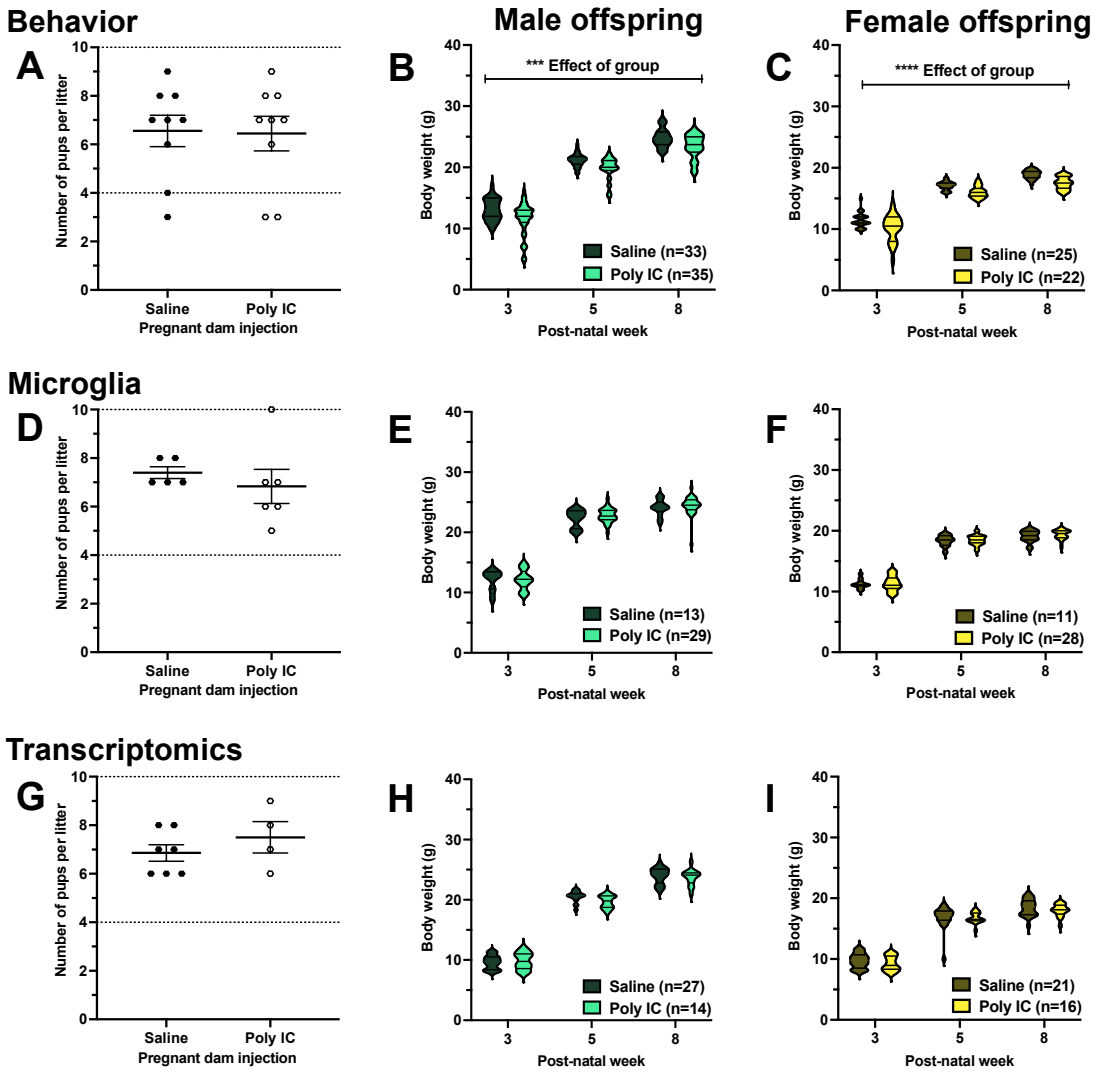
Zhao, X., R. Mohammed, H. Tran, M. Erickson and A. C. Kentner (2021). "Poly (I:C)-induced maternal immune activation modifies ventral hippocampal regulation of stress reactivity: prevention by environmental enrichment." Brain Behav Immun **95**: 203-215.

Zhao, X., H. Tran, H. Derosa, R. C. Roderick and A. C. Kentner (2021). "Hidden talents: Poly (I:C)-induced maternal immune activation improves mouse visual discrimination performance and reversal learning in a sex-dependent manner." Genes, Brain and Behavior **20**(7).

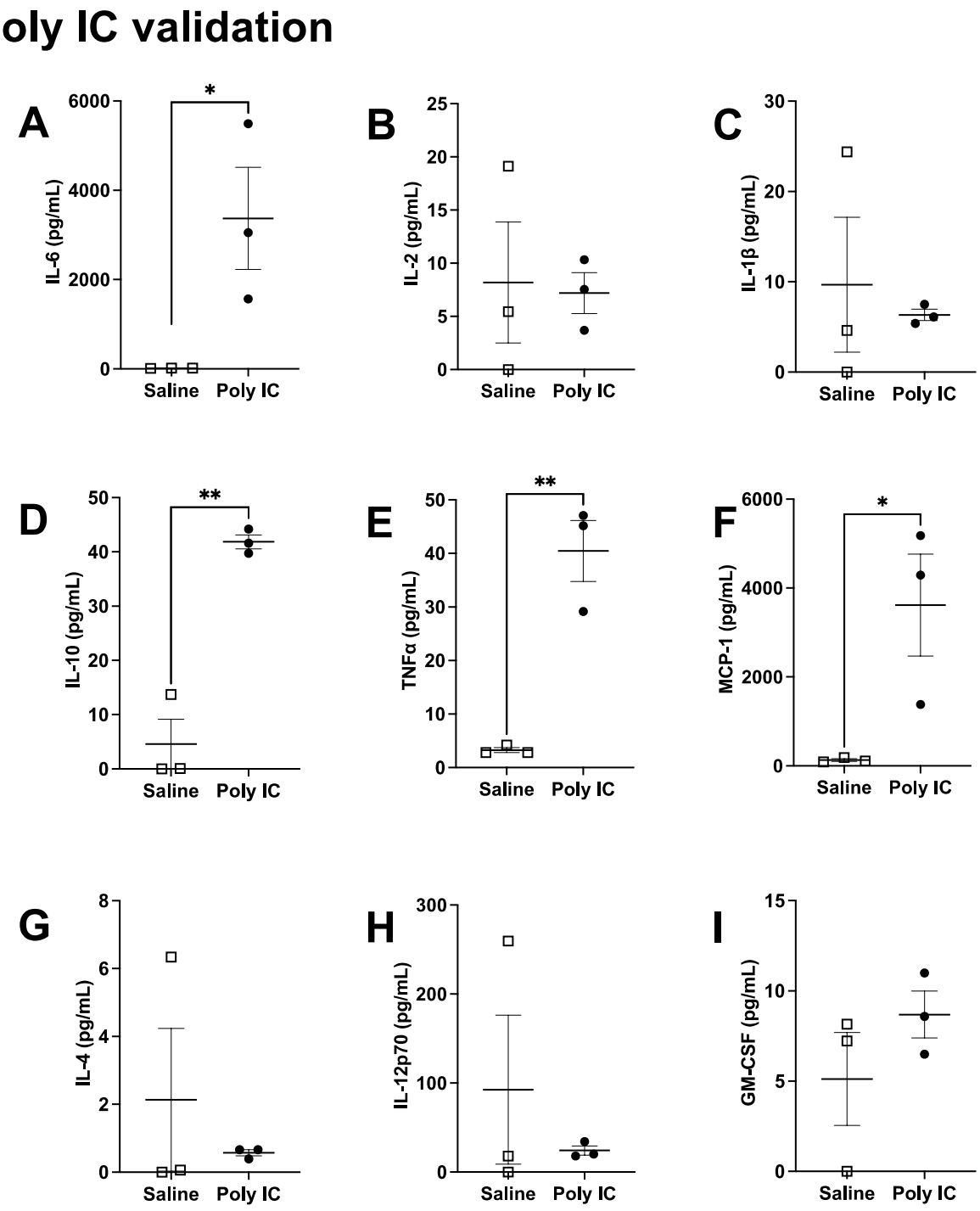
CHAPTER 3: EFFECTS OF PRENATAL MATERNAL IMMUNE ACTIVATION AND EXPOSURE TO CIRCADIAN DISRUPTION DURING ADOLESCENT: EXPLORING THE TWO-HIT MODEL

Tara C. Delorme, Nikki Huebener, Danusa M. Arcego, Nick O'Toole, Patrícia P. Silveira, Lalit K. Srivastava and Nicolas Cermakian

Supplementary Information

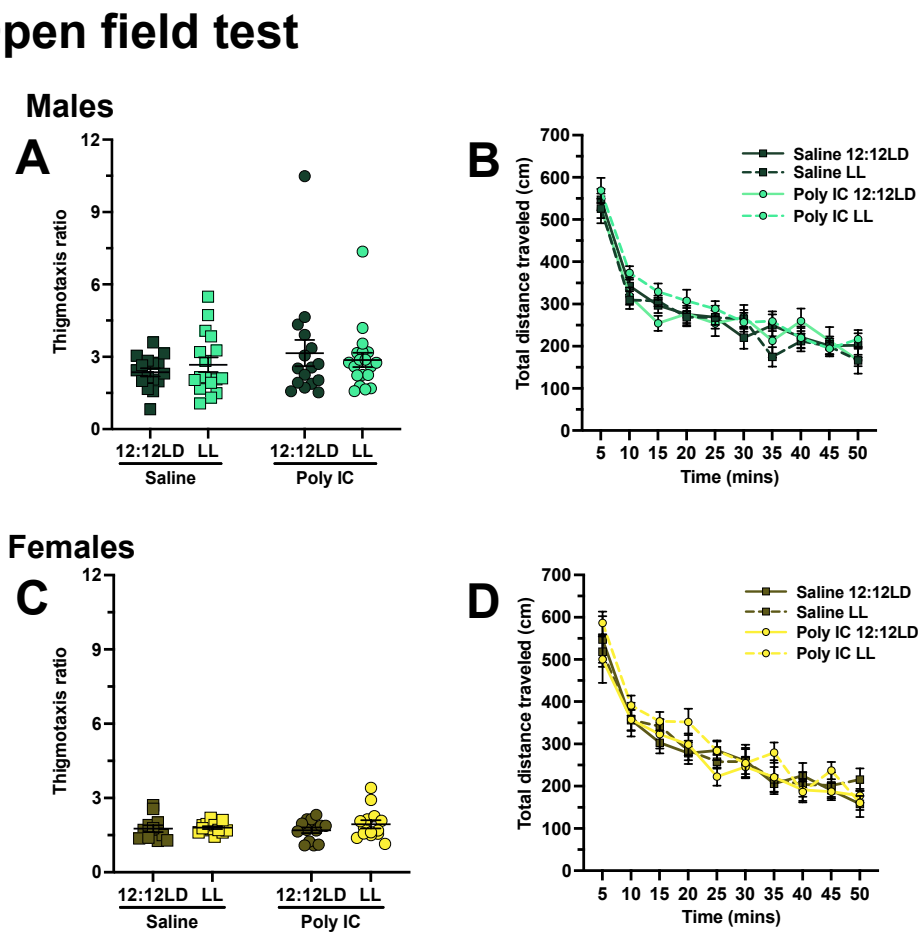


Supplementary Fig. 3.1. Litter details for each cohort of mice. Number of pups per litter **(A, D, G)** was assessed for each cohort and only litters that had 4-10 pups were included. Body weight at 3, 5 and 8 weeks old was assessed in males **(B, E, H)** and females **(C, F, I)** in the cohort used for behavior **(A-C),** microglia **(D-F)** and transcriptomics **(G-I).**

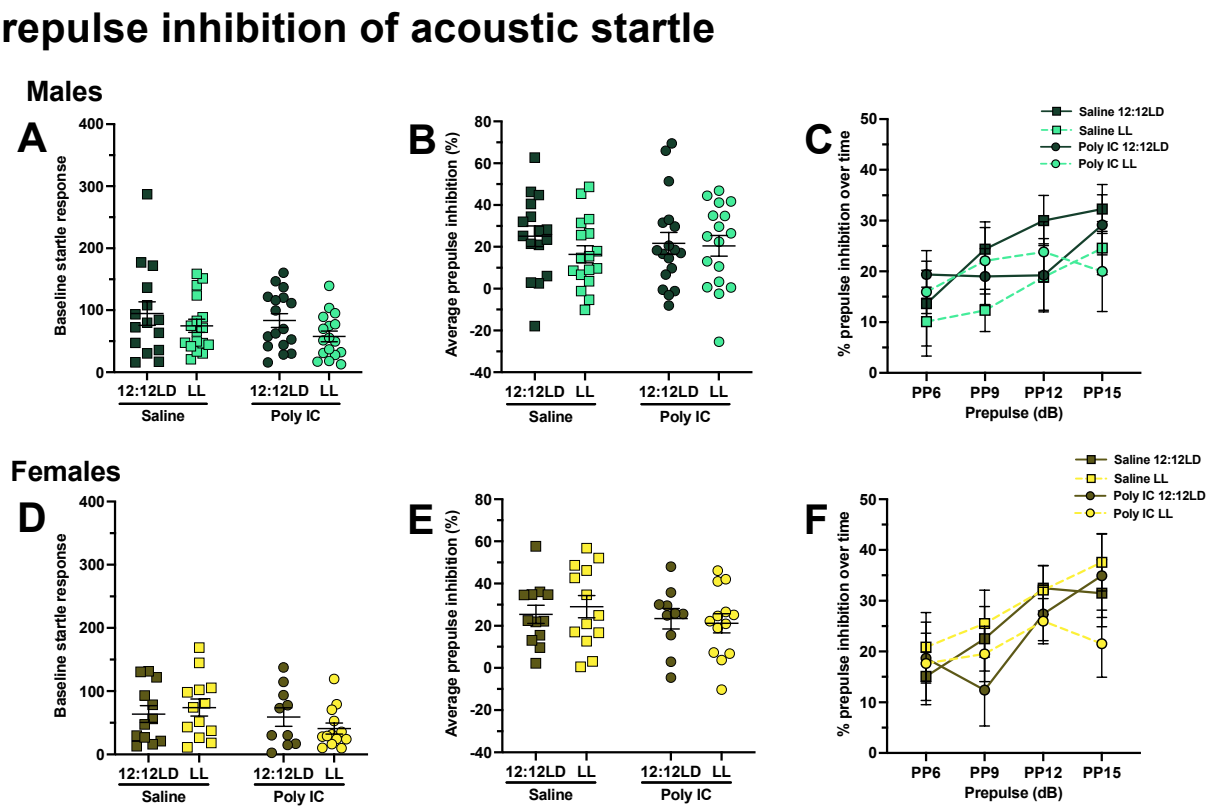


Supplementary Fig. 3.2. Prenatal poly IC induced a cytokine and chemokine response in pregnant dams. The following biomarkers were assessed in the blood plasma of pregnant dams after poly IC or saline injection: IL-6 (**A**), IL-2 (**B**), IL-1β (**C**), IL-10 (**D**), TNFα (**E**), MCP-1 (**F**),

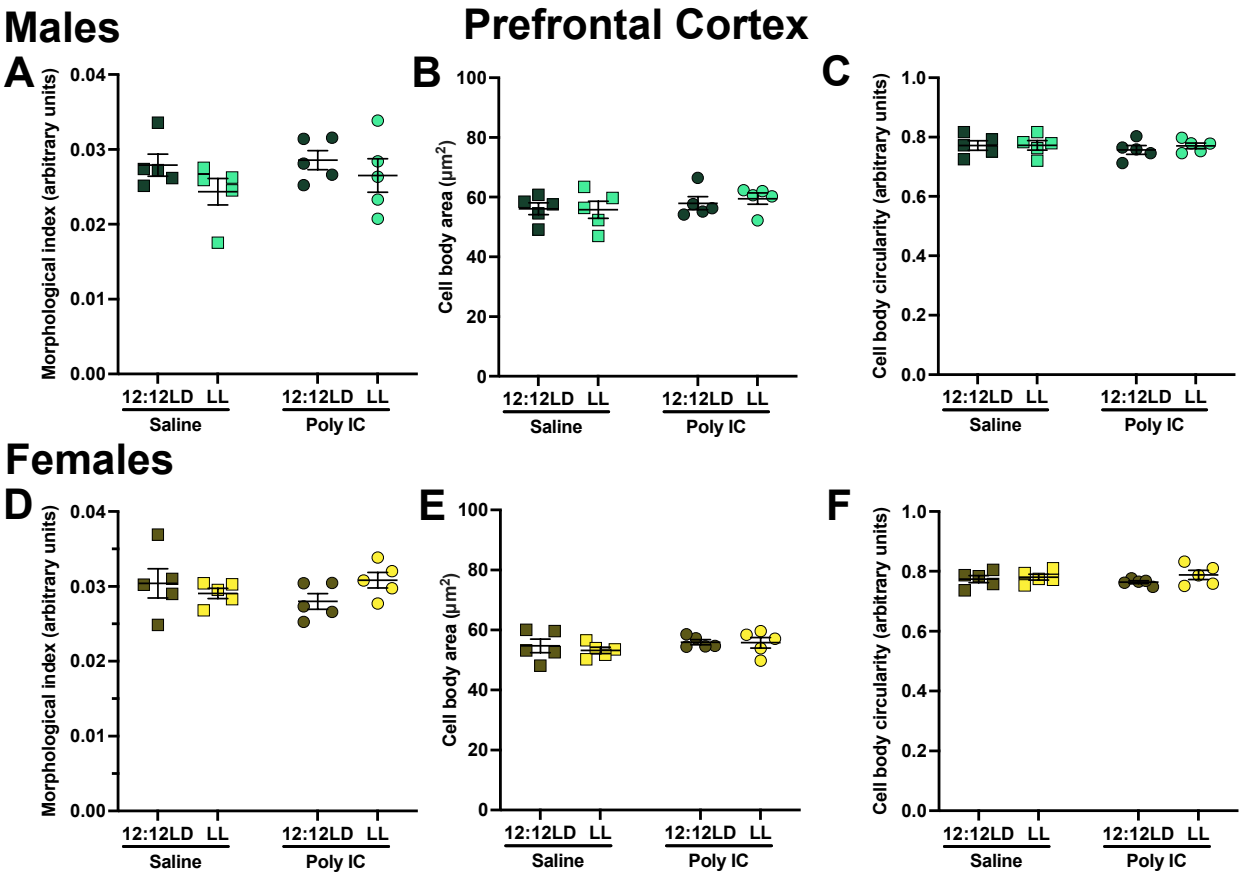
IL-4 (**G**), IL-12p70 (**H**) and GM-CSF (**I**). Individual data points represent independent dams and data were represented as mean ± SEM. An independent samples t-test (with Welsh's correction, or Mann-Whitney U test when appropriate) was used, **p<.01 and *p<.05.



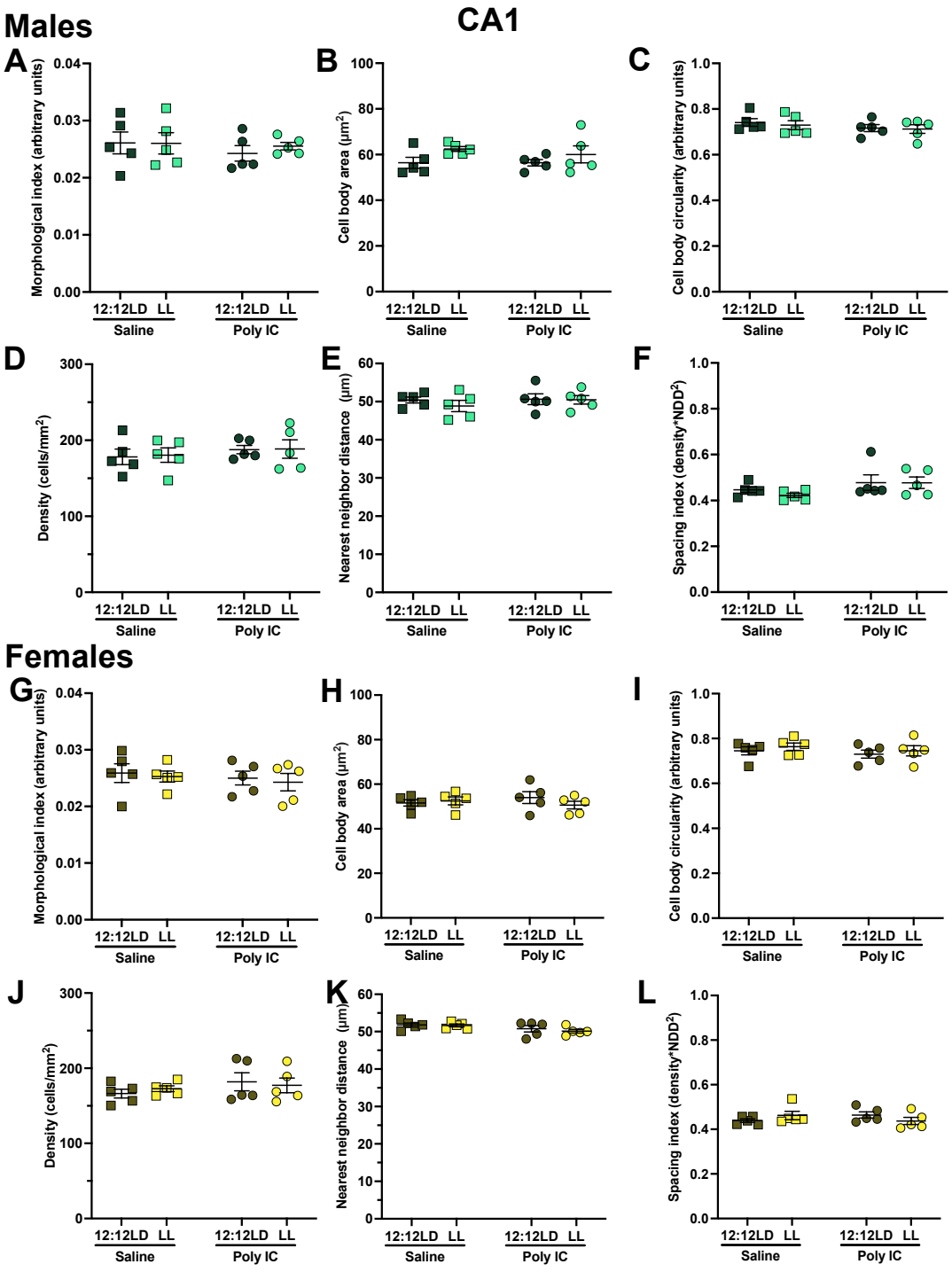
Supplementary Fig. 3.3. Similar spontaneous locomotion in saline and poly IC-exposed mice. Spontaneous locomotor activity was measured in the open field test. Thigmotaxis **(A, C)** and total distance traveled (**B, D**) were assessed in males (**A**-**B**) and females (**C**-**D**). For panels **A and C** data points represent individual mice and are presented as mean ± SEM. Two-way ANOVAs (factors group x lighting with Tukey's post-hoc comparisons) were conducted. For panels **B** and **D,** group averages ± SEM are shown over each 10-minute bin of the test. Three-way ANOVAs (factors group x lighting x time) were conducted.



