DISCOVERING THE IMMEDIATE AND LASTING BENEFITS OF AN AEROBIC INTERVENTION IN ADOLESCENCE ON CORPUS CALLOSUM DEVELOPMENT IN A RAT MODEL OF FETAL ALCOHOL SPECTRUM DISORDERS

by

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A dissertation submitted to the Faculty of the University of Delaware in partial fulfillment of the requirements for the degree of Doctor of Philosophy in Psychological and Brain Sciences

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

AE Alcohol Exposure

ARBD Alcohol-Related Birth Defects

ARND Alcohol-Related Neurodevelopmental Defects

BAC Blood Alcohol Concentration

BGS Brain Growth Spurt

DTI Diffusion Tensor Imaging

FA Fractional Anisotropy

FAS Fetal Alcohol Syndrome

FASD Fetal Alcohol Spectrum Disorders

ICC Interhemispheric Corpus Callosum

MBP Myelin Basic Protein

MRI Magnetic Resonance Imaging

NGS Normal Goat Serum

OD Optical Density

OL Oligodendrocyte

OPC Oligodendrocyte Precursor Cell

PBS Phosphate Buffered Saline

PCC Projecting fibers of Corpus Callosum

PDGF Platelet Derived Growth Factor

PFAS Partial Fetal Alcohol Syndrome

RD Radial Diffusivity

SEM Standard Error of the Mean

SH Socially-Housed

SI Sham-Intubated

TBS Tris-Buffered Saline

WR Wheel Running

ABSTRACT

An estimated 1in 20 infants born annually has been exposed to alcohol prenatally, creating the largest preventable public health crisis in the U.S. (Hoyme et al., 2016). Prenatal alcohol exposure has severe consequences for neurodevelopment and the resulting diagnosable disorders are categorized as Fetal Alcohol Spectrum Disorders (FASD). FASD-related impairments to executive function are the most prevalent among affected youth and contribute to increased risk for substance abuse and employment/legal issues. Importantly, diminished visuospatial processing is linked to disruptions to corpus callosum growth and myelination in affected adolescents. Targeted interventions that support neurodevelopment in FASD-affected youth are nonexistent. To address this issue, this dissertation evaluated the capacity for an adolescent exercise intervention, a known stimulator of myelinogenesis, to upregulate corpus callosum myelination and mitigate lasting deficits to corpus callosum function in a rodent model of FASD.

The studies comprising this dissertation employ an established rodent model of FASD to examine the potential therapeutic effect of adolescent exercise intervention on corpus callosum development in both sexes. In Aim 1 Experiment 1, longitudinal analysis of neuroimaging data collected pre- and post-intervention illustrated that corpus callosum myelination is delayed in alcohol-exposed rats of both sexes, concordant with the clinical literature (Treit et al., 2013). These results were validated using immunocytochemical labelling of brain tissue from rats pre- and post-intervention in Aim 2, Experiments 1 and 2. Quantification of oligoglia and myelin

basic protein density showed that alcohol exposure led to a transient restriction of oligoglia proliferation and differentiation in early adolescence (pre-intervention). Conversely, the number of precursor and mature oligodendrocytes did not differ between alcohol-exposed and control groups in late adolescence. However, the myelinogenic effects of adolescent exercise intervention had a greater effect on myelin plasticity in female rats without previous alcohol exposure.

Several clinical studies have correlated impairments to corpus callosum development and function with deficits in visuospatial processing in youth affected by FASD (Ware et al., 2021; Sowell et al., 2008; Inkelis et al., 2020). Findings from Experiment 2 of Aim 1, illustrate that the functional connectivity between cortical sub-regions comprising the executive control network required for fixating on a particular visual stimulus was impaired in adult female FASD rats. Moreover, electron microscopic analysis of splenium axons revealed that the diameter of axons and thickness of the myelin sheath are reduced these rats. The observed impairments to functional connectivity and ultrastructure were improved by intervention exposure in adolescence.

These results provide clear evidence that increasing aerobic activity in adolescence supports white matter maturation in the FASD brain. This innovative preclinical study was uniquely suited to answer targeted questions concerning sexual dimorphisms in FASD etiology and to aid in the development of a targeted intervention for FASD-affected youth.

PREFACE

I, Katrina Milbocker, hold principle author status for the chapters comprising this dissertation. Each of these chapters have co-authors, whose contributions greatly facilitated my dissertation research as described below.

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Chapter 1

TERATOGENIC IMPACT OF ALCOHOL ON OLIGOGLIA LINEAGE CELL PROLIFERATION AND DEVELOPMENT IN THE MAMMALIAN BRAIN

1.1 Introduction

A 2022 report published by the U.S. Centers for Disease Control and Prevention estimated that 1 in 20 infants in the U.S. is affected by prenatal alcohol exposure annually (reviewed in Popova et al., 2017; Denny et al., 2019; Hoyme et al., 2016), creating one of the largest pediatric public health crises in America. The first published report of developmental abnormalities associated with fetal alcohol exposure was recorded in 1968, giving rise to a new field of research (LeMoine et al., 1968). By 1996, an identifiable spectrum of symptoms had been defined and clinical categorizations were established to distinguish between the similar, yet unique disorders that result from prenatal alcohol exposure (Stratton et al., 1996). These major subgroups included Fetal Alcohol Syndrome (FAS), Partial Fetal Alcohol Syndrome (PFAS), Alcohol-Related Neurodevelopmental Defects (ARND), and Alcohol-Related Birth Defects (ARBD). Distinct diagnostic codes were created for these individual disorders in 2005 (Stratton et al., 1996; Hoyme et al., 2005). Prenatal alcohol exposure may result in characteristic cranial dysmorphologies (e.g., smooth philtrum, thin upper lip, and orbital hypertelorism) and cognitive/behavioral impairment including hyperactivity, reduced executive function, deficits with perceptual learning/abstract conceptualization, reduced visuospatial processing, disrupted language and mathematical learning development, impulsivity, and

disrupted social behaviors (Hoyme et al., 2016). Infants exposed to alcohol in gestation are born much smaller and gain less weight in infancy compared to children without teratogenic exposure (Carter et al., 2012; Mooney et al., 2021). The severity of an individual's physical, behavioral, and cognitive deficits is primarily determined by the timing and amount of the prenatal alcohol exposure (May et al., 2013). Binge drinking sufficient to produce high blood alcohol concentration as well as first trimester exposure are likely to result in the most severe FASD diagnoses (i.e., FAS or PFAS), although exposure at any time in any quantity poses a significant threat to development (Maier & West, 2001; May et al., 2013; Hoyme et al., 2016).

Formally classified as a teratogen, alcohol has varying effects on neurodevelopment resulting from differences in the vulnerability of brain structures, neural tissues, and cell types. The detrimental impact of prenatal alcohol exposure on neuronal proliferation, differentiation, survival and function is well described (Bonthius and West, 1991; Bonthius et al., 1988; Goodlett et al., 1987). More recently, oligodendrocytes (OL), the myelinating glial cells of the central nervous system, were discovered to be particularly vulnerable to the toxicity of alcohol during fetal development. Reductions to OL proliferation and differentiation in early-life can affect late-occurring developmental processes involved in circuit refinement, such as synaptic pruning and adolescent myelination. Indeed, disrupted maturation of major white matter tracts of the central nervous system is observed in adolescents affected by FASD. However, the underlying neurobiological mechanisms contributing to immature white matter growth and myelination require further exploration. Identification of these processes is imperative for advancing therapeutic development. This dissertation research focused on mitigating alterations to white matter tract

development following alcohol exposure coinciding with the mammalian brain growth spurt using a rat model of FASD.

1.2 Overview of Fetal Alcohol Spectrum Disorders (FASD) and description of rodent models of FASD

Shortly following the clinical categorization of Fetal Alcohol Syndrome (FAS) in 1973 (Jones & Smith, 1973), rodent models were developed to identify the lasting consequences of alcohol teratogenesis on brain structure and function as well as to develop novel therapeutic strategies for affected children. Animal model research facilitates the investigation of neurobiological mechanisms underlying alcohol-induced brain damage for targeted intervention development. Indeed, specific neurodevelopmental stages spanning rodent gestation and early postnatal life are well-described in relation to their human analogues (depicted in **Figure 1**). In accordance with this perspective, multiple animal models of FASD have been developed to assess the teratogenicity of alcohol exposure across stages of neurodevelopment in varying animal species. Comparative neuroanatomical studies demonstrate that the development and the rate of growth of different brain structures are species-dependent (Dobbing & Sands, 1979). The advantages of using rodent models of FASD include their highly translational nature, feasibility for controlled experimentation critical for the identification of neuropathological mechanisms, short lifespan, and well-defined patterns of reproduction and development (Cudd, 2005).

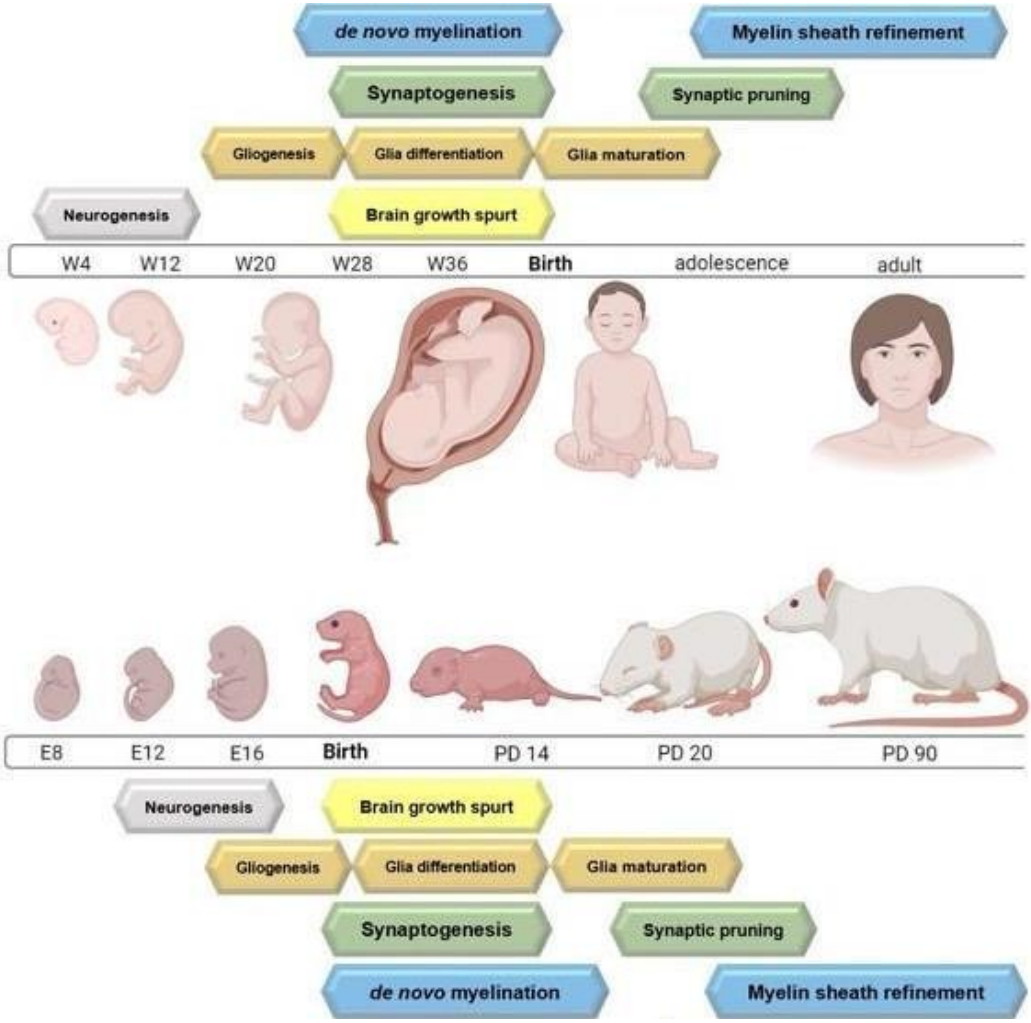

Figure 1. Timelines highlighting the peak of major neurodevelopmental processes occurring from gestation through adulthood in rodents and humans. Figure created with BioRender.com. Modified from Milbocker et al. (2021).

One fundamental discovery gleaned from the use of a rodent model of FASD highlighted the important correlation between the timing of ethanol exposure during fetal development, the peak of blood alcohol concentration (BAC), and their effects on brain region-specific damage (Bonthius et al., 1988; Bonthius & West, 1990, 1991; Ikonomidou et al., 2001). Dose-dependent patterns of damage to neurons and neuroglia are immediately identifiable and may persist into adolescence and adulthood, contributing to the complex etiology of disorders under the FASD continuum (Bielawski & Abel, 2002; Madden et al., 2020; Terasaki & Schwarz, 2016). FAS, resulting from heavy first trimester alcohol exposure, is the most recognizable within the continuum. An FAS diagnosis is based on distinct facial dysmorphologies including shortened palpebral fissures, a smoothened philtrum, and delayed growth that result from extreme cell apoptosis during the first trimester. FAS is also characterized by severe motor skill development and impaired cognitive capacity (Hoyme et al., 2016). A late-term exposure, however, may hinder cellular processes occurring during the brain growth spurt period (BGS); a highly neuroplastic phase marked by increased neuronal migration, gliogenesis, and synaptogenesis which increases the sensitivity of certain structures to the teratogenic effects of alcohol exposure (Dobbing & Sands, 1979; Helfer et al., 2009; Klintsova et al., 2007). Such impairments have been linked to diminished visuospatial processing and attention span; difficulty regulating impulses; and issues with learning, memory, and executive function. Identification of these symptoms along with reported late gestational alcohol exposure may result in ARND or ARBD diagnosis. However, due to the comorbidity of these symptoms with other developmental disorders, ARND and ARBD are difficult to diagnose without apparent facial dysmorphology or documented prenatal

alcohol exposure (Hoyme et al., 2016). Rodent models of FASD are highly advantageous for studying neuroanatomical reorganization following alcohol exposure during the BGS as this neurodevelopmental event occurs during the first two postnatal weeks in rodent pups. In this paradigm, pups are exposed to alcohol through controlled methods (i.e. gas inhalation, subcutaneous injection, or intragastric intubation with formulated milk) to achieve a desired dosage and resulting BAC in each pup that corresponds to daily pup weight. Intragastric intubation administration is arguably the most translational as the alcohol is metabolized in a similar manner to that of a fetus.

1.3 Overview of oligoglia lineage cell proliferation and maturation for initial myelination of white matter tracts during development

Perinatal neurodevelopment represents the period with the greatest neuroplastic potential. Orchestration of several programmed cellular processes during early critical periods is necessary for homeostatic brain development as it leads to the emergence of adaptive behaviors that allow an organism to interact in a species-specific and harmonious way with its environment. Brain tissue growth is marked by massive neuro- and gliogenesis which peaks during late embryogenesis. Following cellular proliferation and neuronal differentiation, axonal connections are established through synaptogenesis to facilitate neurotransmission. Neurotransmission during the BGS is primarily excitatory in nature and results in an overabundance of immature synapses. Neural circuits imperative for survival and early-life functions such as suckling, vision, and audition are immediately myelinated by OLs. Redundant connections are phagocytosed by microglia during the phase of synaptic pruning which occurs during a sensitive period in late childhood and early adolescence, closing the perinatal period of elevated neuroplastic potential. The refined neural circuitry is

then further myelinated to strengthen and optimize salient brain connections which are required for overall functioning in adulthood and efficient cognitive processing. The proliferation, maturation, and putative function of glia are major constituents of proper neurodevelopment and are imperative for sustaining extraordinary neuroplastic potential during the perinatal and adolescent periods.

Critical periods are windows of heightened neuroplasticity during which lack of certain stimuli or experiences has a dramatic, and often irreversible, effect on the development and function of the brain. They are characterized by experience-expectant processes that control the naissance and adequate maturation of brain circuits necessary for vision, hearing, language, and social and emotional development during infancy and early childhood (Nelson and Gabard-Durnam, 2020). As such, critical periods occur in infancy and early childhood. The specific length of critical periods depends on the rate of development of the putative circuit or structure and varies in length from weeks to months (reviewed in Hensch, 2004). Indeed, Nobel-prize winning studies conducted by Drs. David Hubel and Torsten Wiesel were some of the first to demonstrate that the absence of visual stimuli during a perinatal critical period results in lifelong visual impairments (Hubel and Wiesel, 1962). Following the closure of a critical period, the affected brain circuits become more resistant to future neuroplastic alteration. Biological processes occurring during early critical periods include cellular proliferation and differentiation, synaptogenesis, and the onset of myelination.

Excluding microglia, all cells of the central nervous system (CNS) are derived from neuroepithelial cells, which are a type of multipotent neural stem cell (Williams and Price, 1995). Cellular proliferation in the mammalian CNS follows a sequential

order, with the induction of neurogenesis coinciding with the formation of the ventricular zone. In rodents, the induction of neurogenesis begins on embryonic day ED 12, followed by gliogenesis which begins on embryonic day 14 and peaks during the first 2 weeks of life (Levison et al., 1993; Zerlin et al., 1995; Lee et al., 2000). The primary location of cell proliferation in the CNS transitions from the ventricular zone to the subventricular zone between embryonic days 17 and 19, and is the dominant originating site for neuronal and glial progenitors later in gestation and throughout life (Sauvageot and Stiles, 2002). Analogous events occur in human neurodevelopment and are described in time and developmental stage comparison to the rat in **Figure 1**. As the mammalian brain develops from the inside-out (building cortical layers above subcortical structures) radial glia replace neuroepithelial cells as the major progenitors for neurons and glia in the mammalian cortex. Disruption of cellular genesis in the CNS has lasting effects on neurodevelopment and CNS function.

Following cell proliferation, cell differentiation is driven by intrinsic and extrinsic cues occurring during highly regulated phases of gestation and early postnatal development. The ontogenesis of OLs in the mammalian brain is complex and varies by brain region and an organism's age. Briefly, Oligodendrocyte Precursor Cells (OPCs) proliferate in three distinct gestational phases (Cui et al., 2012; Bergles and Richardson, 2016). OPCs are generated in the subventricular zone and migrate along blood vessels to destinations throughout the white and gray matter of the brain during embryogenesis (van Tilborg et al., 2017). Following migration, OPCs continue to proliferate locally and form synapses with neurons to survey local neural activity, ultimately leading to the differentiation of OPC into pre-myelinating OLs in an activity-dependent manner (Kuhn et al., 2019; Newville et al., 2017). Local OPC

proliferation is maintained across the lifespan and creates the OPC pool which is essential for myelin plasticity to modify neuronal connection strength. Growth factors provide important extracellular cues for the differentiation of astrocytes and OLs during gestation and early postnatal life which are required to support cell maturation (for reviews see: Cameron et al., 1998; Sommer, 2006). After differentiation, the new OL is primed to initiate axonal myelination and must do so within 12-18 hours to avoid entering apoptosis (Watkins et al., 2008). The signaling molecules sonic hedgehog, Notch, and Bone Morphogenic Proteins are essential for brain patterning (i.e., ventralization) and for expression of basic helix-loop-helix factors Olig1 and 2 which are required for OL differentiation (Lu et al., 2000; Zhou et al., 2000; Nery et al., 2001; Wang and Almazan, 2016). Secretion of platelet-derived growth factor (PDGF) by astrocytes triggers the differentiation of OPCs to OLs when simultaneous with neuronal firing (Johe et al., 1996; Williams et al., 1997). Additionally, epigenetic modification of certain genes guides the differentiation of neural stem cells to functional glial cells in late gestation (reviewed in Murao et al., 2016). For example, removal of acetyl groups from histone proteins signals the onset for OPC differentiation to OLs (Shen et al., 2005; Ye et al., 2009; Conway et al., 2012). Regulated glial cell genesis and differentiation prepare the neonatal brain for circuit development.

Following neuro- and gliogenesis, synaptogenesis is initiated, and early brain circuits are formed. In the mammalian brain, a massive wave of synaptogenesis occurs during the BGS (Dobbing and Sands, 1979). Glia are essential for modulating synaptogenesis during critical periods. Once an immature synapse is formed, astrocytic processes envelope the synaptic cleft to create the tripartite synapse which

supports effective neurotransmission during synapse maturation (Chung et al., 2015; Farhy-Tselnicker and Allen, 2018). Further, astrocytes secrete cholesterol to stimulate the production of spontaneous excitatory postsynaptic potentials and strengthen immature synapses (Mauch et al., 2001; Nägler et al., 2001; Allen and Barres, 2009; Chung et al., 2015; Reemst et al., 2016; Bosworth and Allen, 2017). An overabundance of synaptic connections is created during this period (e.g., Bourgeois et al., 1994; Huttenlocher and Dabholkar, 1997; Stiles and Jernigan, 2010) and redundant synapses are phagocytosed during the phase of synaptic pruning to support the maturation of putative circuits (Stiles and Jernigan, 2010; Faust et al., 2021). OLs are involved in synaptogenesis, but the direct mechanisms remain unknown (Allen and Barres, 2009). However, once synapses are formed, OLs are primarily responsible for insulating axons with myelin sheaths (myelination) to support the survival and efficiency of relevant neural connections (Simons and Nave, 2016). Astrocytes, OLs, and microglia are thus integral for proper synaptogenesis in early circuit development.