Supplementary Fig. 3.4. No PPI deficits following prenatal poly IC exposure. Prepulse inhibition of acoustic startle (PPI) was used to assess sensory motor gating. Baseline startle response (**A, D)**, average PPI (%) (**B, E**) and PPI (%) across each prepulse levels (**C, F**) were assessed in males (**A-C**) and females (**D-F**). For panels **A, B, D and E** data points represent individual mice and are presented as mean ± SEM. Two-way ANOVAs (factors group x lighting with Tukey's post-hoc comparisons) were conducted. For panels **C** and **F,** group averages ± SEM are shown over prepulse level. Three-way ANOVAs (factors group x lighting x time) were conducted.

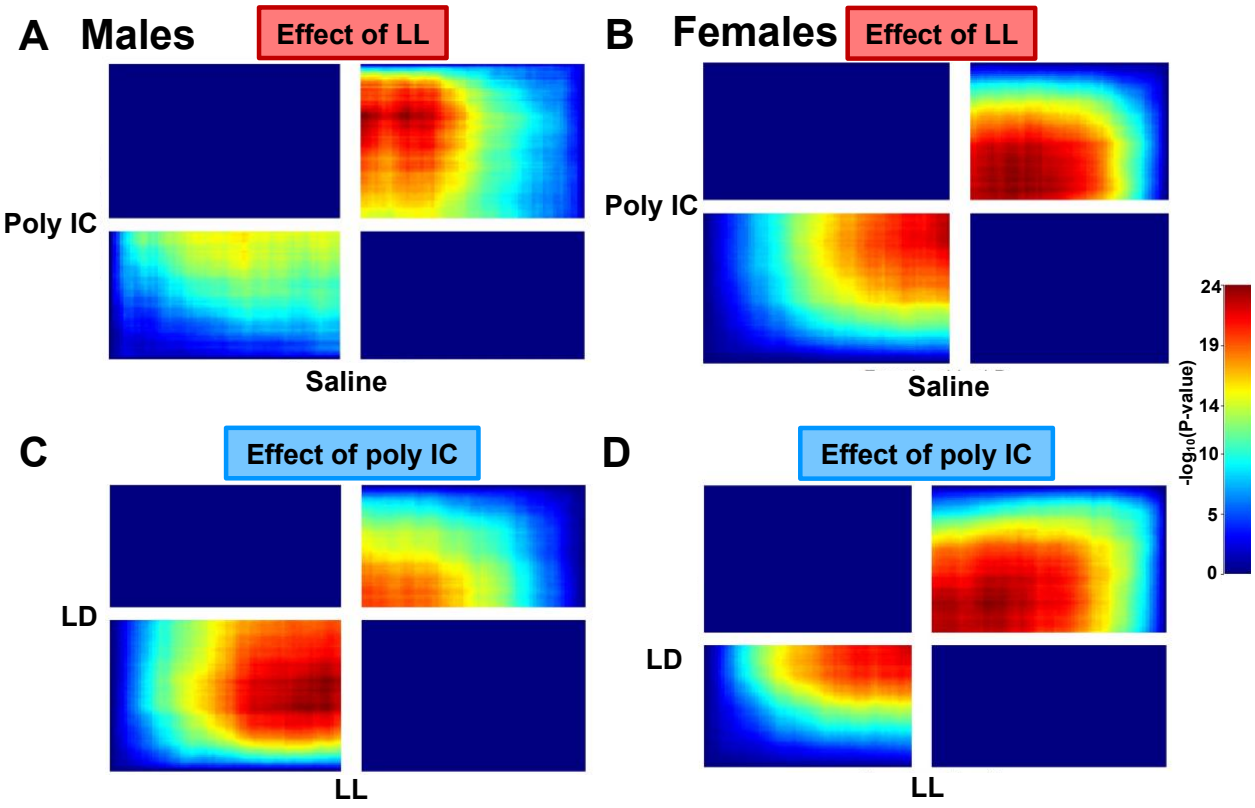


Supplementary Fig. 3.5. No effect of prenatal infection or LL exposure in microglia in prefrontal cortex. Morphological index (**A, G**), cell body area (**B, H**) and cell body circularity (**C, I**), microglial density (**D, J**), nearest neighbor distance (**E, K**), and spacing index (**F, L**) were assessed in males (**A-F**) and females (**G-L**). Data points represent individual mice and are presented as mean ± SEM. Two-way ANOVAs (factors group x lighting with Tukey's post-hoc comparisons) were conducted.



Supplementary Fig. 3.6. No effect of prenatal infection or LL exposure in microglia in CA1. Morphological index (**A, G**), cell body area (**B, H**) and cell body circularity (**C, I**), microglial density (**D, J**), nearest neighbor distance (**E, K**), and spacing index (**F, L**) were assessed in males

(**A-F**) and females (**G-L**). Data points represent individual mice and are presented as mean ± SEM. Two-way ANOVAs (factors group x lighting with Tukey's post-hoc comparisons) were conducted.



Supplementary Fig. 3.7. Rank rank hypergeometric function analysis results in concordant patterns between groups. The effect of LL between poly IC- and saline-exposed mice for males (**A**) and females (**B**), and the effect of poly IC-exposure between mice exposed to LD and LL in males (**C**) and females (**D**).

Supplementary Table 3.1. Statistics of behavioral data of poly IC and saline-exposed under LD or LL.

	Sex	Group x lighting		Lighting		Group		Multiple comparisons *p*-values			
		F	*p* value	F	*p* value	F	*p* value	SAL/LD vs. SAL/LL	SAL/LD vs. POL/LD	POL/LD vs. POL/LL	SAL/LL vs. POL/LL
Open field											
Thigmotaxis ratio	M	0.7201	0.3993	0.0015	0.9691	2.0090	0.1612				
	F	0.6800	0.4135	1.3300	0.2543	0.0771	0.7824				
Elevated plus maze											
Time in open arms	M	2.1210	0.1502	7.5450	**0.0078**	0.2255	0.6365	**0.0237**	NS	NS	NS
	F	1.1150	0.2967	0.0024	0.9606	2.4790	0.1225				
Percent time in open arms	M	1.7960	0.1849	8.6660	**0.0045**	0.0334	0.8556	**0.0204**	NS	NS	NS
	F	1.6000	0.2125	0.0265	0.8714	2.5070	0.1205				
Percent entries in open arms	M	3.4650	***0.0673***	7.2450	**0.0091**	0.0555	0.8145	**0.0121**	NS	NS	NS
	F	2.7260	0.1058	2.2220	0.1432	0.3116	0.5796				
Three-chamber social interaction											
Habituation ratio	M	0.1764	0.6777	0.1344	0.7167	0.8591	0.3619				
	F	0.1258	0.7254	1.2760	0.2681	0.4502	0.5077				
Social preference ratio	M	0.3484	0.5598	0.5073	0.4822	1.7080	0.2019				
	F	0.2168	0.6451	0.2847	0.5979	0.6468	0.4280				
Social memory ratio	M	0.4799	0.4942	0.0019	0.9653	0.0311	0.8612				
	F	0.1146	0.7375	0.1925	0.6642	0.0582	0.8111				
Barnes maze											

Latency to escape box	M	3.3570	***0.0752***	3.1030	***0.0866***	3.0360	***0.0900***	NS	NS	***0.0703***	***0.0534***
Entries in target quadrant	M	0.6772	0.4157	4.8420	**0.0339**	4.3510	**0.0438**	NS	NS	NS	NS
Duration in target quadrant	M	0.3214	0.5741	0.7782	0.3833	4.9410	**0.0322**	NS	NS	NS	NS
Latency to target quadrant	M	3.3570	***0.0752***	3.1030	***0.0866***	3.0360	***0.0900***	NS	NS	***0.0703***	NS
Number of errors	M	1.0140	0.3206	3.8820	***0.0563***	3.5380	***0.0679***	NS	NS	NS	NS
Prepulse inhibition of acoustic startle											
Baseline startle response	M	0.6060	0.4397	2.0250	0.1605	0.1398	0.7100				
	F	0.6529	0.4234	0.0374	0.8475	3.4880	***0.0685***	NS	NS	NS	NS
Percent average prepulse inhibition	M	2.9910	***0.0894***	0.2554	0.6154	0.0266	0.8711	NS	NS	NS	NS
	F	0.0059	0.9390	1.9650	0.1684	0.4194	0.5207				

Notes. Results from two-way ANOVAs (treatment x lighting) for behavioral parameters from poly IC and saline-exposed male and female mice. ANOVA factors include treatment (poly IC, saline) and lighting (LD, LL). P-values in **bold** represent significance, and p-values in ***bold and italics*** represent trending significance.

Supplementary Table 3.2. Sex differences in behavior of poly IC and saline-exposed mice under LD or LL.

	Treatment x Sex x Lighting		Treatment x Sex		Lighting x Sex		Main Effect Sex	
	F	*p-value*	F	*p-value*	F	*p-value*	F	*p-value*
Open field								
Thigmotaxis ratio	0.9472	0.3325	1.2780	0.2606	0.0971	0.7558	22.2300	**0.0001**
Elevated plus maze								
Time in open arms	2.9980	***0.0862***	0.6664	0.4161	3.4900	***0.0644***	0.2026	0.6535
Percent time in open arms	3.2820	***0.0728***	1.0800	0.3010	3.3280	***0.0709***	0.2302	0.6324
Percent entries in open arms	0.0342	0.8537	0.3553	0.5524	0.2089	0.6486	0.0518	0.8294
Three-chamber social interaction								
Habituation ratio	0.0216	0.8837	0.2151	0.6446	0.0015	0.9689	2.4580	0.1227
Social preference ratio	0.6262	0.4321	0.0077	0.9303	0.3275	0.5694	0.2348	0.6299
Social memory ratio	1.8400	0.1804	0.7987	0.3753	0.5268	0.4710	0.0610	0.8057
Prepulse inhibition of acoustic startle								
Baseline startle response	0.3935	0.5318	0.0646	0.7999	1.0500	0.3078	3.9640	**0.0485**
Percent average prepulse inhibition	0.8726	0.3523	0.5551	0.4579	0.6383	0.4161	1.163	0.2833

Notes. Results from ANOVAs (treatment x lighting x sex) for behavioral paramaters from poly IC- and saline mice, while also highlighting the treatment x sex interactions and main effects of sex analysis. ANOVA factors include sex (male, female), treatment (poly IC, saline exposure) and lighting (LD, LL). P-values in **bold** represent significance, and p-values in ***bold and italics*** represent trending significance.

Supplementary Table 3.3. Statistics of microglia data of poly IC and saline-exposed under LD or LL.

	Sex	Group x lighting		Lighting		Group		Multiple comparisons *p*-values			
		F	*p* value	F	*p* value	F	*p* value	SAL/LD vs. SAL/LL	SAL/LD vs. POL/LD	POL/LD vs. POL/LL	SAL/LL vs. POL/LL
Dentate gyrus											
Morphological index	M	0.0506	0.8248	3.6970	***0.0725***	4.4360	***0.0513***	NS	NS	NS	NS
	F	0.3847	0.5438	1.6100	0.2226	2.3580	0.1442				
Cell body area	M	1.0810	0.3139	2.2170	0.1559	0.2469	0.6260				
	F	0.0599	0.8097	0.3382	0.5690	0.0057	0.9408				
Cell body circularity	M	2.5510	0.1297	0.0373	0.8494	1.6190	0.2215				
	F	5.0490	**0.0391**	1.8780	0.1895	1.7150	0.2088	NS	NS	NS	NS
Density	M	2.5320	0.1311	2.5610	0.1291	2.0570	0.1708				
	F	0.3125	0.5839	4.0680	***0.0608***	2.9680	0.1042	NS	NS	NS	NS
Nearest neighbor distance	M	0.8526	0.3695	0.0013	0.9709	0.5134	0.4840				
	F	0.0110	0.9176	4.9750	**0.0404**	0.5851	0.4554	NS	NS	NS	NS
Spacing index	M	0.1856	0.6723	1.9530	0.1814	0.3943	0.5389				
	F	2.2960	0.1492	1.4800	0.2413	1.6790	0.2135				
Prefrontal cortex											
Morphological index	M	0.1877	0.6706	2.628	0.1246	0.6792	0.422				
	F	2.6970	0.1201	0.3404	0.5677	0.0630	0.8049				
Cell body area	M	0.1719	0.6840	0.0706	0.7938	1.5060	0.2376				
	F	0.1785	0.6783	0.2680	0.6118	1.3800	0.2573				

Cell body circularity	M	0.2080	0.6545	0.2560	0.6198	0.3270	0.5754
	F	0.5937	0.4522	1.8660	0.1909	0.0130	0.9105
CA1							
Morphological index	M	0.1904	0.6684	0.1499	0.7037	0.5638	0.4636
	F	0.0014	0.9707	0.2362	0.6336	0.4609	0.5069
Cell body area	M	0.2526	0.6221	4.1390	***0.0588***	0.2341	0.6351
	F	1.2000	0.2896	0.4024	0.5348	0.0146	0.9053
Cell body circularity	M	0.0444	0.8357	0.1963	0.6636	1.3890	0.2558
	F	0.0105	0.9195	0.7553	0.3976	0.7746	0.3918
Density	M	0.0058	0.9399	0.0230	0.8815	0.8295	0.3759
	F	0.4183	0.5270	0.0110	0.9174	1.4130	0.2518
Nearest neighbor distance	M	0.3291	0.5742	0.4810	0.4979	0.5390	0.4735
	F	0.1938	0.6657	0.4014	0.5363	4.5830	**0.0480**
Spacing index	M	0.2937	0.5953	0.3102	0.5853	3.7790	***0.0697***
	F	2.902	0.1078	0.017	0.8983	0.0044	0.9947

Results from two-way ANOVAs (treatment x lighting) for microglial parameters from poly IC and saline-exposed male and female mice. ANOVA factors include treatment (poly IC, saline) and lighting (LD, LL). P-values in **bold** represent significance, and p-values in ***bold and italics*** represent trending significance.

Supplementary Table 3.4. Sex differences in microglia of poly IC and saline-exposed mice under LD or LL.

	Treatment x Sex x Lighting		Treatment x Sex		Lighting x Sex		Main Effect Sex	
	F	*p-value*	F	*p-value*	F	*p-value*	F	*p-value*
Dentate gyrus								
Morphological index	0.1327	0.7180	6.1890	**0.0183**	0.0158	0.9009	4.1630	**0.0496**
Cell body area	0.7995	0.3778	0.0829	0.7752	0.3664	0.5492	10.8500	**0.0024**
Cell body circularity	0.2634	0.6113	3.3340	***0.0772***	1.2580	0.2703	0.0518	0.8213
Density	0.3323	0.5683	5.0250	**0.0320**	0.2974	0.5893	0.0343	0.8542
Nearest neighbor distance	0.3788	0.5426	1.0760	0.3073	3.2630	***0.0803***	0.0439	0.8353
Spacing index	0.4053	0.5289	1.7180	0.1993	3.4310	***0.0732***	0.0087	0.9263
PFC								
Morphological index	0.3848	0.5394	0.6615	0.4220	2.7310	0.1082	6.4700	**0.0160**
Cell body area	0.0093	0.9239	0.1080	0.7446	0.2653	0.6101	3.0760	***0.0890***
Cell body circularity	0.0140	0.9065	0.1434	0.7074	0.2002	0.6576	1.0220	0.3196
CA1								
Morphological index	0.1226	0.7285	0.0115	0.9153	0.3753	0.5445	0.1210	0.7302
Cell body area	0.0922	0.7634	0.2035	0.6549	3.9080	***0.0567***	18.7600	**0.0001**
Cell body circularity	0.0480	0.8280	0.0269	0.8709	0.8782	0.3557	2.6610	0.1127
Density	0.1363	0.7144	0.0100	0.9208	0.0020	0.9649	2.0220	0.1647
Nearest neighbor distance	0.5015	0.4840	2.5400	0.1208	0.1212	0.7300	2.1790	0.1497

Spacing index	1.932	0.1742	2.623	0.1152	0.155	0.6965	0.1436	0.7073

Notes. Results from ANOVAs (treatment x lighting x sex) for microglia paramaters from poly IC- and saline mice, while also highlighting the treatment x sex interactions and main effects of sex analysis. ANOVA factors include sex (male, female), treatment (poly IC, saline exposure) and lighting (LD, LL). P-values in **bold** represent significance, and p-values in ***bold and italics*** represent trending significance.

DISCUSSION

NDDs, such as schizophrenia and ASD are considered multifactorial in nature, caused by genetic risk variants and environmental risk factors, such as prenatal exposure to maternal immune activation (Rapoport, Addington et al. 2005, Brown 2011, Giusti-Rodriguez and Sullivan 2013). Studying how multiple risk factors for NDDs interact with each other is becoming a recurrent theme in research. However, limited work has explored circadian rhythm disruption as a potential risk factor for NDDs. In this thesis, we utilized a combination of behavioral (including running wheel), cellular (immunohistochemistry, fluorescence microscopy), and molecular (RNA sequencing) approaches to uncover the mechanisms underlying the interaction between circadian disruption and NDDs, using a MIA protocol, which models prenatal infection in mice (Chamera, Trojan et al. 2021).

MIA induced an increase in circadian wheel running activity

Many individuals with schizophrenia and ASD experience severe disruptions in sleep, rest/activity, and melatonin rhythms, and exhibit different rhythmic patterns of gene expression in various tissues (Wulff, Dijk et al. 2012, Johansson, Owe-Larsson et al. 2016, Seney, Cahill et al. 2019, Meyer, Faulkner et al. 2020). Disruptions in sleep and circadian rhythms, as measured by wheel running activity, general home cage locomotion recordings, and EEG and EMG setups, have been reported in animal models based on genetic risk factors for schizophrenia (reviewed in: Delorme, Srivastava et al. 2020) and ASD (reviewed in: Wintler, Schoch et al. 2020). However, to our knowledge, disruptions in sleep and circadian rhythms have not been assessed in animal models based on environmental risk factors for schizophrenia and ASD, such as MIA. Thus, as illustrated in **Figure 3**, using the MIA model in mice, we first explored if we could

recapitulate the circadian disruption observed in individuals with schizophrenia and ASD. In **Chapter 1**, we showed that male offspring exposed prenatal poly IC exhibited altered circadian running behavior under LD, DD and LL compared to saline-exposed controls (Delorme, Srivastava et al. 2021). These effects were sex-dependent, as poly IC-exposed females exhibited a milder phenotype.

It is unclear what specific mechanisms are at play for prenatal poly IC to cause the observed phenotype. Strikingly, the increase in (subjective) day activity persisted during all three lighting conditions, LD, DD, and LL, thus in both the presence and absence of light. The acute presence of light typically causes 'masking' (i.e., suppression of activity) in rodents, but the increased (subjective) day activity in poly IC-exposed males persisted under LD and LL. Given that the same phenotype was observed in DD (the absence of light), this provides evidence that these disruptions are not likely caused by deficits in retinal inputs to the brain but represent disruptions in ipRGCs directly impinging upon either the SCN or other brain regions that may or may not be part of circuits involving the SCN. Alternatively, it could involve disruptions in the molecular machinery within the SCN or other brain circuits. Lastly, it also has yet to be determined if and how peripheral oscillators are involved.

Two theories for the underlying causes of this poly IC-induced phenotype in circadian locomotion are presented below. Firstly, although results are conflicting, many studies, including our own (Delorme, Ozell-Landry et al. 2023), found that prenatal poly IC affects microglial development, resulting in adult microglia exhibiting a more inflammatory profile under baseline conditions (reviewed in: Smolders, Notter et al. 2018). Structural and functional differences in adult microglia may then affect circadian locomotor output. Sominsky and colleagues studied transgenic rats containing a diphtheria toxin receptor (Dtr) gene inserted into the fractalkine

receptor (Cx3cr1) promoter, which is expressed on microglia and monocytes. Upon application of diphtheria toxin, microglia and monocytes were acutely depleted. Researchers found that in the absence of microglia, Cx3cr1-Dtr transgenic rats exhibited many disruptions in diurnal rhythms, including dysregulated clock genes and proteins in the SCN and hippocampus, core body temperature (increased during the day) and energy expenditure (decreased during the night) (Sominsky, Dangel et al. 2021). These findings suggested that microglia seem to play a role in regulating diurnal rhythms. In contrast, Matsui and colleagues used PLX3397 (an inhibitor for the colony-stimulating factor 1 receptor (CSF1R), which is found in the membrane of glial cells) to deplete microglia in the mouse brain and found no differences in circadian locomotor activity (counts/day, period, or amplitude) under 12:12LD and DD and no differences in time to re-entrain to a 13-hour phase advance or in interdaily stability (Matsui, Yamaguchi et al. 2023). It is worth noting that researchers in both studies did not measure rhythms in microglia-depleted animals after LL exposure, which may have informed a role of microglia in regulating rhythmicity in a lighting condition known to disrupt rhythms. Matsui and colleagues also found that the density of microglia in the SCN was not affected by a 13-hour phase advance as measured by immunostaining in wildtype mice, but it would have been informative to also have explored morphology. Thus, it is unclear if altered microglia caused by prenatal poly IC are contributing to the phenotype in circadian locomotion. Some experiments to directly test this hypothesis include testing locomotion in 12:12LD, DD, and LL after an LPS challenge which is known to ‘activate’ microglia, or to induce prenatal MIA, deplete and repopulate microglia, and test wheel running activity.

The second proposed mechanism is as follows. Given that prenatal poly IC impairs dopamine release (Luchicchi, Lecca et al. 2016) and dopamine levels modulate the rhythmicity

of circadian activity (Gravotta, Gavrila et al. 2011), we reason that the capacity for poly IC to produce a disrupted circadian phenotype could be underpinned by a dopaminergic mechanism. Dopamine wiring is sensitive to developmental insults, such as prenatal poly IC exposure, which may have long term effects in the organization of dopaminergic circuits in the brain (Reynolds and Flores 2021). Thus, disruptions in circadian locomotion following prenatal poly IC exposure may reflect a maldeveloped dopamine system. To fully understand the mechanism through which prenatal poly IC exposure affects circadian behavior will require careful probing of the circuits thought to be impacted by prenatal insults. For example, treatment with dopamine-targeted interventions, like dopamine receptor agonists and antagonists, may rescue or exacerbate deficits in wheel-running behaviour that emerge in adulthood in prenatal poly IC-exposed mice. Identifying the systems, circuits, and receptors impacted by prenatal poly IC that contribute to disrupted circadian rhythmicity may identify targets for therapeutic intervention in disorders characterized by neurodevelopmental insult and impaired rhythmicity.

Lastly, to our knowledge, there have been no studies that have directly examined clock gene expression in poly IC-exposed offspring, but the altered circadian locomotion phenotype induced by prenatal poly IC may be a result of clock gene or clock-related gene mutations. For example, mutations in the *Opn4* gene, which encodes the photopigment melanopsin, is involved in regulating circadian rhythms, and mutations in this gene have been linked to various changes in mouse activity patterns, including an increase in daytime activity in mice (Lupi, Oster et al. 2008) and zebrafish (Dekens, Fontinha et al. 2022).

Altogether, more evidence is needed to uncover the mechanisms that drives the altered circadian locomotion phenotype caused by prenatal poly IC exposure, which might also shed light on the underlying sex difference observed. Future studies should also assess other

parameters related to circadian rhythms in the MIA model, such as sleep, core body temperature, energy expenditure, food/water intake, and clock gene expression.

Effects of LL in the literature and in our work

Circadian rhythms are endogenously generated and can keep time in the body in the absence of rhythmic environmental cues. However, to stay in synchrony with our ~24-hour external environment, they require daily resetting by light exposure (Patke, Young et al. 2020). Light in the environment is detected by photoreceptor cells in the retina, including intrinsically photosensitive retinal ganglion cells (ipRGCs) (Sonoda, Lee et al. 2018). IpRGCs contain the photopigment melanopsin and they are mainly involved in non-image forming light responses (Fu, Liao et al. 2005). From the retina, this photic input is transmitted through the retinohypothalamic tract to various brain regions, including the SCN, the principal pacemaker in mammals (Fu, Liao et al. 2005). Within the SCN, autoregulatory feedback loops involve key circadian clock genes that contribute to the generation of circadian rhythms. The SCN then sends its outputs through projections to other brain regions, which is how it generates circadian rhythms in physiology and behavior (Hastings, Maywood et al. 2018).

Disruption of the natural light cycle can lead to circadian rhythm disturbances, which can have a wide range of negative effects on health and well-being. For example, shift workers, who experience chronic circadian disruption, often exhibit sleep disturbances, increased risk of certain cancers and increased risk of chronic diseases such as obesity, diabetes, and cardiovascular disease (Foster and Wulff 2005, Evans and Davidson 2013). The mechanism by which environmental circadian disruption produces its effects are not well understood. In this thesis, we utilised LL exposure (200-250 lux, 4 weeks) to induce environmental circadian disruption. Follow up studies

should test different lighting condition that induce circadian disruption, such as chronic jetlag protocols (Castanon-Cervantes, Wu et al. 2010) and dim light at night (Fonken, Weil et al. 2013).

Studies that test the effects of LL exposure often differ in the intensity of the lighting used and the duration of exposure, thus it is difficult to directly compare them. LL may exert its effects by directly targeting the SCN. In the SCN, LL is known to desynchronizes rhythms of cells (Ohta, Yamazaki et al. 2005) and this long term desynchrony may disrupt SCN-driven rhythms in behavior and physiology (Dibner, Schibler et al. 2010). Namely, in mice, exposure to LL (60 lux for 7 days) led to dampened circadian pattern of *PER2* mRNA and reduced amplitude of *Per2* immunoreactive cell number (Sudo, Sasahara et al. 2003). LL exposure (80 lux for 50 days) also led to elevated *PER2* but not *PER1*, *CRY1*, *CRY2* protein levels in mice that remained rhythmic (Muñoz, Peirson et al. 2005). LL exposure leads to a longer circadian period (>24 hours) (Daan and Pittendrigh 1976) and prolonged LL exposed usually leads to arrhythmicity in locomotor activity (Chiesa, Cambras et al. 2010).

The effects seen in our studies might also be a result of LL targeting brain regions other than the SCN. Light information is primarily transmitted to the brain via ipRGCs in the retina (Guler, Ecker et al. 2008, LeGates, Fernandez et al. 2014). Although ipRGCs innervate the SCN, they also innervate various cortical and limbic areas (Schmidt, Do et al. 2011) that can contribute to governing different types of behaviors. For example, we found differences in sociability, cognitive- and anxiety-like behaviors; other studies using LL (200-250 lux for 8 weeks) also reported differences in anxiety-like behaviors, as well as depression-like behaviors (Tapia-Osorio, Salgado-Delgado et al. 2013). Thus, it is likely that other cortical and limbic areas are directly affected by LL exposure and involved in the presentation of these behavioral deficits.

LL exposure is known to affect metabolic systems (Fonken and Nelson 2014). Thus, it can be hypothesized that LL exerts its effects by targeting peripheral tissues. For example, LL (450 lux for 17 days) led to less subjective night melatonin levels, and less food and water intake yet more visceral adiposity in rats (Wideman and Murphy 2009). Clock gene expression in peripheral tissues including the liver, duodenum, and colon was disrupted after LL exposure (50-300 lux) in rats and interestingly time food delivery rescued this behavioral arrhythmicity (Polidarova, Sladek et al. 2011). Given that LL does not affect total 24-hour corticosterone secretion levels (Bhardwaj, Stojkovic et al. 2015), we propose that the behavioral effects induced by LL in our studies are not likely due to stress.

In summary, it is unclear if LL exerts its effects on behavior through circuits involving the SCN, circuits in other brain areas directly impinged upon by ipRGCs, or peripheral effects. Of note, although it is outside the scope of this thesis, it may be informative to study the effects of earlier exposure to LL in prenatal poly IC-exposed mice. For example, melanopsin is expressed in ipRGCs as early as E10.5 (Sekaran, Lupi et al. 2005). In mice, 13% of ipRGCs are already responsive to light at birth, and some photic input is reaching the SCN (Sekaran, Lupi et al. 2005), but will not reach adult levels until P15 (Serón-Ferré, Torres-Farfán et al. 2001). Thus, it would be interesting to expose mice to LL right after birth (e.g., a time while the circadian system is still responsive to light, but also still much in development), and compare this to the effects produced when LL is presented during adolescence or adulthood. Depending on how these experiments would be designed, it would be important in this case to consider any maternal influences on the offspring, independent of light exposure. The next section describes the various effects of adulthood and adolescence LL exposure on poly IC-exposed mice and controls presented in this thesis.

Interaction of MIA and environmental circadian disruption during adulthood and adolescence

As illustrated in **Figure 3**, **Chapter 3 and 4** of the thesis explores if exposure to environmental circadian disruption during adulthood or adolescence interacts with prenatal infection to exacerbate phenotypes related to psychiatric disorders. In **Chapter 3**, we showed a behavioral and cellular interaction between prenatal poly IC exposure and environmental circadian disruption during adulthood (Delorme, Ozell-Landry et al. 2023). One of our main findings was that poly IC-exposed male offspring exhibited decreased sociability specifically after LL. This finding aligned with our hypothesis that adulthood LL exposure interacts with pre-existing risk factors for NDDs, prenatal infection, to exacerbate behaviors related to NDDs. Females had a much milder phenotype than males. We also found that poly IC exposure led to increased microglial morphology index and density in the dentate gyrus, which would indicate a more reactive phenotype, while LL exposure seemed to attenuate these effects.

While LL exposure during adulthood led to significant changes in behavior and microglia morphology and density, many key processes in the brain have reached maturation by the time the mice were exposure to LL. Given that early windows of vulnerability are periods of rapid development, when regions of the developing brain might be most susceptible to environmental influences, we next tested the hypothesis that LL exposure during adolescence in prenatal poly IC-exposed mice would induce more pronounced deficits, which we measured in behavior, microglia, and the transcriptome.

One of our main findings was that LL exposure reduced anxiety-like behavior in saline-exposed males, but not between poly IC-exposed groups. Additionally, prenatal poly IC led to a ‘hidden talent’ phenotype after the Barnes maze and reduced sociability in males under 12:12LD,

but not LL. Similar to the studies in adulthood, adolescent exposure to LL resulted in an increased 'reactive' profile of microglia in the dentate gyrus, while LL exposure seemed to attenuate these effects. Hippocampal gene expression was significantly altered by poly IC exposure in males but not females, although poly IC exposure did have an effect in females under LL exposure. While LL caused a modest change in gene expression in saline-exposed mice of both sexes, it had more of an effect in males exposed to prenatal poly IC. Discordant patterns of gene expression overlap were found between sexes, and concordant patterns were observed between poly IC- and saline-exposed mice and between LD and LL groups, and many clusters of genes were significantly co-expressed due to prenatal poly IC and LL exposure in males and some in females.

Altogether, more deficits were observed when LL exposure was introduced during adolescence, which we interpret to be due to adolescence being a vulnerable developmental period marked by significant changes in the brain, increased vulnerability to stressors, and prominent changes in sleep and circadian rhythms. Nonetheless, LL exposure during adulthood also led to some deficits in poly IC exposed mice, which is notable because circadian disruption occurs for a large part of the population throughout adulthood, and this could directly impact the severity of symptoms for individuals with schizophrenia.

A quick comment on the maternal response to poly IC

An interesting and unexpected finding in this study was that the lot of poly IC we used led to decreased weight in both male and female offspring in the first cohort (used for behavioral tests) but not for the second and third cohorts (used for microglia and transcriptomic analyses respectively). The same dose and lot of poly IC was given to pregnant dams in all cohorts, and in a separate group of pregnant dams, we confirmed that the lot used in our study did induce an

immune response. Factors that could have contributed to the offspring weight difference are the first cohort having more power to detect such differences (more litters thus more pups used) and individual dam differences in response to the poly IC immune challenge. Specifically, in a study by Bronson and colleagues, they tested if MIA-induced deficits in offspring were mediated by the maternal response to poly IC (as measured by whether dams lost weight in response to poly IC). Notably, weight loss in pregnant dams after a E14 poly IC injection predicted whether offspring developed locomotor abnormalities following two pharmacologic challenges MK-801 (a NMDA receptor antagonist) and amphetamine (Bronson, Ahlbrand et al. 2011). While other studies did not report differences in maternal response after administration of different doses of poly IC (Cunningham, Campion et al. 2007), this does not dismiss the fact that individual differences in the maternal response could mediate severity of deficits in offspring and should be considered when interpreting results.

Role of microglia in maternal immune activation model

In this thesis, we assessed microglia morphology and density after LL exposure in adulthood and adolescence in mice subjected to prenatal poly IC. Altered microglia have been observed in the MIA mouse model (Hui, St-Pierre et al. 2018) and they also have been shown to play a role in circadian rhythms. For example, hippocampal microglia were isolated from adult rats every 6 hours and the relative mRNA expression of circadian clock genes (such as Per1, Per2, Rev-erb and BMAL1) were rhythmic over 24 hours. Interestingly, transcripts of pro-inflammatory cytokines (such as IL1, TNFα, IL6 and IL1R1) were also rhythmic over 24 hours, with peak expression during the light phase (Fonken, Frank et al. 2015). In addition, a study by Griffin and colleagues found a time-of-day oscillation in microglial phagocytosis, where microglia engulfed

more presynaptic terminals in the night than the day (Griffin, Sheehan et al. 2020). In a study by Wang and colleagues, when *Bmal1*, a core clock gene, was knocked down in microglia, this increased microglial phagocytosis in mice on a high-fat diet and improved memory on a learning process (Wang, Kooijman et al. 2021). When another clock gene, *Rev-erbα,* was deficient in microglia, this induced a pro-inflammatory phenotype (Griffin, Dimitry et al. 2019).

Due to the intimate relationship between microglia, the brain's immune cells, and the circadian system, it is reasonable to assume that long term exposure to aberrant light cycles has profound effects on microglia. In addition, many studies found that prenatal poly IC affects microglial development, resulting in adult microglia exhibiting a more inflammatory profile under baseline conditions. Thus, the role that microglia play in the interaction between prenatal poly IC and environmental circadian disruption was particularly interesting. Despite replicating some findings that microglia exhibit a more 'reactive' profile in adult offspring exposed to prenatal infection, we also found that subsequent adulthood and also adolescent exposure to LL seemed to attenuate the effects induced by the prenatal poly IC. While LL exposure was originally hypothesized to heighten the activation-related characteristics of MIA-primed microglia, it is possible that the MIA primed microglia exhibited an exhaustive phenotype to subsequent exposure to LL. We also acknowledge that other central nervous system cells are likely involved, such as other glial cells and neurons.

LL exposure during adulthood versus adolescence

LL exposure during adolescence was hypothesized to result in more deficits in prenatal poly IC mice due to adolescence being a sensitive period in development. Firstly, the age of onset of many psychiatric disorders is in adolescence to early adulthood (Kessler, Amminger et al. 2007),

with the caveat that many studies likely underestimate the onset of schizophrenia, by relying on first hospitalization or first signs of psychosis. Additionally, adolescence is also a period of significant changes in the brain (Spear 2000), and a period of increased vulnerability to stressors (McCormick and Green 2013). Structural changes of the brain in adolescence include gray matter volume and cortical thickness, white matter volume and microstructure (reviewed here Foulkes and Blakemore 2018).

Adolescence is marked by a delay in sleep onset, which is partially driven by a delay in the circadian system (Roenneberg, Kuehnle et al. 2004, Randler 2008). A study by Crowley and colleagues studied the sensitivity to light in adolescents during pre-mid puberty (9.1-14.7 years old) and late-post puberty (11.5-15.9 years old), where pubertal status was assessed using the Tanner stages. Researchers found that the pre-mid puberty group exhibited greater melatonin suppression to 15, 150 and 500 lux of evening light exposure compared to the late-post puberty group. Interesting, no differences were seen between groups when light exposure occurred during a morning timepoint, highlighting the finding that young adolescents are particularly sensitive to evening light, even as low as 15 lux (Crowley and et al. 2015). This is important because it is well known that exposure to light at night delays the clock (causing the individual to wake up later the next morning) (Randler 2008). If adolescence is already a period marked by a delay in sleep and circadian rhythms, then heightened sensitivity to light at night would further enforce this delay. These could be reasons for which greater deficits were observed in prenatal poly IC mice who were subjected to LL-exposure during adolescence.

Sex differences

At this level, while we acknowledge the existence of sex differences, it is not fully clear what the underlying cause is. Various factors could contribute to the sex-dependent effects observed in our studies. For example, sex differences could reflect distinct adaptive strategies to prenatal infection and circadian disruption, sex hormones, and genetics. These sex differences may also highlight the ways in which the brain is structured or wired that could affect its functioning. The social environment, and ways in which groups of males interact and socialize versus groups of female mice, may also contribute to the sex differences observed. For human populations, social and cultural factors may also play a role, as cultural beliefs and practices can impact the way these disorders are perceived and managed by individuals in their families (Gopalkrishnan 2018).

Sex differences are apparent in most of the studies conducted in this thesis, as a result of the effect of prenatal poly IC exposure, or the effect of LL exposure or the combined effects of both manipulations. These sex differences appear in all levels studied: on behavior, on microglia and in the transcriptome. Notably, when we directly compared sexes between our groups of interest, we found discordant patterns of gene overlap, which highlight distinct patterns of gene expression in males versus females. The sex differences found in our studies was not surprising, as male and female individuals with schizophrenia and ASD exhibit a number of differences. Both disorders are more prevalent in males (McGrath, Saha et al. 2004, Demily, Poisson et al. 2017) and the symptom profiles, and neuroanatomical and neurocognitive changes in both disorders can vary widely by sex (Volkmar, Siegel et al. 2014, Mendrek and Mancini-Marie 2016).

Additionally, while some studies have explored sleep and circadian rhythms in individuals with schizophrenia and ASD (as discussed above), none of those studies, to our knowledge, had enough power to detect if this disrupted sleep and circadian rhythms phenotype depended on sex

or gender. Similarly, most of the studies exploring animal models of schizophrenia and ASD did not utilize both sexes when assessing the sleep and circadian rhythms phenotype.

Altogether, understanding sex differences in the sleep and circadian rhythms phenotype of individuals with schizophrenia and ASD is critical to better tailor treatment strategies and interventions to support the specific needs of individual patients.

How to target mechanisms underlying the interaction between prenatal infection and circadian disruption

In the transcriptomic data, we identified several differentially regulated genes and co-expressed clusters of genes related to prenatal poly IC exposure and LL exposure in males and females. We also have performed a pathway enrichment analysis (discussed in the general future directions below) to identify the function of the affected gene clusters. Some follow up experiments could be to pharmacologically target one or more of these enriched pathways. For example, if we identify that a significant module positively associated with one of our treatments of interest is related to vesicle fusion, then we can administer pharmacological agents that target cell adhesion molecules.

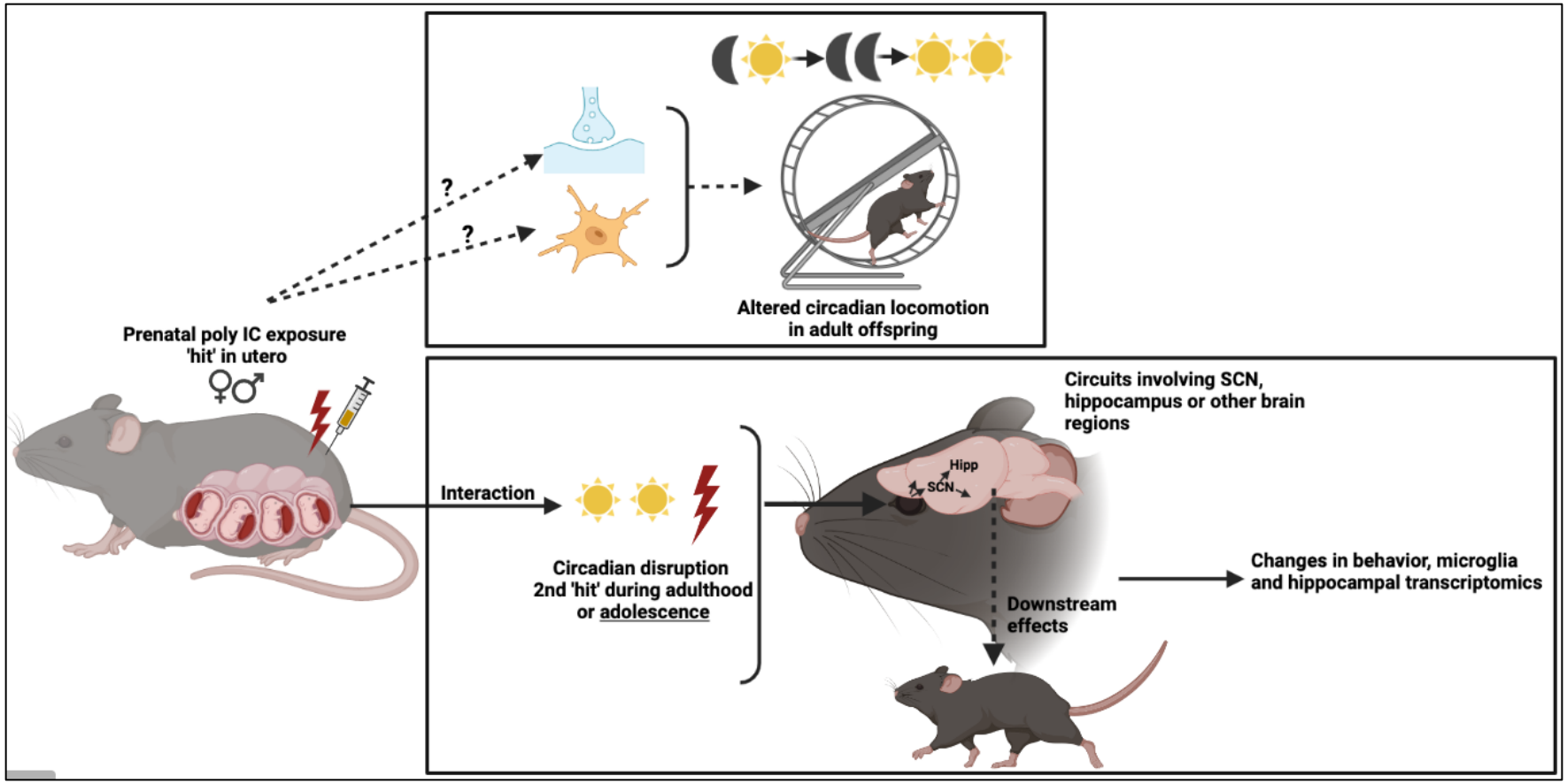


Fig. 4.1. Summary of the interaction between prenatal poly IC exposure and circadian rhythm disruption. We firstly found that prenatal poly IC exposure led to altered circadian locomotion in adult offspring and we propose that disrupted dopamine signalling, or microglia could be mediating this effect. We secondly found that prenatal poly IC exposure interacted with exposure to environmental circadian disruption on a behavioral, microglial, and transcriptomic level. It is unclear if these effects are caused by circuits involving the SCN, hippocampus or other brain regions, or due to peripheral effects.