Central nervous system myelination is one of the longest neurodevelopmental processes and its trajectory is relatively conserved across mammalian species. Corpus callosum is a major white matter tract composed of myelinated axons that connect the cerebral hemispheres and project to the major cortices. The majority of axonal myelination in corpus callosum is complete within the first two years of life; however, myelin sheath development continues through adolescence, when a second myelination peak occurs, following synaptic pruning and connectome refinement (Ganguly et al., 2015). Developmental myelination ceases around 40 years of age, yet myelin ensheathment is continually altered to optimize function of specific neural circuits in adulthood (Yakovlev & Lecours, 1967; Fields, 2015). Similarly, in the rat

brain the initial wave of myelination occurs from postnatal days (PD) 8-24, following the proliferation of OPCs during late gestation and the rodent BGS. A secondary peak in myelin refinement occurs between PD 37-45 and is required to support proper adult cognition (Bergles & Richardson, 2016; Downes & Mullins, 2014; Semple et al., 2013; van Tilborg et al., 2017).

Oligoglia lineage cells are exceptionally sensitive to sex hormone signaling during the BGS. Progesterone is a neurosteroid that is elevated during the final trimester of pregnancy and plays a major role in maintaining placental growth and progressing neurodevelopment when synthesized in the fetal brain (Schumacher et al., 2020). During the third trimester of human pregnancy, the brain parenchyma is expanding exponentially, creating stress on the immature neurovascular system. Maturing OLs, which produce high levels of myelin basic protein (MBP), are at extreme risk for apoptosis during this period as they lack the metabolic support of a mature neurovascular infrastructure. In cases where progesterone exposure during fetal development is limited or completely obliterated, for example, following preterm birth, the fetus may develop periventricular leukomalacia (the neuropathological condition underlying cerebral palsy) characterized by irreversible damage to periventricular white matter due to an arrest of OL maturation (Back et al., 2005). In the infant brain, the ubiquitous OPC pool is not fully formed and remyelination is not possible, leading to lasting damage to white matter following cytotoxic insult during the BGS.

Progesterone is also maintained at an elevated level throughout pregnancy in rodents and plays a major role in coordinating the first peak in white matter myelination during infancy (reviewed in Long et al., 2021). Progesterone receptor

expression is brain region-specific and ligand binding at receptors in the corpus callosum stimulates OPC proliferation and differentiation and increases MBP production during the rodent BGS (Ghoumari et al., 2003, 2005). Rodent oligoglia lineage cells synthesize and metabolize progesterone throughout the BGS (Gago et al., 2001). Notably, progesterone metabolism upregulates the synthesis of myelin basic protein in early-life and adulthood, making it a viable target for therapeutic development for treatment of other myelin-related disorders like Multiple Sclerosis (Schumacher et al., 2014; Palliser et al., 2012; Hussain et al., 2011). Thus, it is evident that neurosteroid signaling is essential for proper white matter development in mammals.

1.4 Effects of alcohol on oligoglia lineage cell development from preclinical and clinical studies

Alcohol exposure during the BGS prevents OPC differentiation, and in extreme cases, reduces oligoglia lineage cell proliferation and causes apoptosis of myelinating OLs. Twenty years following the classification of FAS, Chiappelli and colleagues (1991) were the first to show that fetal alcohol exposure alters the expression of MBP *in vitro* using cell cultures of neural tissue collected from control and alcohol-exposed pups in a rat model of FAS. In 2008, Dalitz and colleagues demonstrated that OL survival was compromised in the fetal sheep brain following alcohol exposure during the BGS. The observed reduction in oligoglia was negatively correlated with the production of cytokines, indicating a link between acute neuroinflammation and white matter degeneration. Five years later, Creeley and colleagues (2013) confirmed that alcohol exposure during the BGS induces apoptosis

of myelin-producing OLs using a nonhuman primate model of FASD. It should be noted that most studies identified such changes in the corpus callosum.

Several controversial studies have sought to determine if alcohol teratogenesis restricts proliferation of OPCs which could contribute to the observed reduction in the OL population. In the studies previously described, there was no evidence of limited OPC proliferation that could have contributed to the reduction in OLs. However, in 2017, Newville and colleagues discovered that vapor inhalation of alcohol during the BGS led to a reduction in the population of OPCs in juvenile male mice. Using cre-recombinase technology, the research team found that OLs differentiating during alcohol exposure (third wave of developmental oligodendrogenesis) were the most vulnerable to apoptosis. Convergent with these findings, Darbinian et al. (2021) reported a reduction in OL number in fetal human tissue extracted from infants with a history of alcohol exposure during the first and second trimesters, prior to the BGS. Interestingly, the authors also noted an upregulation in OPC proliferation that was correlated with cytokine production, likely in response to OL apoptosis during early fetal development. Thus, alcohol-related reductions to OL population may be due in part to apoptosis of OPCs and/or restricted OPC maturation.

Alcohol teratogenesis disrupts the neuroprotective actions of neurosteroids on brain development. In a study conducted by Caldeira et al. (2004), the researchers found that exposing pregnant rat dams to alcohol throughout gestation led to an increase in circulating pregnenolone sulfate in the postnatal infant brain. Alcohol exposure limits cholesterol metabolism which is required for exogenous progesterone synthesis in the placenta and endogenous progesterone development in the fetal brain (Ahluwalia et al., 1992). As aforementioned, proper progesterone signaling is required

for white matter maturation. Thus, alcohol-related reductions to progesterone synthesis and signaling may lead to OL apoptosis, disrupting the trajectory of white matter development in the brain. Importantly, administration of progesterone to infant rats during the BGS upregulated the differentiation of OPCs to OLs to a greater extent in the infant female than male brain (Swamydas et al., 2009), indicating that innate sexual dimorphisms in white matter development occur from parturition and are partially mediated by neurosteroid signaling.

1.5 Summary

Axonal myelination is fundamental for the development of white matter tracts in the mammalian brain. The appropriate maturation of these tracts during gestation and early postnatal life is critical for circuit development. Abnormal neonatal myelination impedes neural signal transduction between cortical and subcortical brain regions, ultimately leading to miscommunication between brain areas and the emergence of maladaptive behaviors in adolescence. Data from over thirty years of research show that fetal alcohol exposure during the mammalian BGS immediately reduces the number of OLs in white matter tracts, predominantly affecting the corpus callosum. If the trajectory of corpus callosum myelination is disrupted by alcohol teratogenesis, is there a way to stimulate myelination in adolescence to improve circuit refinement and quell the emergence of maladaptive behaviors in adulthood? As myelin ensheathment is necessary to promote optimal neuronal signal transduction (Hughes et al., 2018; Gibson et al., 2014), could this capacity be harnessed to improve cognitive and behavioral outcomes in the case of a developmental disorder? In the next chapter, we review the potential for environmental experiences in adolescence to support corpus callosum myelination.

Chapter 2

ADOLESCENT EXPERIENCES INFLUENCE WHITE MATTER MYELINATION: FOCUS ON THE IMPACT OF AEROBIC EXERCISE

2.1 Introduction

Biomolecular processes occurring during perinatal critical periods lay the groundwork for optimizing neural circuitry in adolescence and refining brain circuitry in adulthood. Thus, clinicians hypothesized that a significant reduction to the OL pool may contribute to underdevelopment of white matter in youth diagnosed with FASD. Indeed, a series of studies led by the Wozniak, Kable, Beaulieu, Jacobson, and Mattson teams have generated ample neuroimaging and cognitive assessment data proving that: 1) corpus callosum development is sensitive to the timing and severity of alcohol exposure, and 2) fetal alcohol exposure leads to hypomyelination of white matter tracts in adolescence and this deficit is correlated with impairments to perceptual learning, visuospatial processing, sensory integration, and executive function (Riley et al., 1995; Wozniak et al., 2011). However, the neurobiological underpinnings leading to the alcohol-induced delay in corpus callosum myelination in adolescence are poorly understood. Moreover, the role of sex as a biological factor in white matter refinement in the adolescent FASD-affected brain is underexplored.

2.2 Myelination is one of two critical developmental processes that govern circuit refinement and optimization in adolescence

Sensitive periods begin in late childhood and occur across a longer span of neurodevelopment. Experiences that occur during adolescent sensitive periods alter

brain structure and function in an experience-dependent manner such that input is not required for, but may have a significant effect on, circuit refinement. Unlike critical periods, sensitive periods may be reopened to facilitate the refinement of brain circuitry that occurs as a result of synaptic pruning. However, this residual plasticity is limited (Knudsen, 2004). During this process, redundant synapses are pruned and functionally-relevant connections are conserved and optimized through myelination to support adult cognition (Chechik et al., 1999; Knudsen, 2004). As such, the biological processes surrounding adolescent neurodevelopment necessitate proper glial function.

Following synaptic pruning, myelination thickens around the remaining axons to support axonal survival and fine-tune conduction velocities. Much like neuro- and synaptogenesis, myelination begins first in cortical areas surrounding the central sulcus followed by the progressive myelination of the cortical poles. Myelination is arguably the longest neurodevelopmental process, with the fastest peak of myelination occurring in adolescence; this aligns with and follows the aforementioned process of synaptic pruning (Downes and Mullins, 2014; Kwon et al., 2020). One mature OL can myelinate up to 50 axons in the CNS (Pfeiffer et al., 1993). Thus, abnormal OPC differentiation and OL apoptosis pose a major threat to axon survival and circuit function. Proper onset and progression of these processes are critical for brain circuit optimization. Environmental experiences that alter adolescent myelination are known risk factors for the development of several psychiatric disorders including anxiety, schizophrenia, and depression (Makinodan et al., 2012; Forbes and Gallo, 2017).

All three major types of CNS glia work synergistically to facilitate myelination of axons with terminals forming active synapses. Following synaptic pruning in late childhood and early adolescence, microglia engulf viable OPCs as mediated by

fractalkine signaling. Inadequate reduction of the OPC population prior to the onset of peak myelination in adolescence alters the ratio of OLs to their precursors, favoring mature OLs, and leads to disruptions in typical axonal myelination patterns (i.e., mistargeted ensheathment of neuronal cell bodies and a thinning of the axonal myelin sheath). Thus, precise regulation of myelin content to axonal number is integral for proper myelination, and this balance is directly affected by microglial activity (Almeida et al., 2018; Nemes-Baran et al., 2020). Moreover, microglia are involved in myelin sheath pruning for refinement (Hughes and Appel, 2020) and microglia-OPC interactions mediate precursor cell differentiation to mature OL (Wlodarczyk et al., 2017; Giera et al., 2018). Additionally, in an in vitro study, Pang et al. (2013) showed that astrocytes support long-term survival of OPCs to create the lifelong OPC pool, whereas microglia support OL differentiation and myelination. Astrocyte and OL communication has been shown to play an important role in myelin production. Astrocytes secrete PDGF which supports OPC proliferation and also inhibits immediate precursor cell differentiation (Raff et al., 1988; Richardson et al., 1988; McKinnon et al., 2005; Traiffort et al., 2020). Brain-derived neurotrophic factor is also secreted by astrocytes, and it supports myelination during the BGS (Fletcher et al., 2018; Traiffort et al., 2020). Furthermore, astrocytes control the concentrations of Sonic hedgehog produced which has a direct effect on OPC production throughout the forebrain (Traiffort et al., 2016).

Xin and Chan (2020) elegantly described the forms of plasticity within the oligoglia lineage that are required to accommodate functionally-relevant changes to myelination in adolescence and adulthood. It is evident from **Figure 2** that measuring several features of cytoarchitecture are required to comprehensively investigate

changes to axonal myelination that contribute to the optimization of conduction velocity. Notably, a pool of viable OPCs is maintained throughout the lifespan to support *de novo* myelination. Existing OL myelin remodeling includes altering sheath thickness. However, due to the limited amount of space in the cranium, there exists a limit on sheath thickness, axon diameter, and axon length in the CNS. Chomiak and Hu (2009) were the first to describe the optimal theoretical thickness of the myelin sheath in the forebrain white matter of the adult rat. The g-ratio, a ratio of the diameter of the myelin sheath to the diameter of the fiber, for axons in the adult rat brain was expected to be approximately 0.77. Several studies have confirmed this estimation (Berman et al., 2018; Stikov et al., 2015). The two developmental peaks in mammalian central nervous system myelination – in infancy and then in adolescence – are discretely characterized by robust *de novo* myelination and existing OL myelin remodeling, respectively.

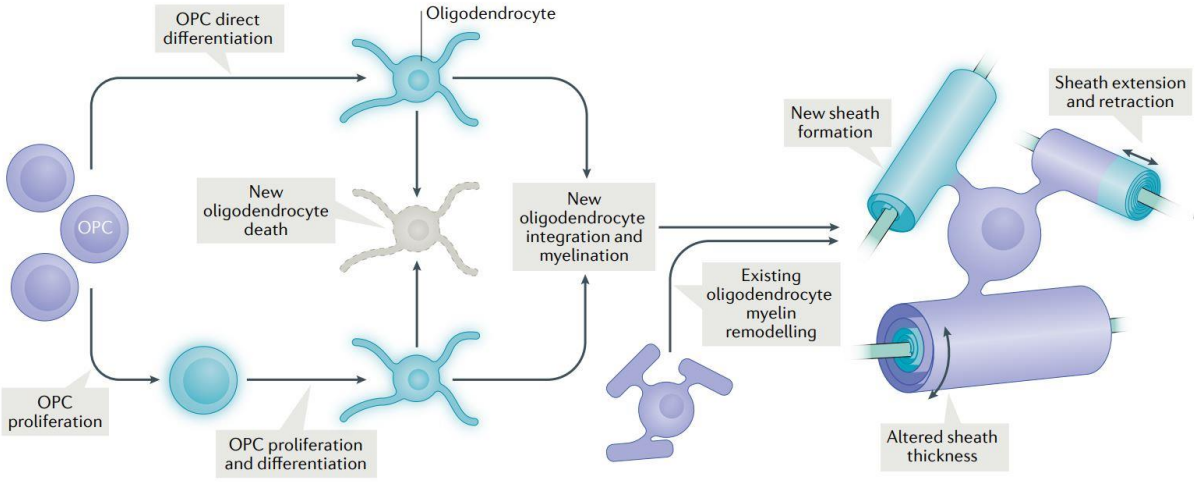

Figure 2. Depiction of some changes made to cytoarchitecture during myelin plasticity and *de novo* myelination. The figure is modified from Xin and Chan (2020).

2.3 Sexual dimorphisms in the onset and progression of white matter myelination in the mammalian brain

Pubertal onset and subsequent hormone cycling contribute to unique patterns of white matter refinement in the male and female adolescent brain. In mammals, corpus callosum maturation begins earlier in the female brain, following pubertal onset. Pubertal changes in myelination can be identified in both male and female youth using noninvasive neuroimaging (Herting et al., 2017). In adult rodents, the lifespan of OLs is about two times shorter in the female brain compared to that in males while the proliferation and differentiation of OPCs is similar in both sexes. As a result, the density of OLs in white matter is 20-40% greater in adult male rats. Importantly, castration of adult male rats increases the turnover rate of OLs in white matter, creating a female phenotype. This suggests that exogenous hormone circulation is in-part responsible for sustaining OL survival (Cerghet et al., 2005). However, local neurosteroid production has also been shown to contribute to OL quantity and activity. OLs synthesize progesterone and express estradiol receptors; these features have been shown to mediate the differentiation and survival of OLs (Marin-Husstege et al., 2004; Takao et al., 2004). Thus, the responsivity of OLs to environmental stimuli in adolescence is sexually dimorphic and altered by hormone signaling in the body and brain.

2.4 FASD delays white matter myelination in the adolescent brain and leads to deficits in spatiotemporal processing, perceptual learning and executive function

Magnetic resonance imaging (MRI) scanning is a powerful tool that can be used to monitor the progress of cerebral white matter myelination noninvasively within-subject. Several clinical studies using neuroimaging provide convergent evidence that CNS myelination is perturbed by prenatal alcohol exposure. Notably,

alcohol exposure during the BGS has been shown to delay the trajectory of white matter myelination in adolescence along several tracts including the corpus callosum, resulting in smaller total brain volumes of youth affected by FASD (Treit et al., 2013). Such impairments have been linked to deficits in spatiotemporal processing, perceptual learning and executive function in FASD-affected youth and young adults (Wozniak et al., 2011; Riley et al., 2016, Kravitz et al., 2011; Ware et al., 2021; Alger et al., 2021). The splenium subregion of corpus callosum was discovered to be of greatest vulnerability to alcohol teratogenesis and disrupted development of this region is correlated with deficits to visuospatial processing, sensory integration, and language and math capacities (Knyazeva, 2013; Lebel et al., 2010).

The neurobiological mechanisms contributing to the delay in corpus callosum myelination in adolescence are largely unknown. Several studies using rodent models of FASD have been imperative for identifying lasting changes to oligoglia lineage cell proliferation and differentiation which contribute to the observed hypomyelination. Newville et al. (2017) demonstrated using a mouse model of FASD that alcohol exposure during the BGS leads to a transient depletion of OLs in juvenile and adolescent male corpus callosum which is recovered in young adulthood. While OL number was only transiently reduced, the researchers did observe a lasting reduction to MBP production which was correlated with atypical ensheathment of axons in adulthood (Newville et al., 2017, 2021).

The cycling of gonadal hormones following pubertal onset regulates white matter myelination progression. Uban et al. (2017) found that typical associations between myelin maturation, measured using diffusion weighted neuroimaging, and neurosteroid levels are altered by prenatal alcohol exposure in FASD affected youth.

In typically developing adolescent boys, there is a positive association between rising testosterone levels and increasing myelination in the cingulum, optic radiation, inferior fronto-occipital fasciculus, and superior fronto-occipital fasciculus which did not exist in their FASD-affected analogues. Conversely, in typically-developing adolescent girls there were no associations discovered between testosterone or DHEA concentration with myelination of white matter tracts. In FASD-affected adolescent girls, several positive correlations between white matter development and testosterone or DHEA levels were apparent in the superior longitudinal fasciculus. Further, higher levels of progesterone were correlated with lower myelination of the fornix in FASD-affected girls. Few alcohol-related alterations to basal hormone levels were recorded in either sex, aside from an increase in basal testosterone levels in males with a history of alcohol exposure. Thus, neurosteroid signaling plays a role in circuit refinement in adolescence as well as in the initial stages of myelination during infancy.

2.5 Aerobic exercise stimulates white matter myelination in the adolescent mammalian brain: potential for mitigating myelin pathology

Prenatal alcohol exposure is one example of a negative effector of the trajectory of myelination. Aerobic exercise is a positive effector of CNS myelination as increased growth factor production stimulates the maturation of OLs (reviewed in Tomlinson et al., 2016, 2018; Xiong et al., 2018; Talukdar et al., 2018). One study demonstrated that two weeks of voluntary aerobic exercise was sufficient to increase OPC proliferation and differentiation and MBP production in mouse motor cortex in adulthood (Zheng et al., 2019). Specifically, voluntary wheel running led to an increase in OPC and OL number, increased MBP production, and reduced the g-ratio. Altogether, these changes demonstrate that myelination was increased in the motor

cortex following exercise intervention. Notably, the same results were not observed in the corpus callosum and all mice used in this study were male (Zheng et al., 2019).

The Klintsova lab and others have previously demonstrated that adolescent exercise intervention via free access to running wheels stimulates hippocampal and cortical neuroplasticity in a rat model of FASD (Schreiber et al., 2013; Hamilton et al., 2014, 2015; Boschen et al., 2017). However, research regarding the influence of aerobic activity on white matter tract myelination and oligodendrocyte morphology in rodent models of FASD is limited. Few clinical studies have implemented physical activity as an intervention for older children and youth diagnosed with FASD. Pritchard Orr and colleagues (2017) assessed executive function capacity following an 8-week exercise program wherein FASD-affected and control participants were asked to participate in two 1.5-hour sessions of aerobic exercise per week. This research team discovered that executive function capacity was improved in FASD-affected participants immediately following and 3 weeks post-exercise intervention, suggesting the efficacy of this intervention for affected youth. Moreover, exercise intervention is free, accessible, and easily implemented into existing youth programs which highlights the importance of this research to refine and implement this strategy to improve cognitive outcomes for youth and young adults with FASD.

Chapter 3

HYPOTHESES FOR CURRENT STUDY

3.1 Gaps in our knowledge

While the benefits of aerobic exercise for proper neurodevelopment in adolescence are clear, the mechanism by which physical activity promotes central nervous system myelination remains unknown. Further, it is if exercise will stimulate myelination in a neural environment with a paucity of viable OLs and with restricted MBP production resulting from alcohol teratogenesis. To address these gaps in our knowledge, the aims of this dissertation have been designed to investigate the impact of an adolescent exercise intervention on corpus callosum myelination in a rodent model of FASD. Specifically, high-resolution in vivo MRI scanning pre- and post-intervention was employed to assess exercise-specific alterations to corpus callosum myelination noninvasively. To validate the neuroimaging findings and to assess nuanced alterations to the cytoarchitecture within the OL matrix, two separate cohorts of rats were generated to provide tissue samples for the quantification of oligoglia lineage cells and MBP density pre- and post-intervention. Of note, cell populations were estimated using unbiased stereology (MBF Bioscience software), a rigorous and established technique that allows experimenters to unbiasedly quantify features of tissue architecture systematically through a 3D region of interest (West et al., 1991; Glasser et al., 2000).

This dissertation included two additional innovative goals: 1) to be the first to examine the impact of sex as a biological factor in central nervous system myelination

in adolescence in alcohol-exposed and control rats, and 2) to evaluate the lasting benefits of an adolescent exercise intervention on central nervous system myelination in adulthood. To our knowledge a comprehensive investigation of how early developmental alcohol exposure affects the trajectory of myelination in the female brain is lacking. Newville et al. (2021) demonstrated that myelin ensheathment of axons continues to be altered in adult male mice following alcohol exposure during the BGS. Thus, it was imperative that we extended the experimental timeline to include an investigation of the long-term impact of adolescent exercise intervention on axonal myelination and functional connectivity between brain regions involved in visuospatial processing. Results from these experiments yielded valuable data that elucidated: 1) whether an alcohol-induced delay in adolescent myelination of white matter tracts contributed to the malfunction of salient neural pathways involved in visuospatial processing, and 2) how adolescent aerobic exercise affected circuit function in adulthood using a rodent model of FASD. Estrus phase was controlled for in all analyses as hormone signaling in the central and peripheral nervous systems is a known effector of myelin ensheathment.