CONCLUSION AND SUMMARY

In this thesis, we examined the effects of prenatal poly IC-exposure on circadian locomotion in both males and females, and then we explored the interaction between prenatal poly IC-exposure and environmental circadian disruption exposure during adolescence and adulthood. We firstly identified that prenatal poly IC-exposure led to altered circadian locomotion in males, which was significant because this was, to our knowledge, the first paper exploring rhythm disruption in an animal model based on an environmental risk factor for schizophrenia and ASD. We secondly identified several interactions on the behavioral, microglial, and transcriptomic level between prenatal poly IC-exposure and LL exposure, a lighting condition that induces circadian disruption.

An obvious follow up to our data is to run a pathway enrichment analysis, which is currently underway, to understand the function of the groups of genes that are changed by our experimental manipulations. Other analyses that are planned for the near future include the establishment of an expression-based polygenic risk score, which utilizes animal gene expression data and human genomic and phenotypic data to predict disease risk in humans (Hari Dass, McCracken et al. 2019). Future studies would benefit from exploring other sequencing techniques such as single nuclei RNA-sequencing to identify which cells are specifically affected by prenatal poly IC exposure and environmental circadian disruption, as well as chromatin immunoprecipitation sequencing, to identify binding sites of DNA-associated proteins and explore protein interactions with DNA. This would aid in the understanding of the mechanism that influences the phenotypes that we observed.

Finally, understanding the role of circadian disruption in psychiatric disorders can inform the development of sleep and circadian based therapies, which can be targeted as a adjunctive treatment for individuals with psychiatric disorders**.**

REFERENCES

Aavani, T., S. A. Rana, R. Hawkes and Q. J. Pittman (2015). "Maternal Immune Activation Produces Cerebellar Hyperplasia and Alterations in Motor and Social Behaviors in Male and Female Mice." The Cerebellum **14**(5): 491-505.

Abel, K. M., R. Drake and J. M. Goldstein (2010). "Sex differences in schizophrenia." International Review of Psychiatry **22**(5): 417-428.

Aishworiya, R., T. Valica, R. Hagerman and B. Restrepo (2022). "An Update on Psychopharmacological Treatment of Autism Spectrum Disorder." Neurotherapeutics **19**(1): 248-262.

Ajami, B., J. L. Bennett, C. Krieger, W. Tetzlaff and F. M. Rossi (2007). "Local self-renewal can sustain CNS microglia maintenance and function throughout adult life." Nat Neurosci **10**(12): 1538-1543.

Alexopoulou, L., A. C. Holt, R. Medzhitov and R. A. Flavell (2001). "Recognition of double-stranded RNA and activation of NF-kappaB by Toll-like receptor 3." Nature **413**(6857): 732-738.

Allswede, D. M., S. L. Buka, R. H. Yolken, E. F. Torrey and T. D. Cannon (2016). "Elevated maternal cytokine levels at birth and risk for psychosis in adult offspring." Schizophr Res **172**(1-3): 41-45.

Alnaes, D., T. Kaufmann, D. van der Meer, A. Cordova-Palomera, J. Rokicki, T. Moberget, F. Bettella, I. Agartz, D. M. Barch, A. Bertolino, C. L. Brandt, S. Cervenka, S. Djurovic, N. T. Doan, S. Eisenacher, H. Fatouros-Bergman, L. Flyckt, A. Di Giorgio, B. Haatveit, E. G. Jonsson, P. Kirsch, M. J. Lund, A. Meyer-Lindenberg, G. Pergola, E. Schwarz, O. B. Smeland, T. Quarto, M. Zink, O. A. Andreassen, L. T. Westlye and C. Karolinska

Schizophrenia Project (2019). "Brain Heterogeneity in Schizophrenia and Its Association With Polygenic Risk." JAMA Psychiatry **76**(7): 739-748.

AmericanPsychiatricAssociation (2013). Diagnostic and statistical manual of mental disorders (5th ed.), https://doi.org/10.1176/appi.books.9780890425596.dsm01.

Arrode-Bruses, G. and J. L. Bruses (2012). "Maternal immune activation by poly I:C induces expression of cytokines IL-1beta and IL-13, chemokine MCP-1 and colony stimulating factor VEGF in fetal mouse brain." J Neuroinflammation **9**: 83.

Aschoff, J. and H. Pohl (1978). "Phase relations between a circadian rhythm and its zeitgeber within the range of entrainment." Naturwissenschaften **65**(2): 80–84.

Association, A. P. (2013). Diagnostic and statistical manual of mental disorders (5th ed.), https://doi.org/10.1176/appi.books.9780890425596.dsm01.

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 **130**(6): e1447-1454.

Atladottir, H. O., P. Thorsen, L. Ostergaard, D. E. Schendel, S. Lemcke, M. Abdallah and E. T. Parner (2010). "Maternal infection requiring hospitalization during pregnancy and autism spectrum disorders." J Autism Dev Disord **40**(12): 1423-1430.

Atladottir, H. O., P. Thorsen, L. Ostergaard, D. E. Schendel, S. Lemcke, M. Abdallah and E. T. Parner (2010). "Maternal infection requiring hospitalization during pregnancy and autism spectrum disorders." J Autism Dev Disord **40**(12): 1423–1430.

Banker, S. M. and et al. (2021). "Hippocampal contributions to social and cognitive deficits in autism spectrum disorder." Trends in Neurosciences **44**(10): 793.

Banker, S. M., X. Gu, D. Schiller and J. H. Foss-Feig (2021). "Hippocampal contributions to social and cognitive deficits in autism spectrum disorder." Trends Neurosci **44**(10): 793-807.

Bargiela, S., R. Steward and W. Mandy (2016). "The Experiences of Late-diagnosed Women with Autism Spectrum Conditions: An Investigation of the Female Autism Phenotype." J Autism Dev Disord **46**(10): 3281-3294.

Baron-Cohen, S. (2017). "Editorial Perspective: Neurodiversity - a revolutionary concept for autism and psychiatry." J Child Psychol Psychiatry **58**(6): 744-747.

Baron-Cohen, S., F. J. Scott, C. Allison, J. Williams, P. Bolton, F. E. Matthews and C. Brayne (2009). "Prevalence of autism-spectrum conditions: UK school-based population study." Br J Psychiatry **194**(6): 500-509.

Baxter, A. J., T. S. Brugha, H. E. Erskine, R. W. Scheurer, T. Vos and J. G. Scott (2015). "The epidemiology and global burden of autism spectrum disorders." Psychol Med **45**(3): 601-613.

Bedrosian, T. A., L. K. Fonken, J. C. Walton, A. Haim and R. J. Nelson (2011). "Dim light at night provokes depression-like behaviors and reduces CA1 dendritic spine density in female hamsters." Psychoneuroendocrinology **36**(7): 1062–1069.

Benson, K. L. (2015). "Sleep in Schizophrenia: Pathology and Treatment." Sleep Med Clin **10**(1): 49–55.

Berg, B. J., 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): 265-282.

Berson, D. M., F. A. Dunn and M. Takao (2002). "Phototransduction by retinal ganglion cells that set the circadian clock." Science **295**(5557): 1070–1073.

Bhardwaj, S. K., M. Baharnoori, B. Sharif-Askari, A. Kamath, S. Williams and L. K. Srivastava (2009). "Behavioral characterization of dysbindin-1 deficient sandy mice." Behav Brain Res **197**(2): 435–441.

Bhardwaj, S. K., K. Stojkovic, S. Kiessling, L. K. Srivastava and N. Cermakian (2015). "Constant light uncovers behavioral effects of a mutation in the schizophrenia risk gene Dtnbp1 in mice." Behav Brain Res **284**: 58–68.

Bhardwaj, S. K., K. Stojkovic, S. Kiessling, L. K. Srivastava and N. Cermakian (2015). "Constant light uncovers behavioral effects of a mutation in the schizophrenia risk gene Dtnbp1 in mice." Behav Brain Res **284**: 58-68.

Bilder, D., E. L. Botts, K. R. Smith, R. Pimentel, M. Farley, J. Viskochil, W. M. McMahon, H. Block, E. Ritvo, R.-A. Ritvo and H. Coon (2013). "Excess Mortality and Causes of Death in Autism Spectrum Disorders: A Follow up of the 1980s Utah/UCLA Autism Epidemiologic Study." Journal of Autism and Developmental Disorders **43**(5): 1196-1204.

Bisht, K., K. P. Sharma, C. Lecours, M. Gabriela Sánchez, H. El Hajj, G. Milior, A. Olmos-Alonso, D. Gómez-Nicola, G. Luheshi, L. Vallières, I. Branchi, L. Maggi, C. Limatola, O. Butovsky and M. È. Tremblay (2016). "Dark microglia: A new phenotype predominantly associated with pathological states." Glia **64**(5): 826-839.

Bitanihirwe, B. K., D. Peleg-Raibstein, F. Mouttet, J. Feldon and U. Meyer (2010). "Late Prenatal Immune Activation in Mice Leads to Behavioral and Neurochemical Abnormalities Relevant to the Negative Symptoms of Schizophrenia." Neuropsychopharmacology **35**(12): 2462-2478.

Block, C. L., O. Eroglu, S. D. Mague, C. J. Smith, A. M. Ceasrine, C. Sriworarat, C. Blount, K. A. Beben, K. E. Malacon, N. Ndubuizu, A. Talbot, N. M. Gallagher, Y. Chan Jo, T.

Nyangacha, D. E. Carlson, K. Dzirasa, C. Eroglu and S. D. Bilbo (2022). "Prenatal environmental stressors impair postnatal microglia function and adult behavior in males." Cell Reports **40**(5): 111161.

Bloomfield, P. S., S. Selvaraj, M. Veronese, G. Rizzo, A. Bertoldo, D. R. Owen, M. A. Bloomfield, I. Bonoldi, N. Kalk, F. Turkheimer, P. McGuire, V. de Paola and O. D. Howes (2016). "Microglial Activity in People at Ultra High Risk of Psychosis and in Schizophrenia: An [(11)C]PBR28 PET Brain Imaging Study." Am J Psychiatry **173**(1): 44-52.

Boden, M. J., T. J. Varcoe and D. J. Kennaway (2013). "Circadian regulation of reproduction: From gamete to offspring." Progress in Biophysics and Molecular Biology **113**(3): 387-397.

Boivin, D. B. (2000). "Influence of sleep-wake and circadian rhythm disturbances in psychiatric disorders." J Psychiatry Neurosci **25**(5): 446-458.

Boivin, D. B., P. Boudreau and A. Kosmadopoulos (2022). "Disturbance of the Circadian System in Shift Work and Its Health Impact." J Biol Rhythms **37**(1): 3-28.

Borbely, A. A. (2009). "Refining sleep homeostasis in the two-process model." J Sleep Res **18**(1): 1–2.

Boulanger-Bertolus, J., C. Pancaro and G. A. Mashour (2018). "Increasing Role of Maternal Immune Activation in Neurodevelopmental Disorders." Front Behav Neurosci **12**: 230.

Braun, A. E., P. A. Carpentier, B. A. Babineau, A. R. Narayan, M. L. Kielhold, H. M. Moon, A. Shankar, J. Su, V. Saravanapandian, U. Haditsch and T. D. Palmer (2019). ""Females Are Not Just 'Protected' Males": Sex-Specific Vulnerabilities in Placenta and Brain after Prenatal Immune Disruption." eNeuro **6**(6).

Bromundt, V., M. Koster, A. Georgiev-Kill, K. Opwis, A. Wirz-Justice, G. Stoppe and C. Cajochen (2011). "Sleep-wake cycles and cognitive functioning in schizophrenia." Br J Psychiatry **198**(4): 269–276.

Bronson, S. L., R. Ahlbrand, P. S. Horn, J. R. Kern and N. M. Richtand (2011). "Individual differences in maternal response to immune challenge predict offspring behavior: Contribution of environmental factors." Behavioural Brain Research **220**(1): 55-64.

Brown, A. (2011). "Exposure to Prenatal Infection and Risk of Schizophrenia." Frontiers in Psychiatry **2**.

Brown, A. S. (2011). "The environment and susceptibility to schizophrenia." Prog Neurobiol **93**(1): 23-58.

Brown, A. S. (2012). "Epidemiologic studies of exposure to prenatal infection and risk of schizophrenia and autism." Dev Neurobiol **72**(10): 1272-1276.

Brown, A. S., M. D. Begg, S. Gravenstein, C. A. Schaefer, R. J. Wyatt, M. Bresnahan, V. P. Babulas and E. S. Susser (2004). "Serologic evidence of prenatal influenza in the etiology of schizophrenia." Arch Gen Psychiatry **61**(8): 774–780.

Brown, A. S., M. D. Begg, S. Gravenstein, C. A. Schaefer, R. J. Wyatt, M. Bresnahan, V. P. Babulas and E. S. Susser (2004). "Serologic evidence of prenatal influenza in the etiology of schizophrenia." Arch Gen Psychiatry **61**(8): 774-780.

Brown, A. S., P. Cohen, J. Harkavy-Friedman, V. Babulas, D. Malaspina, J. M. Gorman and E. S. Susser (2001). "Prenatal rubella, premorbid abnormalities, and adult schizophrenia." Biol Psychiatry **49**(6): 473–486.

Brown, A. S., J. Hooton, C. A. Schaefer, H. Zhang, E. Petkova, V. Babulas, M. Perrin, J. M. Gorman and E. S. Susser (2004). "Elevated maternal interleukin-8 levels and risk of schizophrenia in adult offspring." Am J Psychiatry **161**(5): 889-895.

Brown, A. S. and U. Meyer (2018). "Maternal Immune Activation and Neuropsychiatric Illness: A Translational Research Perspective." Am J Psychiatry **175**(11): 1073-1083.

Brown, J. P., D. Martin, Z. Nagaria, A. C. Verceles, S. L. Jobe and E. M. Wickwire (2020). "Mental Health Consequences of Shift Work: An Updated Review." Curr Psychiatry Rep **22**(2): 7.

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 Behav Immun **15**(4): 411-420.

Butovsky, O., M. P. Jedrychowski, C. S. Moore, R. Cialic, A. J. Lanser, G. Gabriely, T. Koeglsperger, B. Dake, P. M. Wu, C. E. Doykan, Z. Fanek, L. Liu, Z. Chen, J. D. Rothstein, R. M. Ransohoff, S. P. Gygi, J. P. Antel and H. L. Weiner (2014). "Identification of a unique TGF-beta-dependent molecular and functional signature in microglia." Nat Neurosci **17**(1): 131-143.

Buwalda, B., C. Stubbendorff, N. Zickert and J. M. Koolhaas (2013). "Adolescent social stress does not necessarily lead to a compromised adaptive capacity during adulthood: A study on the consequences of social stress in rats." Neuroscience **249**: 258-270.

Cahill, K. M., Z. Huo, G. C. Tseng, R. W. Logan and M. L. Seney (2018). "Improved identification of concordant and discordant gene expression signatures using an updated rank-rank hypergeometric overlap approach." Scientific Reports **8**(1): 9588.

Careaga, M., S. L. Taylor, C. Chang, A. Chiang, K. M. Ku, R. F. Berman, J. A. Van de Water and M. D. Bauman (2018). "Variability in PolyIC induced immune response: Implications for preclinical maternal immune activation models." J Neuroimmunol **323**: 87–93.

Carmassi, C., L. Palagini, D. Caruso, I. Masci, L. Nobili, A. Vita and L. Dell'Osso (2019). "Systematic Review of Sleep Disturbances and Circadian Sleep Desynchronization in Autism Spectrum Disorder: Toward an Integrative Model of a Self-Reinforcing Loop." Front Psychiatry **10**: 366.

Carney, R. S. E. (2019). "Does Prenatal Exposure to Maternal Inflammation Causes Sex Differences in Schizophrenia-Related Behavioral Outcomes in Adult Rats?" eNeuro **6**(6).

Castanon-Cervantes, O., M. Wu, J. C. Ehlen, K. Paul, K. L. Gamble, R. L. Johnson, R. C. Besing, M. Menaker, A. T. Gewirtz and A. J. Davidson (2010). "Dysregulation of inflammatory responses by chronic circadian disruption." J Immunol **185**(10): 5796–5805.

Castro, J. P. M. V., R. Frussa-Filho, D. F. Fukushiro, C. C. Chinen, V. C. Abílio and R. H. Silva (2005). "Effects of long-term continuous exposure to light on memory and anxiety in mice." Physiology & Behavior **86**(1): 218-223.

Chamera, K., E. Trojan, K. Kotarska, M. Szuster-Gluszczak, N. Bryniarska, K. Tylek and A. Basta-Kaim (2021). "Role of Polyinosinic:Polycytidylic Acid-Induced Maternal Immune Activation and Subsequent Immune Challenge in the Behaviour and Microglial Cell Trajectory in Adult Offspring: A Study of the Neurodevelopmental Model of Schizophrenia." Int J Mol Sci **22**(4).

Chang, Y.-C., W.-Y. Li, L. J.-H. Lee and L.-J. Lee (2020). "Interplay of Prenatal and Postnatal Risk Factors in the Behavioral and Histological Features of a “Two-Hit” Non-Genetic

Mouse Model of Schizophrenia." International Journal of Molecular Sciences **21**(22): 8518.

Charlson, F. J., A. J. Ferrari, D. F. Santomauro, S. Diminic, E. Stockings, J. G. Scott, J. J. McGrath and H. A. Whiteford (2018). "Global Epidemiology and Burden of Schizophrenia: Findings From the Global Burden of Disease Study 2016." Schizophr Bull **44**(6): 1195-1203.

Cheng, C. H., P. S. Chan, S. C. Hsu and C. Y. Liu (2018). "Meta-analysis of sensorimotor gating in patients with autism spectrum disorders." Psychiatry Res **262**: 413-419.

Cheroni, C., N. Caporale and G. Testa (2020). "Autism spectrum disorder at the crossroad between genes and environment: contributions, convergences, and interactions in ASD developmental pathophysiology." Mol Autism **11**(1): 69.

Chiesa, J. J., T. Cambras, Á. R. Carpentieri and A. Díez-Noguera (2010). "Arrhythmic rats after SCN lesions and constant light differ in short time scale regulation of locomotor activity." Journal of biological rhythms **25**(1): 37-46.

Chouinard, S., J. Poulin, E. Stip and R. Godbout (2004). "Sleep in untreated patients with schizophrenia: a meta-analysis." Schizophr Bull **30**(4): 957–967.

Cisse, Y. M., J. Peng and R. J. Nelson (2016). "Dim light at night prior to adolescence increases adult anxiety-like behaviors." Chronobiol Int **33**(10): 1473–1480.

Clancy, B., R. B. Darlington and B. L. Finlay (2001). "Translating developmental time across mammalian species." Neuroscience **105**(1): 7–17.

Clancy, B., R. B. Darlington and B. L. Finlay (2001). "Translating developmental time across mammalian species." Neuroscience **105**(1): 7-17.

Clancy, B., B. L. Finlay, R. B. Darlington and K. J. S. Anand (2007). "Extrapolating brain development from experimental species to humans." NeuroToxicology **28**(5): 931-937.

Claustrat, B., J. L. Valatx, C. Harthé and J. Brun (2008). "Effect of Constant Light on Prolactin and Corticosterone Rhythms Evaluated Using a Noninvasive Urine Sampling Protocol in the Rat." Hormone and Metabolic Research **40**(6): 398-403.

Cloutier, M. E., L. K. Srivastava and N. Cermakian (2022). "Exposure to Circadian Disruption During Adolescence Interacts With a Genetic Risk Factor to Modify Schizophrenia-relevant Behaviors in a Sex-dependent Manner." J Biol Rhythms **37**(6): 655-672.

Cohen, S., R. Conduit, S. W. Lockley, S. M. W. Rajaratnam and K. M. Cornish (2014). "The relationship between sleep and behavior in autism spectrum disorder (ASD): a review." Journal of Neurodevelopmental Disorders **6**(1): 44.

Cohrs, S. (2008). "Sleep disturbances in patients with schizophrenia : impact and effect of antipsychotics." CNS Drugs **22**(11): 939-962.

Cohrs, S. (2008). "Sleep disturbances in patients with schizophrenia : impact and effect of antipsychotics." CNS Drugs **22**(11): 939–962.

Coiro, P., R. Padmashri, A. Suresh, E. Spartz, G. Pendyala, S. Chou, Y. Jung, B. Meays, S. Roy, N. Gautam, Y. Alnouti, M. Li and A. Dunaevsky (2015). "Impaired synaptic development in a maternal immune activation mouse model of neurodevelopmental disorders." Brain Behav Immun **50**: 249-258.

Consortium, S. W. G. o. t. P. G. (2014). "Biological insights from 108 schizophrenia-associated genetic loci." Nature **511**(7510): 421–427.

Coomans, C. P., S. A. A. Berg, T. Houben, J. B. Klinken, R. Berg, A. C. M. Pronk, L. M. Havekes, J. A. Romijn, K. W. Dijk, N. R. Biermasz and J. H. Meijer (2013). "Detrimental effects of constant light exposure and high-fat diet on circadian energy metabolism and insulin sensitivity." The FASEB Journal **27**(4): 1721-1732.

Coomans, C. P., S. A. A. Berg, T. Houben, J. B. Klinken, R. Berg, A. C. M. Pronk, L. M. Havekes, J. A. Romijn, K. W. Dijk, N. R. Biermasz and J. H. Meijer (2013). "Detrimental effects of constant light exposure and high-fat diet on circadian energy metabolism and insulin sensitivity." The FASEB Journal **27**(4): 1721–1732.

Cosgrave, J., K. Wulff and P. Gehrman (2018). "Sleep, circadian rhythms, and schizophrenia: where we are and where we need to go." Curr Opin Psychiatry **31**(3): 176-182.

Costa, G. (2010). "Shift Work and Health: Current Problems and Preventive Actions." Safety and Health at Work **1**(2): 112-123.