3.2 Specific Aims

1 in 20 infants born annually in the United States has been exposed to alcohol prenatally, sufficient to produce long-term physical and/or cognitive impairments which are clinically categorized under the umbrella term, Fetal Alcohol Spectrum Disorders (FASD; May et al., 2018). Higher-order cognitive processing requires exchange of information between cerebral hemispheres (e.g., visuospatial processing, verbal learning, memory, processing of abstract concepts) that is critically dependent on the corpus callosum, the largest white matter tract connecting the hemispheres.

Structural abnormalities in corpus callosum are well-described in children and adolescents with prenatal alcohol exposure and are identified with MRI scanning (Adam et al., 2016; Qiang et al., 2002).

In animal models of FASD, persistent alterations to corpus callosum are observed using MRI and histological tissue analysis. Newville et al. (2017) demonstrated that neonatal alcohol exposure prevents the maturation of OLs in infancy which perturbs corpus callosum myelination in adolescent male mice using *ex vivo* MRI scanning and tissue analysis. This *ex vivo* approach, however, prevented the analysis of myelination progression across two or more time points in adolescence/adulthood. We designed an *in vivo* MRI scanning protocol that examined alterations to corpus callosum myelination across adolescence. Moreover, the contribution of sex as a biological factor in alcohol-related myelin pathology is unexplored and was a focus of this research.

Aerobic exercise during adolescence increases myelination of corpus callosum tracts in humans (Talukdar et al., 2018; Xiong et al., 2018), stimulates the differentiation of OPCs in corpus callosum in rats (Tomlinson et al., 2018) and could be used as a powerful intervention strategy. In rodent models of FASD, adolescent exercise intervention via free access to running wheels stimulated hippocampal and cortical plasticity (Helfer et al., 2009; Hamilton et al., 2015). The impact of an adolescent exercise intervention on white matter maturation following neonatal alcohol exposure had not been studied. Thus, in the following two aims, we used complimentary *in vivo* neuroimaging and *ex vivo* tissue analysis techniques to investigate the effect of an adolescent aerobic exercise intervention on restoring the alcohol-induced reduction to corpus callosum size and myelination in male and female

rats. Interpretation of *in vivo* neuroimaging results were validated using *ex vivo* histological analysis of brain tissue. Finally, the impact of alcohol exposure during the BGS and/or adolescent exercise intervention on adult brain resting-state functional connectivity was assessed to better understand how structural alterations to white matter tracts affect brain system function.

Aim 1: Determined the effect of an adolescent exercise intervention on corpus callosum volume, myelination, and connectivity in vivo across adolescence and in adulthood using a rodent model of FASD. We hypothesized that aerobic adolescent exercise intervention would mitigate the impact of alcohol exposure during the BGS on corpus callosum myelination in adolescence and enhance functional connectivity of salient visuospatial pathways in adulthood. To test this, we acquired *in vivo* MRI scans (T1-anatomical and Diffusion Tensor Imaging) of alcohol exposed and control rats of both sexes at two adolescent time points – pre- and post-intervention, i.e. around postnatal days (PD) 30 and 42 (see Chapter 4). Next, a series of cross-sectional multimodal scans was acquired in a second cohort of rats to determine how adolescent exercise intervention impacted functional connectivity between cortical regions involved in visuospatial processing in adult rats in the model of FASD (see Chapter 6). Collectively, these data revealed sex-specific changes to adolescent corpus callosum volume and myelination, as well as changes to the adult connectome in vivo resulting from adolescent intervention in a rat model of FASD.

Aim 2: Investigated the role of mature OL number, MBP density and myelin sheath thickness on alcohol and exercise-related changes to corpus callosum

myelination and volume *ex vivo* across adolescence (see Chapter 5). We hypothesized that neonatal alcohol exposure would reduce OL number, MBP density, and myelin sheath thickness in corpus callosum during adolescence and that these cellular alterations would be reversed by adolescent exercise intervention. To test this, we quantified the number of mature OLs and measured the densitometry of MBP in sub-regions of corpus callosum on PDs 30 and 45 (following scanning) in alcohol-exposed and control animals of both sexes using unbiased stereological microscopy of immunocytochemically-labelled tissue. Further, lasting alterations to myelin sheath thickness were evaluated from electron micrographs of the cross-sections of axons in corpus callosum on PD 70 (see Chapter 6). Collectively, these data elucidated a biomolecular mechanism for potential exercise-induced myelination in a rodent model of FASD.

The goal of the dissertation was to determine (i) how alcohol exposure during the BGS disrupts white matter tract structure and function in adolescence and adulthood, and to (ii) investigate the mitigating impact of an adolescent exercise invention on corpus callosum and global brain connectivity.

3.3 Summary

This dissertation sought to enhance our understanding of the effects of aerobic exercise on myelination, forebrain connectivity and putative function in a rodent model of FASD while exploring a possible mechanistic molecular linkage to mature OL number, MBP density, and myelin sheath thickness. Early alcohol exposure can lead to life-long deficiencies in myelin and OL development. As a result, formation of neurocircuitry and the efficient conduction of neuronal signals are affected due to

impaired survival and maturation of OLs. Although previous findings suggest that alcohol-induced disruptions to developmental myelination may be mitigated by adolescent exercise intervention, there are several important limitations to the literature. The study by Newville et al. (2017) laid the foundation for evaluating the impact of alcohol exposure during the BGS on corpus callosum myelination and OL maturation and density in mice. However, the study lacked an evaluation of alcohol-induced alterations to oligoglia and myelination in both sexes and an investigation on how inefficient circuit function (due to disrupted myelination) affects communication between brain structures at the system level. This study was innovative because it employed three unique approaches – longitudinal structural neuroimaging in adolescence, cross-sectional functional neuroimaging in adulthood, and multiple types of histopathological tissue analysis – to investigate the impact of alcohol exposure and adolescent exercise intervention on brain structure and function from the ultrastructural level to gross connectivity.

Chapter 4

REDUCED AND DELAYED MYELINATION AND VOLUME OF CORPUS CALLOSUM IN AN ANIMAL MODEL OF FETAL ALCOHOL SPECTRUM DISORDERS PARTIALLY BENEFIT FROM VOLUNTARY EXERCISE

4.1 Introduction

Corpus callosum myelination supports efficient neural signaling between brain hemispheres as well as communication between subcortical and cortical subregions. Myelination of this tract is fundamental for supporting brain growth and circuit refinement during childhood and adolescence. OPC proliferation and differentiation lead to the induction of myelination in the mammalian brain and first intensifies during the BGS, a period of rapid brain growth during the third trimester of pregnancy (Dobbing & Sands, 1979; Geeraert et al., 2018; Jakovcevski et al., 2099) during which axon length increases dramatically in a process known as fiber scaling (Yang et al., 2021). During early childhood (first two years of life), axonal myelination of specific brain circuits is directed by putative circuit functions, some of which include nursing, vision, and sound localization (Downes & Mullins, 2014). The rate of myelin development in the brain varies by age and tract as some white matter tracts mature earlier than others. The brain reaches adult size by the end of early childhood (around six years of age), yet regions continue to mature as the ratio of gray to white matter volumes is altered by axonal pruning for homeostatic circuit refinement in late childhood and early adolescence (Pujol et al., 1993; Brenhouse et al., 2011; Williamson et al., 2018). OPCs have been implicated in synaptic pruning coordination

in gray matter regions (Nemes-Baran et al., 2020; Buchanan et al., 2021; Faust et al., 2021). Following synaptic pruning of redundant synapses, the remaining neural circuits become more heavily myelinated to tune the physiological dynamics of salient connections. To achieve this, the organization and thickness of the myelin sheath may be highly variable along a single axon. Functionally relevant adaptations to the myelin sheath occur frequently in the adolescent and adult brain to optimize signal transduction (Salami et al., 2003; Bechler et al., 2015). As a result, early-life and adolescent experiences play a key role in determining the trajectory of tract myelination during circuit refinement. Tomlinson and colleagues (Tomlinson et al., 2016) describe several known effectors of postnatal myelination and their neurobiological substrates. Aerobic exercise is a positive effector of myelination as it stimulates OPC proliferation and myelin basic protein (MBP) production which leads to thickening of the myelin sheath. Contrarily, teratogenic exposure or early-life adversity are characterized as negative effectors of myelination as they prevent the differentiation of OPCs and, in extreme cases, cause apoptosis of myelinating OLs (reviewed in: Milbocker et al. 2021). Failure to properly refine neural circuitry and optimize neuronal communication leads to the emergence of maladaptive behaviors (Green et al., 2010; Ganguly et al., 2015; Paolicelli et al., 2017) and disrupts memory consolidation (Steadman et al., 2020).

A wealth of evidence provided by preclinical and clinical studies indicates that prenatal alcohol exposure (AE) delays the development and myelination of white matter tracts in the adolescent brain. Prenatal AE may result in Fetal Alcohol Spectrum Disorders (FASD), a group of related developmental disorders with a high prevalence around the globe, affecting up to 1 in 20 live births in the U.S. annually

(May et al., 2015; Gosdin et al., 2022). Specific diagnoses within the FASD continuum vary depending on the timing and severity of AE, which give rise to differential symptomologies and physical dysmorphologies (Hoyme et al., 2016). A heavy early-term exposure often results in distinct facial dysmorphologies, delayed growth, and severe cognitive delays in a readily recognizable case of Fetal Alcohol Syndrome. Late-term exposures, however, give rise to executive function and learning deficits without hallmark physical dysmorphologies due to their overlap with the BGS, and are thus more difficult to diagnose. Misdiagnosis with other developmental disorders such as Autism Spectrum Disorder or Attention Deficit Hyperactivity Disorder is common. Therefore, late-term exposure FASDs require the special attention of researchers.

While several behavioral and pharmaceutical therapies exist to support infant and child neurodevelopment and help to resolve some behavioral issues resulting from prenatal AE, fewer therapies have been developed to support FASD-affected youth. To create effective, targeted interventions, it is imperative to identify the underlying neuropathology associated with observed disruptions to behavior. It is well-documented that FASD-affected adolescents exhibit reductions to the growth and myelination of the corpus callosum and this structural anomaly is correlated with impaired perceptual reasoning and executive function (Gautam et al., 2014; Ghazi et al., 2019; Riley et al., 2016; Wozniak et al., 2011). Furthermore, evidence from preclinical studies using animal models of FASD demonstrates that alcohol teratogenicity reduces corpus callosum volume with the highest impact in males (Zimmerberg et al., 1989), and prevents OPC proliferation, induces precocial differentiation of OPCs to mature OLs, and potentiates the apoptosis of myelinating

OLs during infancy and adolescence. Moreover, early-life adversity, in the form of stress or teratogenic exposure, has long-term consequences on glia proliferation and function, ultimately impacting the onset and progression of glia-driven processes such as synaptic pruning and myelination in adolescence (Olney et al., 2006; Darbinian et al., 2021). Indeed, lasting reductions to MBP production are observed in the corpus callosum of adult rats in models of FASD, suggesting that the function of the circuits involved in visuospatial processing, learning, memory, and abstract conceptualization is diminished across the lifespan (Newville et al., 2017, 2021).