Costa, G., E. Haus and R. Stevens (2010). "Shift work and cancer - considerations on rationale, mechanisms, and epidemiology." Scand J Work Environ Health **36**(2): 163-179.

Couturier, J. L., K. N. Speechley, M. Steele, R. Norman, B. Stringer and R. Nicolson (2005). "Parental perception of sleep problems in children of normal intelligence with pervasive developmental disorders: prevalence, severity, and pattern." J Am Acad Child Adolesc Psychiatry **44**(8): 815-822.

Cox, M. M., A. M. Tucker, J. Tang, K. Talbot, D. C. Richer, L. Yeh and S. E. Arnold (2009). "Neurobehavioral abnormalities in the dysbindin-1 mutant, sandy, on a C57BL/6J genetic background." Genes Brain Behav **8**(4): 390–397.

Crowley, S. J. and et al. (2015). "Increased Sensitivity of the Circadian System to Light in Early/Mid-Puberty." Journal of Clinical Endocrinology and Metabolism, The **100**(11): 4067.

Cunningham, C., S. Campion, J. Teeling, L. Felton and V. H. Perry (2007). "The sickness behaviour and CNS inflammatory mediator profile induced by systemic challenge of mice with synthetic double-stranded RNA (poly I:C)." Brain Behav Immun **21**(4): 490-502.

Cunningham, C., S. Campion, J. Teeling, L. Felton and V. H. Perry (2007). "The sickness behaviour and CNS inflammatory mediator profile induced by systemic challenge of mice with synthetic double-stranded RNA (poly I:C)." Brain Behav Immun **21**(4): 490–502.

Daan, S. and C. S. Pittendrigh (1976). "A functional analysis of circadian pacemakers in nocturnal rodents." Journal of Comparative Physiology■ A **106**(3): 253-266.

Davidson, A. J., M. T. Sellix, J. Daniel, S. Yamazaki, M. Menaker and G. D. Block (2006). "Chronic jet-lag increases mortality in aged mice." Curr Biol **16**(21): R914–916.

Davis, J., H. Eyre, F. N. Jacka, S. Dodd, O. Dean, S. McEwen, M. Debnath, J. McGrath, M. Maes, P. Amminger, P. D. McGorry, C. Pantelis and M. Berk (2016). "A review of vulnerability and risks for schizophrenia: Beyond the two hit hypothesis." Neurosci Biobehav Rev **65**: 185-194.

Davis, J., H. Eyre, F. N. Jacka, S. Dodd, O. Dean, S. McEwen, M. Debnath, J. McGrath, M. Maes, P. Amminger, P. D. McGorry, C. Pantelis and M. Berk (2016). "A review of vulnerability and risks for schizophrenia: Beyond the two hit hypothesis." Neurosci Biobehav Rev **65**: 185–194.

De Picker, L. J., M. Morrens, S. A. Chance and D. Boche (2017). "Microglia and Brain Plasticity in Acute Psychosis and Schizophrenia Illness Course: A Meta-Review." Front Psychiatry **8**: 238.

Dekens, M. P. S., B. M. Fontinha, M. Gallach, S. Pflügler and K. Tessmar-Raible (2022). "Melanopsin elevates locomotor activity during the wake state of the diurnal zebrafish." EMBO reports **23**(5).

Delorme, T. C., W. Ozell-Landry, N. Cermakian and L. K. Srivastava (2023). "Behavioral and cellular responses to circadian disruption and prenatal immune activation in mice." Scientific Reports.

Delorme, T. C., L. K. Srivastava and N. Cermakian (2020). "Are Circadian Disturbances a Core Pathophysiological Component of Schizophrenia?" J Biol Rhythms **35**(4): 325–339.

Delorme, T. C., L. K. Srivastava and N. Cermakian (2020). "Are Circadian Disturbances a Core Pathophysiological Component of Schizophrenia?" J Biol Rhythms **35**(4): 325-339.

Delorme, T. C., L. K. Srivastava and N. Cermakian (2021). "Altered circadian rhythms in a mouse model of neurodevelopmental disorders based on prenatal maternal immune activation." Brain Behav Immun **93**: 119-131.

Delorme, T. C., L. K. Srivastava and N. Cermakian (2021). "Altered circadian rhythms in a mouse model of neurodevelopmental disorders based on prenatal maternal immune activation." Brain Behav Immun **93**: 119–131.

Delorme, T. C. W. O.-L., Nicolas Cermakian and Lalit K. Srivastava (In review).

Demily, C., A. Poisson, E. Peyroux, V. Gatellier, A. Nicolas, C. Rigard, C. Schluth-Bolard, D. Sanlaville and M. Rossi (2017). "Autism spectrum disorder associated with 49,XYYYY: case report and review of the literature." BMC Med Genet **18**(1): 9.

Depino, A. M. (2015). "Early prenatal exposure to LPS results in anxiety- and depression-related behaviors in adulthood." Neuroscience **299**: 56-65.

Devnani, P. A. and A. U. Hegde (2015). "Autism and sleep disorders." J Pediatr Neurosci **10**(4): 304-307.

Dibner, C., U. Schibler and U. Albrecht (2010). "The mammalian circadian timing system: organization and coordination of central and peripheral clocks." Annu Rev Physiol **72**: 517–549.

Dickman, D. K. and G. W. Davis (2009). "The schizophrenia susceptibility gene dysbindin controls synaptic homeostasis." Science **326**(5956): 1127–1130.

Ding, S., Y. Hu, B. Luo, Y. Cai, K. Hao, Y. Yang, Y. Zhang, X. Wang, M. Ding, H. Zhang, W. Li and L. Lv (2019). "Age-related changes in neuroinflammation and prepulse inhibition in offspring of rats treated with Poly I:C in early gestation." Behavioral and Brain Functions **15**(1).

Drake, C. L., T. Roehrs, G. Richardson, J. K. Walsh and T. Roth (2004). "Shift work sleep disorder: prevalence and consequences beyond that of symptomatic day workers." Sleep **27**(8): 1453-1462.

Ebling, F. J. (1996). "The role of glutamate in the photic regulation of the suprachiasmatic nucleus." Prog Neurobiol **50**(2-3): 109–132.

Erlenmeyer-Kimling, L., Z. Folnegovic, V. Hrabak-Zerjavic, B. Borcic, V. Folnegovic-Smalc and E. Susser (1994). "Schizophrenia and prenatal exposure to the 1957 A2 influenza epidemic in Croatia." Am J Psychiatry **151**(10): 1496-1498.

Esslinger, M., S. Wachholz, M. P. Manitz, J. Plumper, R. Sommer, G. Juckel and A. Friebe (2016). "Schizophrenia associated sensory gating deficits develop after adolescent microglia activation." Brain Behav Immun **58**: 99-106.

Estes, M. L. and A. K. McAllister (2016). "Maternal immune activation: Implications for neuropsychiatric disorders." Science **353**(6301): 772–777.

Estes, M. L. and A. K. McAllister (2016). "Maternal immune activation: Implications for neuropsychiatric disorders." Science **353**(6301): 772-777.

Evans, J. A. and A. J. Davidson (2013). "Health consequences of circadian disruption in humans and animal models." Prog Mol Biol Transl Sci **119**: 283–323.

Faden, J. (2019). "Treatment-Resistant Schizophrenia: A Brief Overview of Treatment Options." J Clin Psychiatry **80**(4).

Fatemi, S. H. and T. D. Folsom (2009). "The neurodevelopmental hypothesis of schizophrenia, revisited." Schizophr Bull **35**(3): 528-548.

Fekete, M. and et al. (1985). "Disrupting circadian rhythms in rats induces retrograde amnesia." Physiology and Behavior **34**(6): 883.

Feng, Y. Q., Z. Y. Zhou, X. He, H. Wang, X. L. Guo, C. J. Hao, Y. Guo, X. C. Zhen and W. Li (2008). "Dysbindin deficiency in sandy mice causes reduction of snapin and displays behaviors related to schizophrenia." Schizophr Res **106**(2-3): 218–228.

Fernandez de Cossio, L., A. Guzman, S. van der Veldt and G. N. Luheshi (2017). "Prenatal infection leads to ASD-like behavior and altered synaptic pruning in the mouse offspring." Brain Behav Immun **63**: 88–98.

Ferri, S. L., T. Abel and E. S. Brodkin (2018). "Sex Differences in Autism Spectrum Disorder: a Review." Curr Psychiatry Rep **20**(2): 9.

Fishbein, A. B., K. L. Knutson and P. C. Zee (2021). "Circadian disruption and human health." The Journal of clinical investigation **131**(19).

Fisk, A. S., S. K. E. Tam, L. A. Brown, V. V. Vyazovskiy, D. M. Bannerman and S. N. Peirson (2018). "Light and Cognition: Roles for Circadian Rhythms, Sleep, and Arousal." Front Neurol **9**: 56.

Folkes, O. M., R. Baldi, V. Kondev, D. J. Marcus, N. D. Hartley, B. D. Turner, J. K. Ayers, J. J. Baechle, M. P. Misra, M. Altemus, C. A. Grueter, B. A. Grueter and S. Patel (2020). "An endocannabinoid-regulated basolateral amygdala-nucleus accumbens circuit modulates sociability." J Clin Invest **130**(4): 1728-1742.

Fonken, L. K., M. S. Finy, J. C. Walton, Z. M. Weil, J. L. Workman, J. Ross and R. J. Nelson (2009). "Influence of light at night on murine anxiety- and depressive-like responses." Behav Brain Res **205**(2): 349-354.

Fonken, L. K., M. G. Frank, M. M. Kitt, R. M. Barrientos, L. R. Watkins and S. F. Maier (2015). "Microglia inflammatory responses are controlled by an intrinsic circadian clock." Brain Behav Immun **45**: 171–179.

Fonken, L. K., M. G. Frank, M. M. Kitt, R. M. Barrientos, L. R. Watkins and S. F. Maier (2015). "Microglia inflammatory responses are controlled by an intrinsic circadian clock." Brain Behav Immun **45**: 171-179.

Fonken, L. K. and R. J. Nelson (2014). "The effects of light at night on circadian clocks and metabolism." Endocr Rev **35**(4): 648-670.

Fonken, L. K. and R. J. Nelson (2016). "Effects of light exposure at night during development." Current Opinion in Behavioral Sciences **7**: 33-39.

Fonken, L. K., Z. M. Weil and R. J. Nelson (2013). "Mice exposed to dim light at night exaggerate inflammatory responses to lipopolysaccharide." Brain Behav Immun **34**: 159–163.

Fonken, L. K., Z. M. Weil and R. J. Nelson (2013). "Mice exposed to dim light at night exaggerate inflammatory responses to lipopolysaccharide." Brain Behav Immun **34**: 159-163.

Fortier, M. E., G. N. Luheshi and P. Boksa (2007). "Effects of prenatal infection on prepulse inhibition in the rat depend on the nature of the infectious agent and the stage of pregnancy." Behav Brain Res **181**(2): 270–277.

Foster, R. G., S. N. Peirson, K. Wulff, E. Winnebeck, C. Vetter and T. Roenneberg (2013). "Sleep and circadian rhythm disruption in social jetlag and mental illness." Prog Mol Biol Transl Sci **119**: 325-346.

Foster, R. G. and K. Wulff (2005). "The rhythm of rest and excess." Nat Rev Neurosci **6**(5): 407–414.

Foulkes, L. and S. J. Blakemore (2018). "Studying individual differences in human adolescent brain development." Nat Neurosci **21**(3): 315-323.

Frankle, W. G., J. Lerma and M. Laruelle (2003). "The synaptic hypothesis of schizophrenia." Neuron **39**(2): 205–216.

Fromer, M., A. J. Pocklington, D. H. Kavanagh, H. J. Williams, S. Dwyer, P. Gormley, L. Georgieva, E. Rees, P. Palta, D. M. Ruderfer, N. Carrera, I. Humphreys, J. S. Johnson, P. Roussos, D. D. Barker, E. Banks, V. Milanova, S. G. Grant, E. Hannon, S. A. Rose, K. Chambert, M. Mahajan, E. M. Scolnick, J. L. Moran, G. Kirov, A. Palotie, S. A. McCarroll, P. Holmans, P. Sklar, M. J. Owen, S. M. Purcell and M. C. O'Donovan (2014). "De novo mutations in schizophrenia implicate synaptic networks." Nature **506**(7487): 179-184.

Fu, Y., H.-W. Liao, M. T. H. Do and K.-W. Yau (2005). "Non-image-forming ocular photoreception in vertebrates." Current Opinion in Neurobiology **15**(4): 415-422.

Fuhrmann, D., L. J. Knoll and S.-J. Blakemore (2015). "Adolescence as a Sensitive Period of Brain Development." Trends in Cognitive Sciences **19**(10): 558-566.

Fujioka, A., T. Fujioka, R. Tsuruta, T. Izumi, S. Kasaoka and T. Maekawa (2011). "Effects of a constant light environment on hippocampal neurogenesis and memory in mice." Neurosci Lett **488**(1): 41–44.

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." Neurosci Biobehav Rev **37**(8): 1680-1691.

Gachon, F., E. Nagoshi, S. A. Brown, J. Ripperger and U. Schibler (2004). "The mammalian circadian timing system: from gene expression to physiology." Chromosoma **113**: 103-112.

Garay, P. A., E. Y. Hsiao, P. H. Patterson and A. K. McAllister (2013). "Maternal immune activation causes age- and region-specific changes in brain cytokines in offspring throughout development." Brain Behav Immun **31**: 54-68.

Geyer, M. A., K. Krebs-Thomson, D. L. Braff and N. R. Swerdlow (2001). "Pharmacological studies of prepulse inhibition models of sensorimotor gating deficits in schizophrenia: a decade in review." Psychopharmacology (Berl) **156**(2-3): 117-154.

Geyer, M. A. and N. R. Swerdlow (2001). "Measurement of startle response, prepulse inhibition, and habituation." Curr Protoc Neurosci **Chapter 8**: Unit 8 7.

Ginhoux, F., M. Greter, M. Leboeuf, S. Nandi, P. See, S. Gokhan, M. F. Mehler, S. J. Conway, L. G. Ng, E. R. Stanley, I. M. Samokhvalov and M. Merad (2010). "Fate mapping analysis reveals that adult microglia derive from primitive macrophages." Science **330**(6005): 841-845.

Ginhoux, F., S. Lim, G. Hoeffel, D. Low and T. Huber (2013). "Origin and differentiation of microglia." Front Cell Neurosci **7**: 45.

Giovanoli, S., T. Notter, J. Richetto, M. A. Labouesse, S. Vuillermot, M. A. Riva and U. Meyer (2015). "Late prenatal immune activation causes hippocampal deficits in the absence of persistent inflammation across aging." J Neuroinflammation **12**: 221.

Giovanoli, S., U. Weber-Stadlbauer, M. Schedlowski, U. Meyer and H. Engler (2016). "Prenatal immune activation causes hippocampal synaptic deficits in the absence of overt microglia anomalies." Brain Behav Immun **55**: 25-38.

Giusti-Rodriguez, P. and P. F. Sullivan (2013). "The genomics of schizophrenia: update and implications." J Clin Invest **123**(11): 4557-4563.

Gogos, A., A. Sbisa, D. Witkamp and M. van den Buuse (2020). "Sex differences in the effect of maternal immune activation on cognitive and psychosis-like behaviour in Long Evans rats." Eur J Neurosci **52**(1): 2614–2626.

Gogos, A., A. Sbisa, D. Witkamp and M. van den Buuse (2020). "Sex differences in the effect of maternal immune activation on cognitive and psychosis-like behaviour in Long Evans rats." Eur J Neurosci **52**(1): 2614-2626.

Gogtay, N., N. S. Vyas, R. Testa, S. J. Wood and C. Pantelis (2011). "Age of onset of schizophrenia: perspectives from structural neuroimaging studies." Schizophr Bull **37**(3): 504-513.

Goldberg, J. O. and L. A. Schmidt (2001). "Shyness, sociability, and social dysfunction in schizophrenia." Schizophr Res **48**(2-3): 343-349.

Goldman, S. E., A. L. Richdale, T. Clemons and B. A. Malow (2012). "Parental sleep concerns in autism spectrum disorders: variations from childhood to adolescence." J Autism Dev Disord **42**(4): 531-538.

Goldstein, J. M., S. Cherkerzian, L. J. Seidman, J. A. Donatelli, A. G. Remington, M. T. Tsuang, M. Hornig and S. L. Buka (2014). "Prenatal maternal immune disruption and sex-dependent risk for psychoses." Psychol Med **44**(15): 3249-3261.

Gopalkrishnan, N. (2018). "Cultural Diversity and Mental Health: Considerations for Policy and Practice." Front Public Health **6**: 179.

Gravotta, L., A. M. Gavrila, S. Hood and S. Amir (2011). "Global Depletion of Dopamine Using Intracerebroventricular 6-Hydroxydopamine Injection Disrupts Normal Circadian Wheel-Running Patterns and PERIOD2 Expression in the Rat Forebrain." Journal of Molecular Neuroscience **45**(2): 162-171.

Green, M. F., W. P. Horan and J. Lee (2019). "Nonsocial and social cognition in schizophrenia: current evidence and future directions." World Psychiatry **18**(2): 146-161.

Griffin, P., J. M. Dimitry, P. W. Sheehan, B. V. Lananna, C. Guo, M. L. Robinette, M. E. Hayes, M. R. Cedeno, C. J. Nadarajah, L. A. Ezerskiy, M. Colonna, J. Zhang, A. Q. Bauer, T. P. Burris and E. S. Musiek (2019). "Circadian clock protein Rev-erbalpha regulates neuroinflammation." Proc Natl Acad Sci U S A **116**(11): 5102-5107.

Griffin, P., J. M. Dimitry, P. W. Sheehan, B. V. Lananna, C. Guo, M. L. Robinette, M. E. Hayes, M. R. Cedeno, C. J. Nadarajah, L. A. Ezerskiy, M. Colonna, J. Zhang, A. Q. Bauer, T. P. Burris and E. S. Musiek (2019). "Circadian clock protein Rev-erbalpha regulates neuroinflammation." Proc Natl Acad Sci U S A **116**(11): 5102–5107.

Griffin, P., P. W. Sheehan, J. M. Dimitry, C. Guo, M. F. Kanan, J. Lee, J. Zhang and E. S. Musiek (2020). "REV-ERBα mediates complement expression and diurnal regulation of microglial synaptic phagocytosis." eLife **9**.

Guang, S., N. Pang, X. Deng, L. Yang, F. He, L. Wu, C. Chen, F. Yin and J. Peng (2018). "Synaptopathology Involved in Autism Spectrum Disorder." Front Cell Neurosci **12**: 470.

Guler, A. D., J. L. Ecker, G. S. Lall, S. Haq, C. M. Altimus, H. W. Liao, A. R. Barnard, H. Cahill, T. C. Badea, H. Zhao, M. W. Hankins, D. M. Berson, R. J. Lucas, K. W. Yau and S. Hattar (2008). "Melanopsin cells are the principal conduits for rod-cone input to non-image-forming vision." Nature **453**(7191): 102-105.

Guma, E., P. D. C. Bordignon, G. A. Devenyi, D. Gallino, C. Anastassiadis, V. Cvetkovska, A. D. Barry, E. Snook, J. Germann, C. M. T. Greenwood, B. Misic, R. C. Bagot and M. M. Chakravarty (2021). "Early or Late Gestational Exposure to Maternal Immune Activation Alters Neurodevelopmental Trajectories in Mice: An Integrated Neuroimaging, Behavioral, and Transcriptional Study." Biological Psychiatry **90**(5): 328-341.

Guma, E., E. Plitman and M. M. 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." Neurosci Biobehav Rev **104**: 141-157.

Haddad, F. L., S. V. Patel and S. Schmid (2020). "Maternal Immune Activation by Poly I:C as a preclinical Model for Neurodevelopmental Disorders: A focus on Autism and Schizophrenia." Neurosci Biobehav Rev **113**: 546-567.

Hafner, H. and K. Maurer (2006). "Early detection of schizophrenia: current evidence and future perspectives." World Psychiatry **5**(3): 130-138.

Haida, O., T. Al Sagheer, A. Balbous, M. Francheteau, E. Matas, F. Soria, P. O. Fernagut and M. Jaber (2019). "Sex-dependent behavioral deficits and neuropathology in a maternal immune activation model of autism." Transl Psychiatry **9**(1): 124.

Haijma, S. V., N. Van Haren, W. Cahn, P. C. Koolschijn, H. E. Hulshoff Pol and R. S. Kahn (2013). "Brain volumes in schizophrenia: a meta-analysis in over 18 000 subjects." Schizophr Bull **39**(5): 1129-1138.

Hannou, L., P. G. Roy, M. N. Ballester Roig and V. Mongrain (2020). "Transcriptional control of synaptic components by the clock machinery." Eur J Neurosci **51**(1): 241-267.

Hari Dass, S. A., K. McCracken, I. Pokhvisneva, L. M. Chen, E. Garg, T. T. T. Nguyen, Z. Wang, B. Barth, M. Yaqubi, L. M. McEwen, J. L. Macisaac, J. Diorio, M. S. Kobor, K. J. O'Donnell, M. J. Meaney and P. P. Silveira (2019). "A biologically-informed polygenic score identifies endophenotypes and clinical conditions associated with the insulin receptor function on specific brain regions." EBioMedicine **42**: 188-202.

Harrison, P. J. and D. R. Weinberger (2005). "Schizophrenia genes, gene expression, and neuropathology: on the matter of their convergence." Mol Psychiatry **10**(1): 40–68; image 45.

Hastings, M. H., E. S. Maywood and M. Brancaccio (2018). "Generation of circadian rhythms in the suprachiasmatic nucleus." Nat Rev Neurosci **19**(8): 453–469.

Hastings, M. H., E. S. Maywood and M. Brancaccio (2018). "Generation of circadian rhythms in the suprachiasmatic nucleus." Nat Rev Neurosci **19**(8): 453-469.

Hastings, M. H., E. S. Maywood and M. Brancaccio (2019). "The Mammalian Circadian Timing System and the Suprachiasmatic Nucleus as Its Pacemaker." Biology (Basel) **8**(1): 13.

Hayes, J. F., L. Marston, K. Walters, M. B. King and D. P. J. Osborn (2017). "Mortality gap for people with bipolar disorder and schizophrenia: UK-based cohort study 2000-2014." Br J Psychiatry **211**(3): 175-181.

Hayes, L. N., K. An, E. Carloni, F. Li, E. Vincent, C. Trippaers, M. Paranjpe, G. Dölen, L. A. Goff, A. Ramos, S.-I. Kano and A. Sawa (2022). "Prenatal immune stress blunts microglia reactivity, impairing neurocircuitry." Nature **610**(7931): 327-334.

Hornig, M., M. A. Bresnahan, X. Che, A. F. Schultz, J. E. Ukaigwe, M. L. Eddy, D. Hirtz, N. Gunnes, K. K. Lie, P. Magnus, S. Mjaaland, T. Reichborn-Kjennerud, S. Schjolberg, A. S. Oyen, B. Levin, E. S. Susser, C. Stoltenberg and W. I. Lipkin (2018). "Prenatal fever and autism risk." Mol Psychiatry **23**(3): 759-766.

Hoshiko, M., I. Arnoux, E. Avignone, N. Yamamoto and E. Audinat (2012). "Deficiency of the microglial receptor CX3CR1 impairs postnatal functional development of thalamocortical synapses in the barrel cortex." J Neurosci **32**(43): 15106-15111.

Hsiao, E. Y. and P. H. Patterson (2011). "Activation of the maternal immune system induces endocrine changes in the placenta via IL-6." Brain, Behavior, and Immunity **25**(4): 604-615.

Hui, C. W., A. St-Pierre, H. El Hajj, Y. Remy, S. S. Hebert, G. N. Luheshi, L. K. Srivastava and M. E. Tremblay (2018). "Prenatal Immune Challenge in Mice Leads to Partly Sex-Dependent Behavioral, Microglial, and Molecular Abnormalities Associated with Schizophrenia." Front Mol Neurosci **11**: 13.

Hurd, M. W. and M. R. Ralph (1998). "The significance of circadian organization for longevity in the golden hamster." J Biol Rhythms **13**(5): 430–436.

Häfner, H. (2019). "From Onset and Prodromal Stage to a Life-Long Course of Schizophrenia and Its Symptom Dimensions: How Sex, Age, and Other Risk Factors Influence Incidence and Course of Illness." Psychiatry Journal **2019**: 1-15.

Ingiosi, A. M., H. Schoch, T. Wintler, K. G. Singletary, D. Righelli, L. G. Roser, E. Medina, D. Risso, M. G. Frank and L. Peixoto (2019). "Shank3 modulates sleep and expression of circadian transcription factors." Elife **8**.

Jaaro-Peled, H., C. Altimus, T. LeGates, T. Cash-Padgett, S. Zoubovsky, T. Hikida, K. Ishizuka, S. Hattar, V. Mongrain and A. Sawa (2016). "Abnormal wake/sleep pattern in a novel gain-of-function model of DISC1." Neurosci Res **112**: 63-69.

Johansson, A. S., B. Owe-Larsson, J. Hetta and G. B. Lundkvist (2016). "Altered circadian clock gene expression in patients with schizophrenia." Schizophr Res **174**(1-3): 17–23.

Johansson, A. S., B. Owe-Larsson, J. Hetta and G. B. Lundkvist (2016). "Altered circadian clock gene expression in patients with schizophrenia." Schizophr Res **174**(1-3): 17-23.

Juckel, G., M. P. Manitz, M. Brune, A. Friebe, M. T. Heneka and R. J. Wolf (2011). "Microglial activation in a neuroinflammational animal model of schizophrenia--a pilot study." Schizophr Res **131**(1-3): 96–100.

Kabot, S., W. Masi and M. Segal (2003). "Advances in the diagnosis and treatment of autism spectrum disorders." Professional Psychology: Research and Practice **34**: 26-33.

Kahn, R. S., I. E. Sommer, R. M. Murray, A. Meyer-Lindenberg, D. R. Weinberger, T. D. Cannon, M. O'Donovan, C. U. Correll, J. M. Kane, J. van Os and T. R. Insel (2015). "Schizophrenia." Nat Rev Dis Primers **1**: 15067.

Kaidanovich-Beilin, O., T. Lipina, I. Vukobradovic, J. Roder and J. R. Woodgett (2011). "Assessment of Social Interaction Behaviors." Journal of Visualized Experiments(48).

Karatsoreos, I. N. (2012). "Effects of circadian disruption on mental and physical health." Curr Neurol Neurosci Rep **12**(2): 218–225.

Karatsoreos, I. N. (2012). "Effects of circadian disruption on mental and physical health." Curr Neurol Neurosci Rep **12**(2): 218-225.

Karatsoreos, I. N., S. Bhagat, E. B. Bloss, J. H. Morrison and B. S. McEwen (2011). "Disruption of circadian clocks has ramifications for metabolism, brain, and behavior." Proc Natl Acad Sci U S A **108**(4): 1657-1662.

Karatsoreos, I. N., S. Bhagat, E. B. Bloss, J. H. Morrison and B. S. McEwen (2011). "Disruption of circadian clocks has ramifications for metabolism, brain, and behavior." Proc Natl Acad Sci U S A **108**(4): 1657–1662.

Kaskie, R. E., B. Graziano and F. Ferrarelli (2017). "Schizophrenia and sleep disorders: links, risks, and management challenges." Nat Sci Sleep **9**: 227-239.

Kendell, R. E. and I. W. Kemp (1989). "Maternal influenza in the etiology of schizophrenia." Arch Gen Psychiatry **46**(10): 878-882.

Kessler, R. C., G. P. Amminger, S. Aguilar-Gaxiola, J. Alonso, S. Lee and T. B. St??N (2007). "Age of onset of mental disorders: a review of recent literature." Current Opinion in Psychiatry **20**(4): 359-364.

Kessler, R. C., G. P. Amminger, S. Aguilar-Gaxiola, J. Alonso, S. Lee and T. B. Ustun (2007). "Age of onset of mental disorders: a review of recent literature." Current Opinion in Psychiatry **20**(4): 359-364.

Kierdorf, K., D. Erny, T. Goldmann, V. Sander, C. Schulz, E. G. Perdiguero, P. Wieghofer, A. Heinrich, P. Riemke, C. Holscher, D. N. Muller, B. Luckow, T. Brocker, K. Debowski, G. Fritz, G. Opdenakker, A. Diefenbach, K. Biber, M. Heikenwalder, F. Geissmann, F. Rosenbauer and M. Prinz (2013). "Microglia emerge from erythromyeloid precursors via Pu.1- and Irf8-dependent pathways." Nat Neurosci **16**(3): 273-280.

Kim-Cohen, J., A. Caspi, T. E. Moffitt, H. Harrington, B. J. Milne and R. Poulton (2003). "Prior Juvenile Diagnoses in Adults With Mental Disorder." Archives of General Psychiatry **60**(7): 709.

Klebanoff, M. A. (2009). "The Collaborative Perinatal Project: a 50-year retrospective." Paediatr Perinat Epidemiol **23**(1): 2-8.

Knuesel, I., L. Chicha, M. Britschgi, S. A. Schobel, M. Bodmer, J. A. Hellings, S. Toovey and E. P. Prinssen (2014). "Maternal immune activation and abnormal brain development across CNS disorders." Nat Rev Neurol **10**(11): 643–660.

Knuesel, I., L. Chicha, M. Britschgi, S. A. Schobel, M. Bodmer, J. A. Hellings, S. Toovey and E. P. Prinssen (2014). "Maternal immune activation and abnormal brain development across CNS disorders." Nat Rev Neurol **10**(11): 643-660.

Kobayashi, Y., H. Inaba, Y. Iwakura, H. Namba, H. Sotoyama, Y. Murata, K. Iwamoto and H. Nawa (2021). "Inter-breeder differences in prepulse inhibition deficits of C57BL/6J mice in a maternal immune activation model." Neuropsychopharmacology Reports **41**(3): 416-421.

Konstantareas, M. M. and T. Hewitt (2001). "Autistic disorder and schizophrenia: diagnostic overlaps." J Autism Dev Disord **31**(1): 19-28.

Korenic, S. A., E. A. Klingaman, E. M. Wickwire, F. E. Gaston, H. Chen, S. A. Wijtenburg and L. M. Rowland (2019). "Sleep quality is related to brain glutamate and symptom severity in schizophrenia." J Psychiatr Res **120**: 14–20.

Korenic, S. A., E. A. Klingaman, E. M. Wickwire, F. E. Gaston, H. Chen, S. A. Wijtenburg and L. M. Rowland (2019). "Sleep quality is related to brain glutamate and symptom severity in schizophrenia." J Psychiatr Res **120**: 14-20.

Koyama, R. and Y. Ikegaya (2015). "Microglia in the pathogenesis of autism spectrum disorders." Neurosci Res **100**: 1-5.

Kraepelin, E., R. M. Barclay and G. M. Robertson (1919). Dementia praecox and paraphrenia.

Krakowiak, P., B. Goodlin-Jones, I. Hertz-Picciotto, L. A. Croen and R. L. Hansen (2008). "Sleep problems in children with autism spectrum disorders, developmental delays, and typical development: a population-based study." J Sleep Res **17**(2): 197-206.

Krakowiak, P., B. Goodlin-Jones, I. Hertz-Picciotto, L. A. Croen and R. L. Hansen (2008). "Sleep problems in children with autism spectrum disorders, developmental delays, and typical development: a population-based study." J Sleep Res **17**(2): 197–206.

Kumamoto, N., S. Matsuzaki, K. Inoue, T. Hattori, S. Shimizu, R. Hashimoto, A. Yamatodani, T. Katayama and M. Tohyama (2006). "Hyperactivation of midbrain dopaminergic system in schizophrenia could be attributed to the down-regulation of dysbindin." Biochem Biophys Res Commun **345**(2): 904–909.

Kwon, H. K., G. B. Choi and J. R. Huh (2022). "Maternal inflammation and its ramifications on fetal neurodevelopment." Trends Immunol **43**(3): 230-244.

Lai, M. C., M. V. Lombardo, B. Auyeung, B. Chakrabarti and S. Baron-Cohen (2015). "Sex/gender differences and autism: setting the scene for future research." J Am Acad Child Adolesc Psychiatry **54**(1): 11-24.

Lally, J. and J. H. MacCabe (2015). "Antipsychotic medication in schizophrenia: a review." Br Med Bull **114**(1): 169-179.

Langfelder, P. and S. Horvath (2008). "WGCNA: an R package for weighted correlation network analysis." BMC bioinformatics **9**(1): 1-13.

Laskemoen, J. F., C. Simonsen, C. Buchmann, E. A. Barrett, T. Bjella, T. V. Lagerberg, T. J. Vedal, O. A. Andreassen, I. Melle and M. Aas (2019). "Sleep disturbances in schizophrenia spectrum and bipolar disorders - a transdiagnostic perspective." Compr Psychiatry **91**: 6–12.

Laskemoen, J. F., C. Simonsen, C. Buchmann, E. A. Barrett, T. Bjella, T. V. Lagerberg, T. J. Vedal, O. A. Andreassen, I. Melle and M. Aas (2019). "Sleep disturbances in schizophrenia spectrum and bipolar disorders - a transdiagnostic perspective." Compr Psychiatry **91**: 6-12.

Lee, B. K., C. Magnusson, R. M. Gardner, A. Blomstrom, C. J. Newschaffer, I. Burstyn, H. Karlsson and C. Dalman (2015). "Maternal hospitalization with infection during pregnancy and risk of autism spectrum disorders." Brain Behav Immun **44**: 100-105.

Lee, B. K., C. Magnusson, R. M. Gardner, A. Blomstrom, C. J. Newschaffer, I. Burstyn, H. Karlsson and C. Dalman (2015). "Maternal hospitalization with infection during pregnancy and risk of autism spectrum disorders." Brain Behav Immun **44**: 100–105.

Lee, F. Y., H. B. Wang, O. N. Hitchcock, D. H. Loh, D. S. Whittaker, Y. S. Kim, A. Aiken, C. Kokikian, E. C. Dell'Angelica, C. S. Colwell and C. A. Ghiani (2018). "Sleep/Wake Disruption in a Mouse Model of BLOC-1 Deficiency." Front Neurosci **12**: 759.

LeGates, T. A., C. M. Altimus, H. Wang, H. K. Lee, S. Yang, H. Zhao, A. Kirkwood, E. T. Weber and S. Hattar (2012). "Aberrant light directly impairs mood and learning through melanopsin-expressing neurons." Nature **491**(7425): 594–598.

LeGates, T. A., D. C. Fernandez and S. Hattar (2014). "Light as a central modulator of circadian rhythms, sleep and affect." Nat Rev Neurosci **15**(7): 443-454.

Li, Q. and B. A. Barres (2018). "Microglia and macrophages in brain homeostasis and disease." Nat Rev Immunol **18**(4): 225-242.

Li, W., M. Chen, X. Feng, M. Song, M. Shao, Y. Yang, L. Zhang, Q. Liu, L. Lv and X. Su (2021). "Maternal immune activation alters adult behavior, intestinal integrity, gut microbiota and the gut inflammation." Brain and Behavior **11**(5).

Ling, Z.-Q., Q. Tian, L. Wang, Z.-Q. Fu, X.-C. Wang, Q. Wang and J.-Z. Wang (2009). "Constant Illumination Induces Alzheimer-Like Damages with Endoplasmic Reticulum Involvement and the Protection of Melatonin." Journal of Alzheimer's Disease **16**: 287-300.

Logan, R. W. and C. A. McClung (2019). "Rhythms of life: circadian disruption and brain disorders across the lifespan." Nat Rev Neurosci **20**(1): 49–65.

Loomes, R., L. Hull and W. P. L. Mandy (2017). "What Is the Male-to-Female Ratio in Autism Spectrum Disorder? A Systematic Review and Meta-Analysis." J Am Acad Child Adolesc Psychiatry **56**(6): 466-474.

Lord, C., T. S. Brugha, T. Charman, J. Cusack, G. Dumas, T. Frazier, E. J. H. Jones, R. M. Jones, A. Pickles, M. W. State, J. L. Taylor and J. Veenstra-VanderWeele (2020). "Autism spectrum disorder." Nat Rev Dis Primers **6**(1): 5.

Love, M., W. Huber and S. Anders (2014). "Moderated estimation of fold changes and dispersion for RNA-seq data with Deseq2. vol. 15." Genome Biol.

Luchicchi, A., S. Lecca, M. Melis, M. De Felice, F. Cadeddu, R. Frau, A. L. Muntoni, P. Fadda, P. Devoto and M. Pistis (2016). "Maternal Immune Activation Disrupts Dopamine System in the Offspring." International Journal of Neuropsychopharmacology **19**(7).

Lunsford-Avery, J. R., B. Goncalves, E. Brietzke, R. A. Bressan, A. Gadelha, R. P. Auerbach and V. A. Mittal (2017). "Adolescents at clinical-high risk for psychosis: Circadian rhythm disturbances predict worsened prognosis at 1-year follow-up." Schizophr Res **189**: 37–42.

Lupi, D., H. Oster, S. Thompson and R. G. Foster (2008). "The acute light-induction of sleep is mediated by OPN4-based photoreception." Nature Neuroscience **11**(9): 1068-1073.

Luthringer, R., L. Staner, N. Noel, M. Muzet, C. Gassmann-Mayer, K. Talluri, A. Cleton, M. Eerdekens, W. P. Battisti and J. M. Palumbo (2007). "A double-blind, placebo-controlled, randomized study evaluating the effect of paliperidone extended-release tablets on sleep architecture in patients with schizophrenia." Int Clin Psychopharmacol **22**(5): 299–308.

Ma, W.-P., J. Cao, M. Tian, M.-H. Cui, H.-L. Han, Y.-X. Yang and L. Xu (2007). "Exposure to chronic constant light impairs spatial memory and influences long-term depression in rats." Neuroscience Research **59**(2): 224-230.

Madore, C., Z. Yin, J. Leibowitz and O. Butovsky (2020). "Microglia, Lifestyle Stress, and Neurodegeneration." Immunity **52**(2): 222-240.

Malkova, N. V., C. Z. Yu, E. Y. Hsiao, M. J. Moore and P. H. Patterson (2012). "Maternal immune activation yields offspring displaying mouse versions of the three core symptoms of autism." Brain Behav Immun **26**(4): 607–616.

Malkova, N. V., C. Z. Yu, E. Y. Hsiao, M. J. Moore and P. H. Patterson (2012). "Maternal immune activation yields offspring displaying mouse versions of the three core symptoms of autism." Brain Behav Immun **26**(4): 607-616.

Maple, A. M., R. K. Rowe, J. Lifshitz, F. Fernandez and A. L. Gallitano (2018). "Influence of Schizophrenia-Associated Gene Egr3 on Sleep Behavior and Circadian Rhythms in Mice." J Biol Rhythms **33**(6): 662-670.

Mariani, M. M. and T. Kielian (2009). "Microglia in infectious diseases of the central nervous system." J Neuroimmune Pharmacol **4**(4): 448-461.

Martin, J., D. V. Jeste, M. P. Caliguiri, T. Patterson, R. Heaton and S. Ancoli-Israel (2001). "Actigraphic estimates of circadian rhythms and sleep/wake in older schizophrenia patients." Schizophr Res **47**(1): 77–86.

Martin, J. L., D. V. Jeste and S. Ancoli-Israel (2005). "Older schizophrenia patients have more disrupted sleep and circadian rhythms than age-matched comparison subjects." J Psychiatr Res **39**(3): 251–259.

Martin, J. L., D. V. Jeste and S. Ancoli-Israel (2005). "Older schizophrenia patients have more disrupted sleep and circadian rhythms than age-matched comparison subjects." J Psychiatr Res **39**(3): 251-259.

Massrali, A., D. Adhya, D. P. Srivastava, S. Baron-Cohen and M. R. Kotter (2022). "Virus-Induced Maternal Immune Activation as an Environmental Factor in the Etiology of Autism and Schizophrenia." Front Neurosci **16**: 834058.

Matsui, F., S. T. Yamaguchi, R. Kobayashi, S. Ito, S. Nagashima, Z. Zhou and H. Norimoto (2023). "Ablation of microglia does not alter circadian rhythm of locomotor activity." Molecular Brain **16**(1).

Mattei, D., A. Ivanov, C. Ferrai, P. Jordan, D. Guneykaya, A. Buonfiglioli, W. Schaafsma, P. Przanowski, W. Deuther-Conrad, P. Brust, S. Hesse, M. Patt, O. Sabri, T. L. Ross, B. J. L. Eggen, E. Boddeke, B. Kaminska, D. Beule, A. Pombo, H. Kettenmann and S. A. Wolf (2017). "Maternal immune activation results in complex microglial transcriptome signature in the adult offspring that is reversed by minocycline treatment." Transl Psychiatry **7**(5): e1120.

McCormick, C. M. and M. R. Green (2013). "From the stressed adolescent to the anxious and depressed adult: investigations in rodent models." Neuroscience **249**: 242-257.

McCormick, C. M., D. L. Mongillo and J. J. Simone (2013). "Age and adolescent social stress effects on fear extinction in female rats." Stress **16**(6): 678-688.

McCutcheon, R. A., R. S. E. Keefe and P. K. McGuire (2023). "Cognitive impairment in schizophrenia: aetiology, pathophysiology, and treatment." Molecular Psychiatry.

McGrath, J., S. Saha, D. Chant and J. Welham (2008). "Schizophrenia: a concise overview of incidence, prevalence, and mortality." Epidemiol Rev **30**: 67-76.

McGrath, J., S. Saha, D. Chant and J. Welham (2008). "Schizophrenia: a concise overview of incidence, prevalence, and mortality." Epidemiol Rev **30**: 67–76.

McGrath, J., S. Saha, J. Welham, O. El Saadi, C. MacCauley and D. Chant (2004). "A systematic review of the incidence of schizophrenia: the distribution of rates and the influence of sex, urbanicity, migrant status and methodology." BMC Med **2**: 13.

Mednick, S. A., R. A. Machon and M. O. Huttunen (1990). "An update on the Helsinki Influenza Project." Arch Gen Psychiatry **47**(3): 292.

Mednick, S. A., R. A. Machon, M. O. Huttunen and D. Bonett (1988). "Adult schizophrenia following prenatal exposure to an influenza epidemic." Arch Gen Psychiatry **45**(2): 189–192.

Meier, M. H., A. Caspi, A. Ambler, H. Harrington, R. Houts, R. S. Keefe, K. McDonald, A. Ward, R. Poulton and T. E. Moffitt (2012). "Persistent cannabis users show neuropsychological decline from childhood to midlife." Proc Natl Acad Sci U S A **109**(40): E2657-2664.

Mena, A., J. C. Ruiz-Salas, A. Puentes, I. Dorado, M. Ruiz-Veguilla and L. G. De la Casa (2016). "Reduced Prepulse Inhibition as a Biomarker of Schizophrenia." Front Behav Neurosci **10**: 202.

Mendes, M. S. and A. K. Majewska (2021). "An overview of microglia ontogeny and maturation in the homeostatic and pathological brain." Eur J Neurosci **53**(11): 3525-3547.

Mendrek, A. and A. Mancini-Marie (2016). "Sex/gender differences in the brain and cognition in schizophrenia." Neurosci Biobehav Rev **67**: 57-78.

Menninger, K. A. (1994). "Influenza and schizophrenia. An analysis of post-influenzal "dementia precox," as of 1918, and five years later further studies of the psychiatric aspects of influenza. 1926." Am J Psychiatry **151**(6 Suppl): 182-187.

Meyer, N., S. M. Faulkner, R. A. McCutcheon, T. Pillinger, D. J. Dijk and J. H. MacCabe (2020). "Sleep and Circadian Rhythm Disturbance in Remitted Schizophrenia and Bipolar Disorder: A Systematic Review and Meta-analysis." Schizophr Bull.