To our knowledge, few if any studies have investigated the potential beneficial effect of a behavioral intervention on supporting adolescent myelination in the AE corpus callosum. Behavioral interventions are effective neuroplastic inducers and are more accessible and affordable to implement when compared to pharmaceutical therapies. Previous work has demonstrated that voluntary exercise in adolescence or adulthood restores neuroplasticity in rodent models of FASD (Boschen et al., 2017; Hamilton et al., 2012; Klintsova et al., 2002, 2013; Milbocker et al., 2020; Gursky et al., 2020). Alterations to cerebral tissue growth, connectivity, and physiology are supported by several neurobiological mechanisms that are stimulated by increased aerobic activity. These include increased cerebral perfusion and metabolism via angiogenesis, upregulations to growth factor production and anti-apoptotics (i.e. antioxidants), as well as alterations to neurotransmitter and hormone signaling (reviewed in: Klintsova et al., 2013). As a result, deficits in executive function, anxiety and depressive-like behaviors, coordination and balance, fine motor skills, and spatial memory are partially mitigated (Klintsova et al., 2013). Thus, the benefits of aerobic exercise on brain plasticity, neurogenesis and synaptic remodeling are well-

established, yet evidence supporting changes to white matter remain underinvestigated (Fields et al., 2008). The few studies investigating the impact of aerobic exercise on white matter structure have revealed that exercise has a positive impact on the maturation of multiple white matter tracts, as evidenced by increases in tract volume, myelin sheath thickness and stimulation of oligoglia lineage cell proliferation and differentiation (Tomlinson et al., 2016; Chaddock-Heyman et al., 2018; Ruotsalainen et al., 2020). Importantly, these structural changes benefit executive function capacity and motor learning in typically-developing animals (Ruotsalainen et al., 2020; Lehmann et al., 2020; McKenzie et al., 2014).

Diffusion tensor imaging (DTI) scanning is a powerful tool that allows for noninvasive longitudinal assessment of structural alterations in myelination. Specifically, DTI scanning measures differences in water movement, or diffusivity, in various biological tissues. The direction of water movement through different tissues is quantified into its 3-dimensional vector components—primary ($\lambda 1$), secondary ($\lambda 2$), and tertiary ($\lambda 3$)—and these values are used to calculate fractional anisotropy (FA), axial diffusivity (AD), and radial diffusivity (RD). FA is calculated on a scale from 0 to 1 and describes the degree to which water molecule movement is restricted by surrounding tissue cytoarchitecture. As expected, FA is lowest when measured in fluid-filled ventricles and highest within tightly-packed fiber bundles of the neural white matter. Therefore, it is not surprising that in the typically-developing brain FA values increase across neurodevelopment (Deoni et al., 2011; Treit et al., 2013). AD and RD values describe differences in the directionality of water diffusivity which can be interpreted as alterations to tissue microstructure. AD is the primary direction of water movement and tracks the movement of water molecules along fiber bundles in

white matter tracts. Changes to AD (λ1) occur in cases of altered axonal integrity (i.e. during early development or in cases of axon degeneration). RD represents the average water movement that is perpendicular to the fiber bundles ((λ2 + λ3)/2). There is an inverse correlation between RD and myelination: RD values decrease across adolescence in the typically-developing brain as water diffusivity is restricted by de novo myelination and thickening of myelin sheaths (Deoni et al., 2011). FA values represent the foil of the sum of AD and RD.

To investigate the effect of voluntary exercise on myelination in a rodent model of FASD, we conducted a longitudinal experiment using noninvasive neuroimaging pre- and post-intervention exposure. Combining the knowledge of developmental myelination with our understanding of FASD-associated neuropathology, we hypothesized that exposure to a positive effector of myelination targeting the peak of adolescent myelination might mitigate the impact of alcohol exposure during the BGS on the trajectory of corpus callosum growth and myelination in adolescence. To test our hypothesis, we have collected two sets of DTI scans (pre- and post- exercise intervention, within-subject) in AE and control rats with and without intervention exposure to investigate changes to myelination by comparing values of FA, AD, and RD in corpus callosum. Importantly, sex was included as a biological factor in this study. This is particularly significant as we are the first to investigate this in the female FASD brain and evidence indicates that the typical progression of myelination across the lifespan is sexually-dimorphic (Berman et al., 2018; Shaw et al., 2020).

4.2 Methods

4.2.1 Rat model of Fetal Alcohol Spectrum Disorders

All rats were acquired, cared for, and methodology was in accordance with the guidelines provided by the University of Delaware's Institutional Animal Care and Use Committee, the NIH Guide for the Care and Use of Laboratory Animals, and comply with the ARRIVE guidelines (https:// arriveguidelines.org), and were approved by the University of Delaware's Institutional Animal Care and Use Committee (animal use protocol #1134).

Ten timed-pregnant Long-Evans dams were obtained from Charles River Laboratories (Boston, MA) and litters were culled to ten pups each three days after birth to counterbalance for sex and postnatal treatment group. On the same day, rat pup paws were tattooed using India Ink for identification of each pup in the litter across the duration of the experiment. To investigate the teratogenic impact of binge-like AE during the BGS on adolescent neurodevelopment, half of each litter was exposed to alcohol across six days within the first two postnatal weeks of life as described in greater detail in Milbocker and Klintsova (Milbocker et al., 2020) and Gursky and Klintsova (Gursky et al., 2017). A 2020 report discovered that 4% of pregnant women across the United States participate in binge drinking (4 or more drinks during one occasion; Umer et al., 2020). Moreover, England et al. (2020) analyzed national survey data on drug use and health from 2015 to 2018 and found that 1.4% of pregnant women reported binge drinking during the third trimester of pregnancy, highlighting the relevance of studying late-term alcohol exposure using the established rat model of FASD described below (England et al., 2020).

Briefly, on postnatal days (PD) 4-9, AE pups received a 5.25 g/kg/day (11.9% v/v, divided into two administrations two hours apart) of alcohol in milk substitute via intragastric intubation. An additional milk-only intubation was given to AE pups two hours after the second alcohol dose to compensate for reduced suckling during intoxication. The remaining half of each litter, procedural control pups, was sham-intubated (SI) without liquid administration during the same postnatal period. A 60 μL blood sample was collected from the tail vein of each pup 90 min after the second dose on the first day of treatment (PD 4) to measure blood alcohol concentration (BAC; Bonthius et al., 1990). Blood samples collected from AE rats were analyzed using an Analox GL5 Alcohol Analyzer (Analox Instruments, Boston, MA) and resulted in an average daily BAC of 321 ± 22.8 mg/ dL, indicating high AE during the postnatal treatment period. Measuring peak BAC during specific periods of development not only confirms receipt of alcohol but is used as a significant indicator of brain damage (Dobbing & Sands, 1979; Bonthius et al., 1990, 1988). The moderate to high BACs resulting from our exposure procedure model the BAC of a pregnant woman during a one-occasion binge-like event, and thus, increase translatability. Patten et al. (2014) provide a complete review of animal models of FASD and their relation to the clinical setting (Patten et al., 2014). All animals were weighed daily during the treatment period. Following the last dose on PD 9, all pups were earpunched according to litter number for increased ease of identification during the remainder of the longitudinal study. On PD23, all rats were weaned into same-sex cages of 2–3.

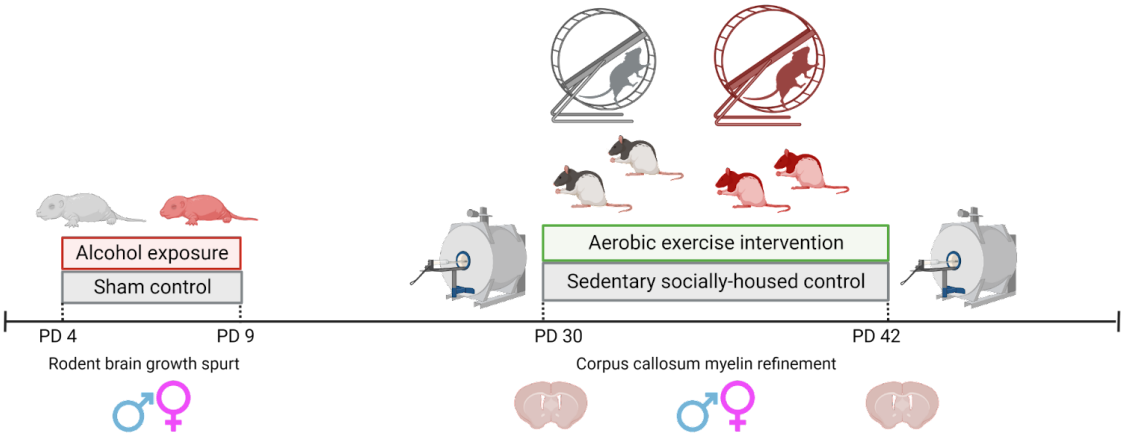

Figure 3. Experimental timeline depicting postnatal treatment (AE/SI) and intervention exposures (WR/SH). Scans were collected twice from each rat pre- and post-intervention. Graphic representation of the timeline is created with BioRender.com and published in Milbocker et al. (2022).

4.2.2 Voluntary adolescent exercise intervention

On PD30, adolescent rats were randomly re-housed into either modified wheel running cages (WR) or into socially-housed (SH) cages as sedentary controls until PD 42–45. Previous studies from our lab and others have demonstrated that a 12-day duration of voluntary exercise is optimal to produce significant structural alterations in the brain and that WR in adolescence and adulthood results in neuroplastic remodeling in the cortex, hippocampus, and cerebellum in rat models of FASD (Lehmann et al., 2020; Helfer et al., 2009). Social housing was maintained for all rats during the entirety of the study to prevent the confounding effects of social isolation on OL maturation and myelination (Liu et al., 2012; Makinodan et al., 2012). The number of wheel rotations per cage was recorded daily at 09:00 to compare the total running distance between cages containing AE and SI rats of each sex. The circumference of the wheels was used to convert the number of rotations to kilometers run. All rats were

weighed at the beginning, middle, and end of the intervention period in order to assess correlations between weight and brain volume. Based on a priori power analyses, a total of 100 pups from 10 litters were generated for this study resulting in an expected sample size of 12 animals per sex/postnatal treatment group/intervention group (8 total groups: F/SI/SH, F/AE/SH, M/SI/SH, M/AE/SH, F/SI/WR, F/AE/WR, M/SI/WR, M/AE/WR).

4.2.3 Longitudinal magnetic resonance imaging (MRI) scanning

A novel *in vivo* neuroimaging protocol was developed to scan the rats once they reached adolescence, pre- and post-intervention in a within-subjects longitudinal study design. Pre-intervention scans were collected from PD27–30 while post-intervention scans were collected from PD42–45 as was feasible given scanner availability. Brain tissue was fixed and collected from all rats between PD45–47 following the final scanning session. An experimental timeline is depicted in **Fig. 3.**

A series of multimodal scans was captured using a 9.4 Tesla Bruker BioSpec scanner (Bruker; Bellirica, MA) with Paravision 6.2 housed in the Center for Biomedical and Brain Imaging at the University of Delaware to examine structural alterations to corpus callosum across adolescence. A 4-channel rat brain surface array coil was used to collect the images. First, a high resolution 2D T2 weighted RARE anatomical scan was collected for each rat with the following parameters: TR/TE = 8500/30 ms; matrix size = 120 × 120; field of view = 30 × 30 mm; and voxel size = 250 × 250 × 500 μm. Sixty axial slices were collected with a slice gap of 0 mm and an excitation angle of 90 degrees. Next, a high resolution spin echo 2D DTI scan was acquired for each rat with the following parameters: TR/TE = 3025/19 ms; four EPI segments; bandwidth of 300 kHz; and 30 non-collinear gradient directions with a

single b-value shell at 1000 s/mm^2 and five images with a b-value of 0 s/mm^2 (referred to as B0). Comparable to the acquired T1 scans, sixty axial slices were collected for DTI with a slice gap of 0mm; matrix size = 120 × 120; field of view = 30 × 30mm; and voxel size = 250 × 250 × 500μm. A single shot PRESS shimming protocol was applied to increase field homogeneity around the rodent brain and collect a baseline B0 map prior to scan acquisition. Total scan time did not exceed one hour per session for each animal. During scanning, rats were anesthetized under 1–3% isoflurane in oxygen, maintained a body temperature of 34–36 degrees Celsius on a heated water bed, and were monitored for respiratory rates between 40 and 60 beats/min. Representative scans are depicted in **Fig. 4**. DTI data are available via request to the authors contingent upon potential co-authorship agreement and the creation of a formal data sharing agreement plan.

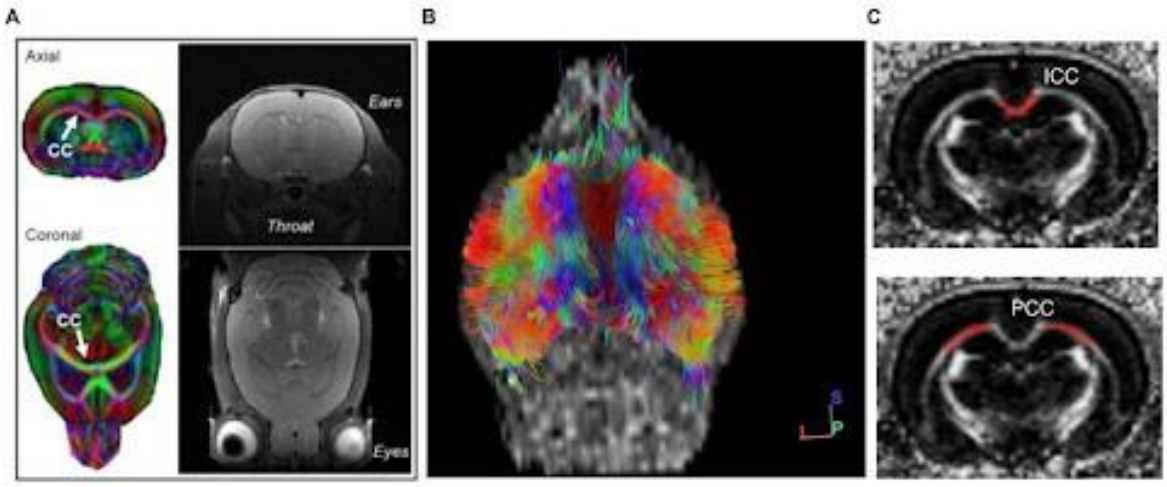

Figure 4. (A) Representative images from diffusion tensor imaging (left) and T2-weighted anatomical (right) scanning from an anesthetized male rat on PD30. Scans were analyzed in axial orientation for this study and an arrow indicates the location of the CC in the DTI scans. Reconstruction of DTI images yields data of water diffusivity within 3D tracts as depicted in (B, ventral view). Red-blue-green color schema represents fiber orientation with red indicating fibers traveling right to left, blue indicating fibers traveling dorsal to ventral, and green indicating fibers traveling in the rostrocaudal direction. (C) Regions of interest (interhemispheric commissural fibers, ICC and cortically-projecting fibers, PCC) contoured in red on grayscale diffusion tensor imaging scans to collect fractional anisotropy, axial and radial diffusivity data. Published in Milbocker et al. (2022).

4.2.4 Processing and statistical analysis

Once collected, all DTI scans were converted to NIFTI files and uploaded into MedInria v.1.9.2 (2010 INRIA—Asclepios Research Team) for scan analysis. Two experimenters blind to animal condition outlined two distinct sub-regions of the corpus callosum for analysis: the interhemispheric sub-region that is predominantly composed of commissural fibers (ICC) and the cortically-projecting lateral sub-regions (PCC). PCC was further subdivided into the left and right PCC to account for any lateralization in the neuroimaging data. Regions of interest were hand drawn due

to a lack of early and late adolescent rat MRI atlases and to examine alcohol-related neuropathology appropriately. Reliable scanning data was collected for 7–12 rats per sex/postnatal treatment/intervention group. For three regions (forebrain, ICC, and left/ right PCC), the following data were extracted from the two sets of scans and analyzed using SPSS statistical software (IBM): volume, FA, and AD/RD. Mixed repeated measures ANOVAs were employed to investigate alterations to the rate of change to weight and brain structure within-animal across adolescence while cross-sectional two and three-way ANOVAs were conducted at each time point to explore significant main effects of sex, treatment, and intervention on corpus callosum myelination on PD 30 or 42 only. Regression-based mediation analysis was performed to investigate relationships between brain-body growth. Collectively, these analyses aid in the evaluation of alcohol- or intervention-specific alterations to the trajectory of myelination in the adolescent brain.

4.3 Results

4.3.1 Alcohol exposure during the BGS prevents normative weight gain during neonatal and adolescent development

Analysis using a mixed repeated measures ANOVA (within-subject variable: weight/day; between- subject variables: sex, postnatal treatment group; covariate: litter number) shows that rat pups from each postnatal treatment group gained weight during the treatment period (PD 4-9; **Fig. 5A**). However, there is a significant interaction between treatment day and treatment group ($F_{5, 380} = 31.6$, $p < .001$, $\eta p^2 = 0.30$). Further investigation using two-way ANOVAs (sex × postnatal treatment group; covariate: litter number) confirm that AE pups weighed consistently less than SI control pups on PD 5-9 ($p < .001$). The negative impact of AE on pups' weight

during the brain growth spurt was dampened by delivering an additional daily milk-only intubation to AE pups. There were no main or interactive effects of sex on weight gain during the postnatal treatment period. Additionally, we did not discover any effect of litter on neonatal weight gain despite culling and cross-fostering procedures. This is in line with other preclinical studies which have determined that such procedures have little to no impact on development or behavior (Denenberg et al., 1963; Phillips et al., 2019; Roth et al., 2009).

Analysis using a mixed repeated measures ANOVA (within-subject variable: weight/day; between-subject variables: postnatal treatment and intervention groups; covariate: sex) shows that juvenile rats from all treatment and intervention groups gained weight across adolescence (PD 30, 36 and 42; **Fig. 5B, C**). We have found that on average female rats ran more than male rats during the intervention period ($F_{1, 17}$ = 40.73, $p < .001$, $\eta p^2 = 0.71$; see Fig. 5D). In line with this finding, there exist significant interactions between intervention day and sex or intervention group on weight gain, longitudinally ($F_{2, 148} = 51.4$, $p < .001$, $\eta p^2 = 0.41$; $F_{2, 148} = 4.5$, $p = .01$, $\eta p^2 = 0.06$, respectively). Collectively, these results show that female rats gained less weight across adolescence when com- pared to their male counterparts, as expected, and that access to a running wheel further prevented weight gain and disproportionately affected females, potentially due to the fact that female rats ran the most. While we did not directly measure caloric intake across the intervention period, it is well-established that male rats weigh more than their female counterparts during adolescence (Pahl et al., 1969).

Despite demonstrating that AE and SI control rats have similar rates of body growth across adolescence, two and three-way ANOVA analyses (postnatal treatment

group × sex; postnatal treatment group × intervention group with sex as a covariate, respectively) conducted at each time point indicate that AE rats weighed consistently less than SI control rats on PD 30, 36, and 42 ($p < .001$). To further probe these relationships, an analysis of the percentage of weight gained across adolescence was conducted using a three-way ANOVA (postnatal treatment group × intervention group with sex as a covariate). This analysis shows that there were main effects of sex ($F_{1,74} = 17.8$, $p < .001$, $\eta p^2 = 0.20$) and intervention exposure ($F_{1,74} = 6.7$, $p = .012$, $\eta p^2 = 0.10$) on the percentage of weight gain across adolescence wherein female WR rats had the smallest increase in weight gain between PD30 and 42. Finally, we find that pup weight on PD 9 is positively correlated with weight on PD30 ($r^2 = 0.71$) in both AE and SI control rats. Together, these findings indicate that AE during the BGS prevents age-appropriate weight gain in the neonatal period, ultimately affecting the growth trajectory of these rodents in adolescence. Finally, exercise intervention had no main or interactive effects on weight when analyzed cross-sectionally at each adolescent time point.