Meyer, N., S. M. Faulkner, R. A. McCutcheon, T. Pillinger, D. J. Dijk and J. H. MacCabe (2020). "Sleep and Circadian Rhythm Disturbance in Remitted Schizophrenia and Bipolar Disorder: A Systematic Review and Meta-analysis." Schizophr Bull **46**(5): 1126–1143.

Meyer, U. (2014). "Prenatal poly(i:C) exposure and other developmental immune activation models in rodent systems." Biol Psychiatry **75**(4): 307-315.

Meyer, U. (2019). "Neurodevelopmental Resilience and Susceptibility to Maternal Immune Activation." Trends Neurosci **42**(11): 793-806.

Meyer, U. and J. 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): 1308-1321.

Meyer, U., J. Feldon, M. Schedlowski and B. K. Yee (2005). "Towards an immuno-precipitated neurodevelopmental animal model of schizophrenia." Neurosci Biobehav Rev **29**(6): 913-947.

Meyer, U., J. Feldon, M. Schedlowski and B. K. Yee (2005). "Towards an immuno-precipitated neurodevelopmental animal model of schizophrenia." Neurosci Biobehav Rev **29**(6): 913–947.

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." Mol Psychiatry **13**(2): 208–221.

Meyer, U., M. Nyffeler, A. Engler, A. Urwyler, M. Schedlowski, I. Knuesel, B. K. Yee and J. Feldon (2006). "The time of prenatal immune challenge determines the specificity of inflammation-mediated brain and behavioral pathology." J Neurosci **26**(18): 4752-4762.

Meyer, U., M. Nyffeler, A. Engler, A. Urwyler, M. Schedlowski, I. Knuesel, B. K. Yee and J. Feldon (2006). "The time of prenatal immune challenge determines the specificity of inflammation-mediated brain and behavioral pathology." J Neurosci **26**(18): 4752–4762.

Meyer, U., M. Nyffeler, B. K. Yee, I. Knuesel and J. Feldon (2008). "Adult brain and behavioral pathological markers of prenatal immune challenge during early/middle and late fetal development in mice." Brain Behav Immun **22**(4): 469-486.

Meyer, U., M. Nyffeler, B. K. Yee, I. Knuesel and J. Feldon (2008). "Adult brain and behavioral pathological markers of prenatal immune challenge during early/middle and late fetal development in mice." Brain Behav Immun **22**(4): 469–486.

Meyer, U., B. K. Yee and J. Feldon (2007). "The neurodevelopmental impact of prenatal infections at different times of pregnancy: the earlier the worse?" Neuroscientist **13**(3): 241–256.

Minakova, E. and B. B. Warner (2018). "Maternal immune activation, central nervous system development and behavioral phenotypes." Birth Defects Research **110**(20): 1539-1550.

Mishra, A., R. Maiti, B. R. Mishra, M. Jena, S. Nath and P. Sahu (2020). "Effect of add-on ramelteon therapy on sleep and circadian rhythm disruption in patients with schizophrenia: A randomized controlled trial." Eur Neuropsychopharmacol **31**: 109–118.

Missig, G., C. J. McDougle and W. A. Carlezon, Jr. (2020). "Sleep as a translationally-relevant endpoint in studies of autism spectrum disorder (ASD)." Neuropsychopharmacology **45**(1): 90-103.

Missig, G., E. L. Mokler, J. O. Robbins, A. J. Alexander, C. J. McDougle and W. A. Carlezon, Jr. (2018). "Perinatal Immune Activation Produces Persistent Sleep Alterations and Epileptiform Activity in Male Mice." Neuropsychopharmacology **43**(3): 482-491.

Miyamoto, S., G. E. Duncan, C. E. Marx and J. A. Lieberman (2005). "Treatments for schizophrenia: a critical review of pharmacology and mechanisms of action of antipsychotic drugs." Mol Psychiatry **10**(1): 79–104.

Mohrmann, R., H. de Wit, M. Verhage, E. Neher and J. B. Sorensen (2010). "Fast vesicle fusion in living cells requires at least three SNARE complexes." Science **330**(6003): 502–505.

Mondelli, V., A. C. Vernon, F. Turkheimer, P. Dazzan and C. M. Pariante (2017). "Brain microglia in psychiatric disorders." Lancet Psychiatry **4**(7): 563-572.

Monji, A., T. Kato and S. Kanba (2009). "Cytokines and schizophrenia: Microglia hypothesis of schizophrenia." Psychiatry Clin Neurosci **63**(3): 257–265.

Morgan, J. T., G. Chana, I. Abramson, K. Semendeferi, E. Courchesne and I. P. Everall (2012). "Abnormal microglial-neuronal spatial organization in the dorsolateral prefrontal cortex in autism." Brain Res **1456**: 72-81.

Morgan, J. T., G. Chana, I. Abramson, K. Semendeferi, E. Courchesne and I. P. Everall (2012). "Abnormal microglial-neuronal spatial organization in the dorsolateral prefrontal cortex in autism." Brain Res **1456**: 72–81.

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." Schizophr Res **26**(1): 25-39.

Morrissey, M. D., I. Z. Mathews and C. M. McCormick (2011). "Enduring deficits in contextual and auditory fear conditioning after adolescent, not adult, social instability stress in male rats." Neurobiology of Learning and Memory **95**(1): 46-56.

Mortensen, P. B., B. Norgaard-Pedersen, B. L. Waltoft, T. L. Sorensen, D. Hougaard and R. H. Yolken (2007). "Early infections of Toxoplasma gondii and the later development of schizophrenia." Schizophr Bull **33**(3): 741-744.

Mosner, M. G., J. L. Kinard, J. S. Shah, S. McWeeny, R. K. Greene, S. C. Lowery, C. A. Mazefsky and G. S. Dichter (2019). "Rates of Co-occurring Psychiatric Disorders in Autism Spectrum Disorder Using the Mini International Neuropsychiatric Interview." J Autism Dev Disord **49**(9): 3819-3832.

Mouridsen, S. E. (2013). "Mortality and Factors Associated with Death in Autism Spectrum Disorders - a Review." American Journal of Archaeology.

Moyer, C. E., M. A. Shelton and R. A. Sweet (2015). "Dendritic spine alterations in schizophrenia." Neurosci Lett **601**: 46-53.

Mueller, F. S., J. Richetto, L. N. Hayes, A. Zambon, D. D. Pollak, A. Sawa, U. Meyer and U. Weber-Stadlbauer (2019). "Influence of poly(I:C) variability on thermoregulation, immune responses and pregnancy outcomes in mouse models of maternal immune activation." Brain Behav Immun **80**: 406–418.

Mueller, F. S., J. Richetto, L. N. Hayes, A. Zambon, D. D. Pollak, A. Sawa, U. Meyer and U. Weber-Stadlbauer (2019). "Influence of poly(I:C) variability on thermoregulation, immune responses and pregnancy outcomes in mouse models of maternal immune activation." Brain Behav Immun **80**: 406-418.

Murray, B. G., D. A. Davies, J. J. Molder and J. G. Howland (2017). "Maternal immune activation during pregnancy in rats impairs working memory capacity of the offspring." Neurobiol Learn Mem **141**: 150-156.

Muñoz, M., S. N. Peirson, M. W. Hankins and R. G. Foster (2005). "Long-Term Constant Light Induces Constitutive Elevated Expression of mPER2 Protein in the Murine SCN: A Molecular Basis for Aschoff's Rule?" Journal of Biological Rhythms **20**(1): 3–14.

Najjar, S. and D. M. Pearlman (2015). "Neuroinflammation and white matter pathology in schizophrenia: systematic review." Schizophr Res **161**(1): 102-112.

O'Callaghan, E., P. Sham, N. Takei, G. Glover and R. M. Murray (1991). "Schizophrenia after prenatal exposure to 1957 A2 influenza epidemic." Lancet **337**(8752): 1248-1250.

Ochoa, S., J. Usall, J. Cobo, X. Labad and J. Kulkarni (2012). "Gender differences in schizophrenia and first-episode psychosis: a comprehensive literature review." Schizophr Res Treatment **2012**: 916198.

Oh-Nishi, A., S. Obayashi, I. Sugihara, T. Minamimoto and T. 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 Res **1363**: 170-179.

Ohta, H., S. Yamazaki and D. G. McMahon (2005). "Constant light desynchronizes mammalian clock neurons." Nat Neurosci **8**(3): 267–269.

Ohta, H., S. Yamazaki and D. G. McMahon (2005). "Constant light desynchronizes mammalian clock neurons." Nat Neurosci **8**(3): 267-269.

Oliver, P. L., M. V. Sobczyk, E. S. Maywood, B. Edwards, S. Lee, A. Livieratos, H. Oster, R. Butler, S. I. Godinho, K. Wulff, S. N. Peirson, S. P. Fisher, J. E. Chesham, J. W. Smith, M. H. Hastings, K. E. Davies and R. G. Foster (2012). "Disrupted circadian rhythms in a mouse model of schizophrenia." Curr Biol **22**(4): 314-319.

Organization, W. H. (2019). "6A20 Schizophrenia." In International statistical classification of diseases and related health problems (11th ed.).

Orsmond, G. I., P. T. Shattuck, B. P. Cooper, P. R. Sterzing and K. A. Anderson (2013). "Social participation among young adults with an autism spectrum disorder." J Autism Dev Disord **43**(11): 2710-2719.

Orsmond, G. I., P. T. Shattuck, B. P. Cooper, P. R. Sterzing and K. A. Anderson (2013). "Social participation among young adults with an autism spectrum disorder." J Autism Dev Disord **43**(11): 2710–2719.

Ozawa, K. and et al. (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 **59**(6): 546.

Paolicelli, R. C. and M. T. Ferretti (2017). "Function and Dysfunction of Microglia during Brain Development: Consequences for Synapses and Neural Circuits." Front Synaptic Neurosci **9**: 9.

Paolicelli, R. C., A. Sierra, B. Stevens, M. E. Tremblay, A. Aguzzi, B. Ajami, I. Amit, E. Audinat, I. Bechmann, M. Bennett, F. Bennett, A. Bessis, K. Biber, S. Bilbo, M. Blurton-Jones, E. Boddeke, D. Brites, B. Brone, G. C. Brown, O. Butovsky, M. J. Carson, B. Castellano, M. Colonna, S. A. Cowley, C. Cunningham, D. Davalos, P. L. De Jager, B. de Strooper, A. Denes, B. J. L. Eggen, U. Eyo, E. Galea, S. Garel, F. Ginhoux, C. K. Glass, O. Gokce, D. Gomez-Nicola, B. Gonzalez, S. Gordon, M. B. Graeber, A. D. Greenhalgh, P. Gressens, M. Greter, D. H. Gutmann, C. Haass, M. T. Heneka, F. L. Heppner, S. Hong, D. A. Hume, S. Jung, H. Kettenmann, J. Kipnis, R. Koyama, G. Lemke, M. Lynch, A. Majewska, M. Malcangio, T. Malm, R. Mancuso, T. Masuda, M. Matteoli, B. W. McColl, V. E. Miron, A. V. Molofsky, M. Monje, E. Mracsko, A. Nadjar, J. J. Neher, U. Neniskyte, H. Neumann, M. Noda, B. Peng, F. Peri, V. H. Perry, P. G. Popovich, C. Pridans, J. Priller, M. Prinz, D. Ragozzino, R. M. Ransohoff, M. W. Salter, A. Schaefer, D. P. Schafer, M. Schwartz, M. Simons, C. J. Smith, W. J. Streit, T. L. Tay, L. H. Tsai, A. Verkhratsky, R. von Bernhardi, H. Wake, V. Wittamer, S. A. Wolf, L. J. Wu and T. Wyss-Coray (2022). "Microglia states and nomenclature: A field at its crossroads." Neuron **110**(21): 3458-3483.

Papaleo, F., F. Yang, S. Garcia, J. Chen, B. Lu, J. N. Crawley and D. R. Weinberger (2012). "Dysbindin-1 modulates prefrontal cortical activity and schizophrenia-like behaviors via dopamine/D2 pathways." Mol Psychiatry **17**(1): 85–98.

Park, N. and et al. (2012). "Chronic circadian disturbance by a shortened light-dark cycle increases mortality." Neurobiology of Aging **33**(6): 11.

Patel, K. R., J. Cherian, K. Gohil and D. Atkinson (2014). "Schizophrenia: overview and treatment options." P T **39**(9): 638–645.

Patke, A., M. W. Young and S. Axelrod (2020). "Molecular mechanisms and physiological importance of circadian rhythms." Nat Rev Mol Cell Biol **21**(2): 67–84.

Patke, A., M. W. Young and S. Axelrod (2020). "Molecular mechanisms and physiological importance of circadian rhythms." Nat Rev Mol Cell Biol **21**(2): 67-84.

Patterson, P. H. (2009). "Immune involvement in schizophrenia and autism: Etiology, pathology and animal models." Behavioural Brain Research **204**(2): 313-321.

Paxinos, G. and K. B. Franklin (2019). Paxinos and Franklin's the mouse brain in stereotaxic coordinates, Academic press.

Perez-Cerda, F., M. V. Sanchez-Gomez and C. Matute (2015). "Pio del Rio Hortega and the discovery of the oligodendrocytes." Front Neuroanat **9**: 92.

Perkinson-Gloor, N., S. Lemola and A. Grob (2013). "Sleep duration, positive attitude toward life, and academic achievement: The role of daytime tiredness, behavioral persistence, and school start times." Journal of Adolescence **36**(2): 311-318.

Perry, W., A. Minassian, B. Lopez, L. Maron and A. Lincoln (2007). "Sensorimotor gating deficits in adults with autism." Biol Psychiatry **61**(4): 482-486.

Pickard, G. E., M. R. Ralph and M. Menaker (1987). "The intergeniculate leaflet partially mediates effects of light on circadian rhythms." Journal of Biological Rhythms **2**(1): 35-56.

Pinato, L., C. S. Galina Spilla, R. P. Markus and S. da Silveira Cruz-Machado (2019). "Dysregulation of Circadian Rhythms in Autism Spectrum Disorders." Curr Pharm Des **25**(41): 4379–4393.

Pittendrigh, C. S. and D. H. Minis (1972). "Circadian systems: longevity as a function of circadian resonance in Drosophila melanogaster." Proc Natl Acad Sci U S A **69**(6): 1537–1539.

Poe, S. L., G. Brucato, N. Bruno, L. Y. Arndt, S. Ben-David, K. E. Gill, T. Colibazzi, J. T. Kantrowitz, C. M. Corcoran and R. R. Girgis (2017). "Sleep disturbances in individuals at clinical high risk for psychosis." Psychiatry Res **249**: 240–243.

Polidarova, L., M. Sladek, M. Sotak, J. Pacha and A. Sumova (2011). "Hepatic, duodenal, and colonic circadian clocks differ in their persistence under conditions of constant light and in their entrainment by restricted feeding." Chronobiol Int **28**(3): 204–215.

Pratt, L., L. Ni, N. M. Ponzio and G. M. Jonakait (2013). "Maternal inflammation promotes fetal microglial activation and increased cholinergic expression in the fetal basal forebrain: role of interleukin-6." Pediatr Res **74**(4): 393-401.

Presser, H. B. (2003). "Race-Ethnic and Gender Differences in Nonstandard Work Shifts." Work and Occupations **30**(4): 412–439.

Presser, H. B. (2003). "Race-Ethnic and Gender Differences in Nonstandard Work Shifts." Work and Occupations **30**(4): 412-439.

Pritchett, D., A. Jagannath, L. A. Brown, S. K. Tam, S. Hasan, S. Gatti, P. J. Harrison, D. M. Bannerman, R. G. Foster and S. N. Peirson (2015). "Deletion of Metabotropic Glutamate Receptors 2 and 3 (mGlu2 & mGlu3) in Mice Disrupts Sleep and Wheel-Running Activity, and Increases the Sensitivity of the Circadian System to Light." PLoS One **10**(5): e0125523.

Pritchett, D., K. Wulff, P. L. Oliver, D. M. Bannerman, K. E. Davies, P. J. Harrison, S. N. Peirson and R. G. Foster (2012). "Evaluating the links between schizophrenia and sleep and circadian rhythm disruption." J Neural Transm (Vienna) **119**(10): 1061–1075.

Puttonen, S., M. Harma and C. Hublin (2010). "Shift work and cardiovascular disease - pathways from circadian stress to morbidity." Scand J Work Environ Health **36**(2): 96–108.

Puttonen, S., M. Harma and C. Hublin (2010). "Shift work and cardiovascular disease - pathways from circadian stress to morbidity." Scand J Work Environ Health **36**(2): 96-108.

Quagliato, L. A., U. De Matos and A. E. Nardi (2021). "Maternal immune activation generates anxiety in offspring: A translational meta-analysis." Translational Psychiatry **11**(1).

Randler, C. (2008). "Morningness-Eveningness Comparison in Adolescents from Different Countries around the World." Chronobiology International **25**(6): 1017-1028.

Rapoport, J. L., A. M. Addington, S. Frangou and M. R. Psych (2005). "The neurodevelopmental model of schizophrenia: update 2005." Mol Psychiatry **10**(5): 434-449.

Rapoport, J. L., J. N. Giedd and N. Gogtay (2012). "Neurodevelopmental model of schizophrenia: update 2012." Mol Psychiatry **17**(12): 1228-1238.

Rapoport, J. L., J. N. Giedd and N. Gogtay (2012). "Neurodevelopmental model of schizophrenia: update 2012." Mol Psychiatry **17**(12): 1228–1238.

Ratnayake, U., T. Quinn, D. W. Walker and H. Dickinson (2013). "Cytokines and the neurodevelopmental basis of mental illness." Frontiers in neuroscience **7**: 180.

Reisinger, S., D. Khan, E. Kong, A. Berger, A. Pollak and D. D. Pollak (2015). "The Poly(I:C)-induced maternal immune activation model in preclinical neuropsychiatric drug discovery." Pharmacology & Therapeutics **149**: 213-226.

Reppert, S. M. and D. R. Weaver (2002). "Coordination of circadian timing in mammals." Nature **418**(6901): 935–941.

Reynolds, L. M. and C. Flores (2021). Chapter 26 - Adolescent dopamine development: connecting experience with vulnerability or resilience to psychiatric disease. Diagnosis,

Management and Modeling of Neurodevelopmental Disorders. C. R. Martin, V. R. Preedy and R. Rajendram, Academic Press**:** 295-304.

Rich-Edwards, J. W., U. B. Kaiser, G. L. Chen, J. E. Manson and J. M. Goldstein (2018). "Sex and Gender Differences Research Design for Basic, Clinical, and Population Studies: Essentials for Investigators." Endocr Rev **39**(4): 424-439.

Roenneberg, T., T. Kuehnle, P. P. Pramstaller, J. Ricken, M. Havel, A. Guth and M. Merrow (2004). "A marker for the end of adolescence." Current Biology **14**(24): R1038-R1039.

Roenneberg, T., L. K. Pilz, G. Zerbini and E. C. Winnebeck (2019). "Chronotype and Social Jetlag: A (Self-) Critical Review." Biology (Basel) **8**(3).

Roenneberg, T., A. Wirz-Justice and M. Merrow (2003). "Life between clocks: daily temporal patterns of human chronotypes." J Biol Rhythms **18**(1): 80–90.

Salter, M. W. and B. Stevens (2017). "Microglia emerge as central players in brain disease." Nat Med **23**(9): 1018-1027.

Saper, C. B., T. E. Scammell and J. Lu (2005). "Hypothalamic regulation of sleep and circadian rhythms." Nature **437**(7063): 1257–1263.

Schaafsma, S. M., K. Gagnidze, A. Reyes, N. Norstedt, K. Månsson, K. Francis and D. W. Pfaff (2017). "Sex-specific gene–environment interactions underlying ASD-like behaviors." Proceedings of the National Academy of Sciences **114**(6): 1383-1388.

Schmidt, T. M., M. T. Do, D. Dacey, R. Lucas, S. Hattar and A. Matynia (2011). "Melanopsin-positive intrinsically photosensitive retinal ganglion cells: from form to function." J Neurosci **31**(45): 16094-16101.

Schmidt, T. M., M. T. Do, D. Dacey, R. Lucas, S. Hattar and A. Matynia (2011). "Melanopsin-positive intrinsically photosensitive retinal ganglion cells: from form to function." J Neurosci **31**(45): 16094–16101.

Schmitt, A., P. Falkai and S. Papiol (2022). "Neurodevelopmental disturbances in schizophrenia: evidence from genetic and environmental factors." J Neural Transm (Vienna): 1-11.

Schmitt, A., P. Falkai and S. Papiol (2022). "Neurodevelopmental disturbances in schizophrenia: evidence from genetic and environmental factors." J Neural Transm (Vienna): 1–11.

Seibenhener, M. L. and M. C. Wooten (2015). "Use of the Open Field Maze to Measure Locomotor and Anxiety-like Behavior in Mice." Journal of Visualized Experiments(96).

Sekaran, S., D. Lupi, S. L. Jones, C. J. Sheely, S. Hattar, K. W. Yau, R. J. Lucas, R. G. Foster and M. W. Hankins (2005). "Melanopsin-Dependent Photoreception Provides Earliest Light Detection in the Mammalian Retina." Current Biology **15**(12): 1099-1107.

Seney, M. L., K. Cahill, J. F. Enwright, 3rd, R. W. Logan, Z. Huo, W. Zong, G. Tseng and C. A. McClung (2019). "Diurnal rhythms in gene expression in the prefrontal cortex in schizophrenia." Nat Commun **10**(1): 3355.

Serón-Ferré, M., C. Torres-Farfán, M. L. Forcelledo and G. J. Valenzuela (2001). "The development of circadian rhythms in the fetus and neonate." Seminars in Perinatology **25**(6): 363-370.

Sharma, V. K. and M. Chandrashekaran (2005). "Zeitgebers (time cues) for biological clocks." Current Science: 1136-1146.

Sheaves, B., D. Freeman, L. Isham, J. McInerney, A. Nickless, L. M. Yu, S. Rek, J. Bradley, S. Reeve, C. Attard, C. A. Espie, R. Foster, A. Wirz-Justice, E. Chadwick and A. Barrera (2018). "Stabilising sleep for patients admitted at acute crisis to a psychiatric hospital

(OWLS): an assessor-blind pilot randomised controlled trial." Psychol Med **48**(10): 1694–1704.

Shi, L., S. H. Fatemi, R. W. Sidwell and P. H. Patterson (2003). "Maternal Influenza Infection Causes Marked Behavioral and Pharmacological Changes in the Offspring." The Journal of Neuroscience **23**(1): 297-302.

Shi, L., S. H. Fatemi, R. W. Sidwell and P. H. Patterson (2003). "Maternal influenza infection causes marked behavioral and pharmacological changes in the offspring." J Neurosci **23**(1): 297-302.

Slawecki, C. J., A. Thorsell and C. L. Ehlers (2004). "Long-term neurobehavioral effects of alcohol or nicotine exposure in adolescent animal models." Ann N Y Acad Sci **1021**: 448-452.

Smith, S. E., J. Li, K. Garbett, K. Mirnics and P. H. Patterson (2007). "Maternal immune activation alters fetal brain development through interleukin-6." J Neurosci **27**(40): 10695–10702.

Smith, S. E., J. Li, K. Garbett, K. Mirnics and P. H. Patterson (2007). "Maternal immune activation alters fetal brain development through interleukin-6." J Neurosci **27**(40): 10695-10702.

Smolders, S., T. Notter, S. M. T. Smolders, J.-M. Rigo and B. Brône (2018). "Controversies and prospects about microglia in maternal immune activation models for neurodevelopmental disorders." Brain, Behavior, and Immunity **73**: 51-65.

Sominsky, L., T. Dangel, S. Malik, S. N. De Luca, N. Singewald and S. J. Spencer (2021). "Microglial ablation in rats disrupts the circadian system." The FASEB Journal **35**(2).

Sonoda, T., S. K. Lee, L. Birnbaumer and T. M. Schmidt (2018). "Melanopsin Phototransduction Is Repurposed by ipRGC Subtypes to Shape the Function of Distinct Visual Circuits." Neuron **99**(4): 754-767.e754.

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." Schizophr Bull **35**(3): 631-637.

Souders, M. C., T. B. Mason, O. Valladares, M. Bucan, S. E. Levy, D. S. Mandell, T. E. Weaver and J. Pinto-Martin (2009). "Sleep behaviors and sleep quality in children with autism spectrum disorders." Sleep **32**(12): 1566–1578.

Souders, M. C., T. B. Mason, O. Valladares, M. Bucan, S. E. Levy, D. S. Mandell, T. E. Weaver and J. Pinto-Martin (2009). "Sleep behaviors and sleep quality in children with autism spectrum disorders." Sleep **32**(12): 1566-1578.

Spear, L. P. (2000). "The adolescent brain and age-related behavioral manifestations." Neuroscience & Biobehavioral Reviews **24**(4): 417-463.

Spisska, V., D. Pacesova, H. Mikova, P. Pohanova, P. Telensky, J. Novotny and Z. Bendova (2020). "Prenatal exposure to lipopolysaccharide induces changes in the circadian clock in the SCN and AA-NAT activity in the pineal gland." Brain Res **1743**: 146952.

Stilo, S. A. and R. M. Murray (2019). "Non-Genetic Factors in Schizophrenia." Curr Psychiatry Rep **21**(10): 100.

Strohmaier, S., E. E. Devore, Y. Zhang and E. S. Schernhammer (2018). "A Review of Data of Findings on Night Shift Work and the Development of DM and CVD Events: a Synthesis of the Proposed Molecular Mechanisms." Curr Diab Rep **18**(12): 132.

Sudo, M., K. Sasahara, T. Moriya, M. Akiyama, T. Hamada and S. Shibata (2003). "Constant light housing attenuates circadian rhythms of mPer2 mRNA and mPER2 protein expression in the suprachiasmatic nucleus of mice." Neuroscience **121**(2): 493-499.

Sudo, M., K. Sasahara, T. Moriya, M. Akiyama, T. Hamada and S. Shibata (2003). "Constant light housing attenuates circadian rhythms of mPer2 mRNA and mPER2 protein expression in the suprachiasmatic nucleus of mice." Neuroscience **121**(2): 493–499.

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." Am J Psychiatry **151**(6): 922-924.

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." Schizophr Bull **26**(2): 257–273.

Tahara, Y. and S. Shibata (2016). "Circadian rhythms of liver physiology and disease: experimental and clinical evidence." Nature Reviews Gastroenterology & Hepatology **13**(4): 217-226.

Talbot, K. (2009). "The sandy (sdy) mouse: a dysbindin-1 mutant relevant to schizophrenia research." Prog Brain Res **179**: 87–94.

Tan, H. Y. and Y. G. Ang (2001). "First-episode psychosis in the military: a comparative study of prodromal symptoms." Aust N Z J Psychiatry **35**(4): 512-519.

Tan, H. Y. and Y. G. Ang (2001). "First-episode psychosis in the military: a comparative study of prodromal symptoms." Aust N Z J Psychiatry **35**(4): 512–519.

Tandon, R., W. Gaebel, D. M. Barch, J. Bustillo, R. E. Gur, S. Heckers, D. Malaspina, M. J. Owen, S. Schultz, M. Tsuang, J. Van Os and W. Carpenter (2013). "Definition and description of schizophrenia in the DSM-5." Schizophr Res **150**(1): 3-10.

Tandon, R., J. E. Shipley, S. Taylor, J. F. Greden, A. Eiser, J. DeQuardo and J. Goodson (1992). "Electroencephalographic sleep abnormalities in schizophrenia. Relationship to

positive/negative symptoms and prior neuroleptic treatment." Arch Gen Psychiatry **49**(3): 185–194.

Tandon, R., J. E. Shipley, S. Taylor, J. F. Greden, A. Eiser, J. DeQuardo and J. Goodson (1992). "Electroencephalographic sleep abnormalities in schizophrenia. Relationship to positive/negative symptoms and prior neuroleptic treatment." Arch Gen Psychiatry **49**(3): 185-194.

Tapia-Osorio, A., R. Salgado-Delgado, M. Angeles-Castellanos and C. Escobar (2013). "Disruption of circadian rhythms due to chronic constant light leads to depressive and anxiety-like behaviors in the rat." Behavioural Brain Research **252**: 1-9.

Tartaglione, A. M., A. Villani, M. A. Ajmone-Cat, L. Minghetti, L. Ricceri, V. Pazienza, R. De Simone and G. Calamandrei (2022). "Maternal immune activation induces autism-like changes in behavior, neuroinflammatory profile and gut microbiota in mouse offspring of both sexes." Translational Psychiatry **12**(1).

Tetreault, N. A., A. Y. Hakeem, S. Jiang, B. A. Williams, E. Allman, B. J. Wold and J. M. Allman (2012). "Microglia in the cerebral cortex in autism." J Autism Dev Disord **42**(12): 2569-2584.

Tordjman, S., G. M. Anderson, E. Bellissant, M. Botbol, H. Charbuy, F. Camus, R. Graignic, S. Kermarrec, C. Fougerou, D. Cohen and Y. Touitou (2012). "Day and nighttime excretion of 6-sulphatoxymelatonin in adolescents and young adults with autistic disorder." Psychoneuroendocrinology **37**(12): 1990-1997.

Town, T., V. Nikolic and J. Tan (2005). "The microglial "activation" continuum: from innate to adaptive responses." J Neuroinflammation **2**: 24.

Tremblay, M. E., R. L. Lowery and A. K. Majewska (2010). "Microglial interactions with synapses are modulated by visual experience." PLoS Biol **8**(11): e1000527.

Tremblay, M. E., B. Stevens, A. Sierra, H. Wake, A. Bessis and A. Nimmerjahn (2011). "The role of microglia in the healthy brain." J Neurosci **31**(45): 16064-16069.

Tripathi, A., S. K. Kar and R. Shukla (2018). "Cognitive Deficits in Schizophrenia: Understanding the Biological Correlates and Remediation Strategies." Clinical Psychopharmacology and Neuroscience **16**(1): 7-17.

Valle, F. P. (1970). "Effects of strain, sex, and illumination on open-field behavior of rats." Am J Psychol **83**(1): 103-111.

Van Erp, T. G. M., E. Walton, D. P. Hibar, L. Schmaal, W. Jiang, D. C. Glahn, G. D. Pearlson, N. Yao, M. Fukunaga, R. Hashimoto, N. Okada, H. Yamamori, J. R. Bustillo, V. P. Clark, I. Agartz, B. A. Mueller, W. Cahn, S. M. C. De Zwarte, H. E. Hulshoff Pol, R. S. Kahn, R. A. Ophoff, N. E. M. Van Haren, O. A. Andreassen, A. M. Dale, N. T. Doan, T. P. Gurholt, C. B. Hartberg, U. K. Haukvik, K. N. Jørgensen, T. V. Lagerberg, I. Melle, L. T. Westlye, O. Gruber, B. Kraemer, A. Richter, D. Zilles, V. D. Calhoun, B. Crespo-Facorro, R. Roiz-Santiañez, D. Tordesillas-Gutiérrez, C. Loughland, V. J. Carr, S. Catts, V. L. Cropley, J. M. Fullerton, M. J. Green, F. A. Henskens, A. Jablensky, R. K. Lenroot, B. J. Mowry, P. T. Michie, C. Pantelis, Y. Quidé, U. Schall, R. J. Scott, M. J. Cairns, M. Seal, P. A. Tooney, P. E. Rasser, G. Cooper, C. Shannon Weickert, T. W. Weickert, D. W. Morris, E. Hong, P. Kochunov, L. M. Beard, R. E. Gur, R. C. Gur, T. D. Satterthwaite, D. H. Wolf, A. Belger, G. G. Brown, J. M. Ford, F. Macciardi, D. H. Mathalon, D. S. O'Leary, S. G. Potkin, A. Preda, J. Voyvodic, K. O. Lim, S. McEwen, F. Yang, Y. Tan, S. Tan, Z. Wang, F. Fan, J. Chen, H. Xiang, S. Tang, H. Guo, P. Wan, D. Wei, H. J. Bockholt, S. Ehrlich, R. P. F.

Wolthusen, M. D. King, J. M. Shoemaker, S. R. Sponheim, L. De Haan, L. Koenders, M. W. Machielsen, T. Van Amelsvoort, D. J. Veltman, F. Assogna, N. Banaj, P. De Rossi, M. Iorio, F. Piras, G. Spalletta, P. J. McKenna, E. Pomarol-Clotet, R. Salvador, A. Corvin, G. Donohoe, S. Kelly, C. D. Whelan, E. W. Dickie, D. Rotenberg, A. N. Voineskos, S. Ciufolini, J. Radua, P. Dazzan, R. Murray, T. Reis Marques, A. Simmons, S. Borgwardt, L. Egloff, F. Harrisberger, A. Riecher-Rössler, R. Smieskova, K. I. Alpert, L. Wang, E. G. Jönsson, S. Koops, I. E. C. Sommer, A. Bertolino, A. Bonvino, A. Di Giorgio, E. Neilson, A. R. Mayer, J. M. Stephen, J. S. Kwon, J.-Y. Yun, D. M. Cannon, C. McDonald, I. Lebedeva, A. S. Tomyshev, T. Akhadov, V. Kaleda, H. Fatouros-Bergman, L. Flyckt, G. F. Busatto, P. G. P. Rosa, M. H. Serpa, M. V. Zanetti, C. Hoschl, A. Skoch, F. Spaniel, D. Tomecek, S. P. Hagenaars, A. M. McIntosh, H. C. Whalley, S. M. Lawrie, C. Knöchel, V. Oertel-Knöchel, M. Stäblein, F. M. Howells, D. J. Stein, H. S. Temmingh, A. Uhlmann, C. Lopez-Jaramillo, D. Dima, A. McMahon, J. I. Faskowitz, B. A. Gutman, N. Jahanshad, P. M. Thompson, J. A. Turner, L. Farde, L. Flyckt, G. Engberg, S. Erhardt, H. Fatouros-Bergman, S. Cervenka, L. Schwieler, F. Piehl, I. Agartz, K. Collste, P. Victorsson, A. Malmqvist, M. Hedberg and F. Orhan (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): 644-654.

Vargas, D. L., C. Nascimbene, C. Krishnan, A. W. Zimmerman and C. A. Pardo (2005). "Neuroglial activation and neuroinflammation in the brain of patients with autism." Ann Neurol **57**(1): 67-81.

Vargas, D. L., C. Nascimbene, C. Krishnan, A. W. Zimmerman and C. A. Pardo (2005). "Neuroglial activation and neuroinflammation in the brain of patients with autism." Ann Neurol **57**(1): 67–81.

Vaze, K. M. and V. K. Sharma (2013). "On the Adaptive Significance of Circadian Clocks for Their Owners." Chronobiology International **30**(4): 413-433.

Ver Hoeve, E. S., G. Kelly, S. Luz, S. Ghanshani and S. Bhatnagar (2013). "Short-term and long-term effects of repeated social defeat during adolescence or adulthood in female rats." Neuroscience **249**: 63-73.

Vetter, C., E. E. Devore, C. A. Ramin, F. E. Speizer, W. C. Willett and E. S. Schernhammer (2015). "Mismatch of Sleep and Work Timing and Risk of Type 2 Diabetes." Diabetes Care **38**(9): 1707–1713.

Vigli, D., G. Palombelli, S. Fanelli, G. Calamandrei, R. Canese, L. Mosca, M. L. Scattoni and L. Ricceri (2020). "Maternal Immune Activation in Mice Only Partially Recapitulates the Autism Spectrum Disorders Symptomatology." Neuroscience **445**: 109-119.

Vohr, B. R., E. Poggi Davis, C. A. Wanke and N. F. Krebs (2017). "Neurodevelopment: The Impact of Nutrition and Inflammation During Preconception and Pregnancy in Low-Resource Settings." Pediatrics **139**(Suppl 1): S38-S49.

Volkmar, F., M. Siegel, M. Woodbury-Smith, B. King, J. McCracken, M. State, C. American Academy of and I. Adolescent Psychiatry Committee on Quality (2014). "Practice parameter for the assessment and treatment of children and adolescents with autism spectrum disorder." J Am Acad Child Adolesc Psychiatry **53**(2): 237-257.

Walder, D. J., D. P. Laplante, A. Sousa-Pires, F. Veru, A. Brunet and S. King (2014). "Prenatal maternal stress predicts autism traits in 6(1/2) year-old children: Project Ice Storm." Psychiatry Res **219**(2): 353-360.

Walf, A. A. and C. A. Frye (2007). "The use of the elevated plus maze as an assay of anxiety-related behavior in rodents." Nat Protoc **2**(2): 322-328.

Walker, C. D., S. Deschamps, K. Proulx, M. Tu, C. Salzman, B. Woodside, S. Lupien, N. Gallo-Payet and D. Richard (2004). "Mother to infant or infant to mother? Reciprocal regulation of responsiveness to stress in rodents and the implications for humans." J Psychiatry Neurosci **29**(5): 364-382.

Walker, W. H., 2nd, J. C. Walton, A. C. DeVries and R. J. Nelson (2020). "Circadian rhythm disruption and mental health." Transl Psychiatry **10**(1): 28.

Wang, M., L. Zhang and F. H. Gage (2019). "Microglia, complement and schizophrenia." Nat Neurosci **22**(3): 333-334.

Wang, X.-L., S. Kooijman, Y. Gao, L. Tzeplaeff, B. Cosquer, I. Milanova, S. E. C. Wolff, N. Korpel, M.-F. Champy, B. Petit-Demoulière, I. Goncalves Da Cruz, T. Sorg-Guss, P. C. N. Rensen, J.-C. Cassel, A. Kalsbeek, A.-L. Boutillier and C.-X. Yi (2021). "Microglia-specific knock-down of Bmal1 improves memory and protects mice from high fat diet-induced obesity." Molecular Psychiatry.

Watts, A. G., L. W. Swanson and G. Sanchez-Watts (1987). "Efferent projections of the suprachiasmatic nucleus: I. Studies using anterograde transport of Phaseolus vulgaris leucoagglutinin in the rat." Journal of Comparative Neurology **258**(2): 204-229.

Weber-Stadlbauer, U. and U. Meyer (2019). "Challenges and opportunities of a-priori and a-posteriori variability in maternal immune activation models." Current Opinion in Behavioral Sciences **28**: 119-128.

Weittenhiller, L. P., M. E. Mikhail, J. Mote, T. R. Campellone and A. M. Kring (2021). "What gets in the way of social engagement in schizophrenia?" World Journal of Psychiatry **11**(1): 13-26.

Welsh, D. K., D. E. Logothetis, M. Meister and S. M. Reppert (1995). "Individual neurons dissociated from rat suprachiasmatic nucleus express independently phased circadian firing rhythms." Neuron **14**(4): 697-706.

Whiteford, H. A., L. Degenhardt, J. Rehm, A. J. Baxter, A. J. Ferrari, H. E. Erskine, F. J. Charlson, R. E. Norman, A. D. Flaxman, N. Johns, R. Burstein, C. J. Murray and T. Vos (2013). "Global burden of disease attributable to mental and substance use disorders: findings from the Global Burden of Disease Study 2010." Lancet **382**(9904): 1575-1586.

Wideman, C. H. and H. M. Murphy (2009). "Constant light induces alterations in melatonin levels, food intake, feed efficiency, visceral adiposity, and circadian rhythms in rats." Nutritional Neuroscience **12**(5): 233–240.

Wideman, C. H. and H. M. Murphy (2009). "Constant light induces alterations in melatonin levels, food intake, feed efficiency, visceral adiposity, and circadian rhythms in rats." Nutritional Neuroscience **12**(5): 233-240.

Wiggs, L. and G. Stores (2004). "Sleep patterns and sleep disorders in children with autistic spectrum disorders: insights using parent report and actigraphy." Dev Med Child Neurol **46**(6): 372-380.

Williams, C. (2008). "Work-life balance of shift workers." Statistics Canada Perspectives **9**: 5-16.

Williams, C. (2008). "Work-life balance of shift workers."

Wintler, T., H. Schoch, M. G. Frank and L. Peixoto (2020). "Sleep, brain development, and autism spectrum disorders: Insights from animal models." J Neurosci Res **00**: 1–13.

Wintler, T., H. Schoch, M. G. Frank and L. Peixoto (2020). "Sleep, brain development, and autism spectrum disorders: Insights from animal models." J Neurosci Res **00**: 1-13.

Wulff, K., D. J. Dijk, B. Middleton, R. G. Foster and E. M. Joyce (2012). "Sleep and circadian rhythm disruption in schizophrenia." Br J Psychiatry **200**(4): 308-316.

Wulff, K., D. J. Dijk, B. Middleton, R. G. Foster and E. M. Joyce (2012). "Sleep and circadian rhythm disruption in schizophrenia." Br J Psychiatry **200**(4): 308–316.

Wulff, K. and E. Joyce (2011). "Circadian rhythms and cognition in schizophrenia." Br J Psychiatry **198**(4): 250–252.

Xuan, I. C. and D. R. Hampson (2014). "Gender-dependent effects of maternal immune activation on the behavior of mouse offspring." PLoS One **9**(8): e104433.

Yan, J., H. Wang, Y. Liu and C. Shao (2008). "Analysis of Gene Regulatory Networks in the Mammalian Circadian Rhythm." PLoS Computational Biology **4**(10): e1000193.

Yee, N., A. Ribic, C. C. de Roo and E. Fuchs (2011). "Differential effects of maternal immune activation and juvenile stress on anxiety-like behaviour and physiology in adult rats: No evidence for the “double-hit hypothesis”." Behavioural Brain Research **224**(1): 180-188.

Yerushalmi, S. and R. M. Green (2009). "Evidence for the adaptive significance of circadian rhythms." Ecology Letters **12**(9): 970.

Yin, D. M., Y. J. Chen, A. Sathyamurthy, W. C. Xiong and L. Mei (2012). "Synaptic dysfunction in schizophrenia." Adv Exp Med Biol **970**: 493-516.

Yoo, S.-H., S. Yamazaki, P. L. Lowrey, K. Shimomura, C. H. Ko, E. D. Buhr, S. M. Siepka, H.-K. Hong, W. J. Oh, O. J. Yoo, M. Menaker and J. S. Takahashi (2004). "PERIOD2::LUCIFERASE real-time reporting of circadian dynamics reveals persistent circadian oscillations in mouse peripheral tissues." Proceedings of the National Academy of Sciences **101**(15): 5339-5346.

Zanini, M., J. Castro, F. M. Coelho, L. Bittencourt, R. A. Bressan, S. Tufik and E. Brietzke (2013). "Do sleep abnormalities and misaligned sleep/circadian rhythm patterns represent early clinical characteristics for developing psychosis in high risk populations?" Neurosci Biobehav Rev **37**(10 Pt 2): 2631–2637.

Zhang, J., Y. Jing, H. Zhang, D. K. Bilkey and P. Liu (2019). "Maternal immune activation altered microglial immunoreactivity in the brain of postnatal day 2 rat offspring." Synapse **73**(2): e22072.

Zhang, Z. and H. van Praag (2015). "Maternal immune activation differentially impacts mature and adult-born hippocampal neurons in male mice." Brain Behav Immun **45**: 60-70.

Zhao, X., M. Erickson, R. Mohammed and A. C. Kentner (2022). "Maternal immune activation accelerates puberty initiation and alters mechanical allodynia in male and female C57BL6/J mice." Developmental Psychobiology **64**(5).

Zhao, X., R. Mohammed, H. Tran, M. Erickson and A. C. Kentner (2021). "Poly (I:C)-induced maternal immune activation modifies ventral hippocampal regulation of stress reactivity: prevention by environmental enrichment." Brain Behav Immun **95**: 203-215.

Zhao, X., H. Tran, H. Derosa, R. C. Roderick and A. C. Kentner (2021). "Hidden talents: Poly (I:C)-induced maternal immune activation improves mouse visual discrimination

performance and reversal learning in a sex-dependent manner." Genes, Brain and Behavior **20**(7).

Zou, J., T. Kawai, T. Tsuchida, T. Kozaki, H. Tanaka, K. S. Shin, H. Kumar and S. 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): 717-728.