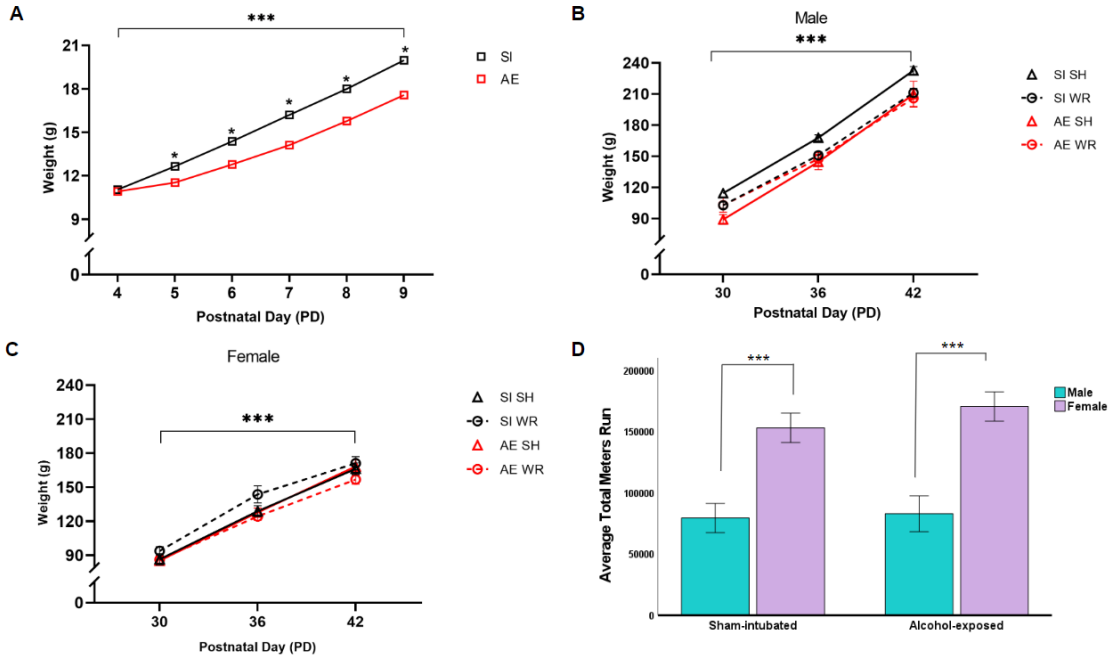

Figure 5. (A) Daily recorded mean body weight of AE (red) and SI (black) rat pups during the postnatal treatment period which targets the BGS. Mixed repeated measures ANOVA indicates that all rats gained weight across the treatment period (***$p < .01$). Cross-sectional analysis shows that AE pups weighed less than SI pups from PD 5-9 during the treatment period (*$p < .01$). Data were collapsed across sex as there was no main effect or interaction of weight gain with sex. (B–C) Recorded mean body weight of male and female AE and SI rats from both intervention groups (WR and SH control) before, during, and after the intervention period. Mixed repeated measures ANOVAs indicate that all rats gained weight across the adolescent intervention period (***$p < .001$). There were significant main effects of intervention exposure and sex wherein intervention-exposed females gained the least amount of weight across the adolescent intervention period. Cross-sectional analysis shows that AE pups weighed less than SI rats at each time point during the intervention period. D) Average total kilometers run recorded from WR cages containing same-sex AE or SI rats. Female rats ran the most during the adolescent intervention period. There was no main effect of postnatal treatment group on distance run, suggesting that AE did not lead to significant motor deficits that impeded the capacity to run. Published in Milbocker et al. (2022). n = 7–12 rats per sex/postnatal treatment/intervention group. Error bars are mean ± SEM.

4.3.2 Alcohol exposure during the BGS delays the trajectory of forebrain and corpus callosum growth in adolescence and is mediated by body weight

To investigate the main and interactive effects of AE during the BGS and exercise intervention exposure on corpus callosum growth between juvenile and adolescence periods, mixed repeated measures ANOVAs were used (within-subjects variable: weight/day; between-subjects variables: postnatal treatment and intervention groups; covariate: sex). We found that forebrain volume increased in all juvenile rats in this study, as predicted (PD30–42; $F_{1, 75} = 250.9$, $p < .001$, $\eta p^2 = 0.77$). Additionally, there is a significant interaction between age and intervention group ($F_{1, 75} = 7.9$, $p = .006$, $\eta p^2 = 0.10$) indicating that exercising increases the rate of forebrain growth across adolescence in all rats that had free access to a running wheel. Similar analyses show that ICC and PCC volumes increased with age in all rats ($F_{1, 75} = 29.0$, $p < .001$, $\eta p^2 = 0.28$; $F_{1, 75} = 79.4$, $p < .001$, $\eta p^2 = 0.51$, respectively). Yet, there was no interaction of ICC or PCC growth with postnatal

treatment exposure, exercise intervention, or sex. Collectively, these data show that the rate of forebrain, ICC and PCC growth across adolescence was unchanged by AE, but that the rate of forebrain growth was immediately increased by aerobic exercise intervention in all rats. All within-subjects analyses are depicted in the graphs in **Fig. 6**.

Further analysis using two and three-way ANOVAs (postnatal treatment × sex; postnatal treatment × intervention exposure with sex as a covariate, respectively) were conducted for data collected on PD30 or 42 to investigate group differences in forebrain, ICC, and PCC volumes at each time point. First, we discovered that there exists a main effect of postnatal treatment on the volumes of forebrain, ICC, and PCC when measured at either time point ($p < .05$), illustrating that AE during the BGS

results in a lasting reduction to the volumes of the brain and corpus callosum subregions in adolescence. Importantly, the previously described longitudinal analysis confirms that AE does not perturb the rate of growth of these structures between the chosen time points. Second, we found a main effect of sex on forebrain, ICC, and PCC volumes when measured at both time points. This relationship shows that the female brain and its subregions are smaller than its male counterparts in adolescence, consistent with the literature (Treit et al., 2017; Ieraci et al., 2020; Inkelis et al., 2020). Moreover, there is a significant interactive effect between sex and postnatal treatment group on ICC and PCC volumes which is depicted in the graphs in Fig. 6B and E (male comparison) and Fig. 6C and F (female comparison). These data indicate that AE during the BGS disproportionately affects the volumes of ICC and PCC in males compared to females across late development. Indeed, the male brain is more vulnerable to AE-related impairments to growth and maturation. Since the female ICC and PCC are smaller at baseline and AE does not change ICC or PCC volume in adolescence, the volumes of male AE CC are comparable to those in the AE and SI female brain on PD30 and 42.

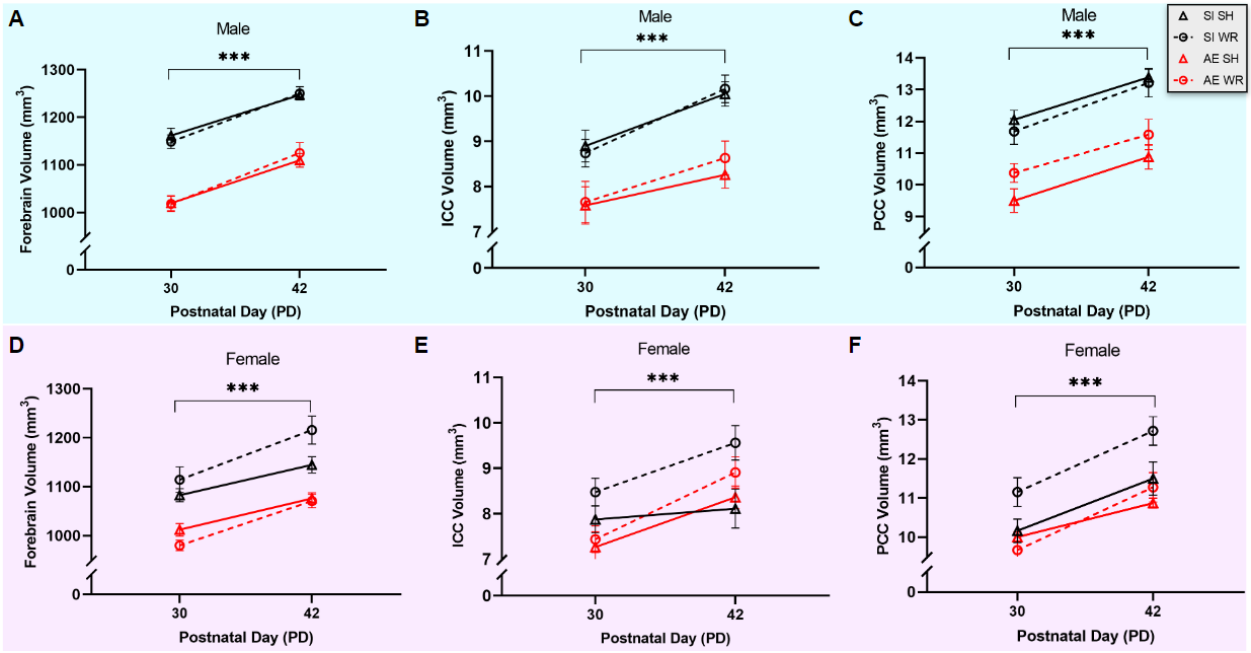

Figure 6. Average within-animal forebrain volume growth in male (A) and female (D) AE/SH, AE/WR, SI/SH, and SI/WR rats during the intervention period. Mixed repeated-measures ANOVA indicates that forebrain volume increases between PD30–42 in all rats (***$p < .001$) and there is a significant interaction with intervention exposure. Average within-animal ICC volume growth in male (B) and female (E) AE/SH, AE/WR, SI/SH, and SI/WR rats during the intervention period. Mixed repeated-measures ANOVA indicates that ICC volume increases between PD30–42 in all rats (***$p < .001$). Average within-animal PCC volume growth in male (C) and female (F) AE/SH, AE/WR, SI/SH, and SI/WR rats during the intervention period. Mixed repeated-measures ANOVA indicates that PCC volume increases between PD30–42 in all rats (***$p < .001$). Published in Milbocker et al. (2022). n = 7–12 rats per sex/postnatal treatment/intervention group. Error bars are mean ± SEM.

Finally, we observed a positive effect of exercise intervention exposure on ICC volume in late adolescence for female rats. Our data show that wheel running exposure significantly increased the volume of ICC on PD42 in AE and control SI female rats only. However, given the fact that AE did not lead to reductions in ICC

volume in the female adolescent brain, we cannot conclude that the observed intervention-related findings indicate a restoration of alcohol-induced damage to corpus callosum. Nevertheless, when considering the elevated distance run by female rats compared to males in this study, it can be predicted that increasing exercise exposure for male rats in future studies might mitigate the alcohol-induced reduction to ICC and PCC volume observed in the male adolescent brain. Cross-sectional results are summarized in **Table 1**.

Regression-based mediation analysis with bootstrapping using the PROCESS v3.5 function for SPSS confirms that the impact of postnatal treatment on ICC (**Fig. 7A**) and PCC (**Fig. 7B**) volume is mediated by alterations to forebrain volume on PD30 and 42. When sex is included as a biological factor (covariate) in the analysis, it is evident that sex also has an effect on forebrain volume and, thus, indirectly affects ICC/PCC volume in adolescence.

		Forebrain Volume		ICC Volume		PCC Volume	
		PD30	PD42	PD30	PD42	PD30	PD42
Main effects	Sex	***** F < M**	n/a	***** F < M**	n/a	**** F < M**	n/a
	PN Treatment	***** AE < SI**	***** AE < SI**	***** AE < SI**	***** AE < SI**	***** AE < SI**	***** AE < SI**
	Intervention	n/a	*n.s.*	n/a	*** SI < WR**	n/a	*n.s.*
Covariates	Sex	n/a	***** F < M**	n/a	*** F < M**	n/a	*** F < M**
Interactions		*n.s*	*n.s.*	***sex x PN treatment**	***sex x intervention *sex x PN treatment**	***sex x PN treatment**	***sex x PN treatment**

Table 1. Significant values are in bold. ***$p < .001$, **$p < .01$, *$p < .05$; n/a variable not included in analysis, n.s. non-significant difference. Published in Milbocker et al. (2022).

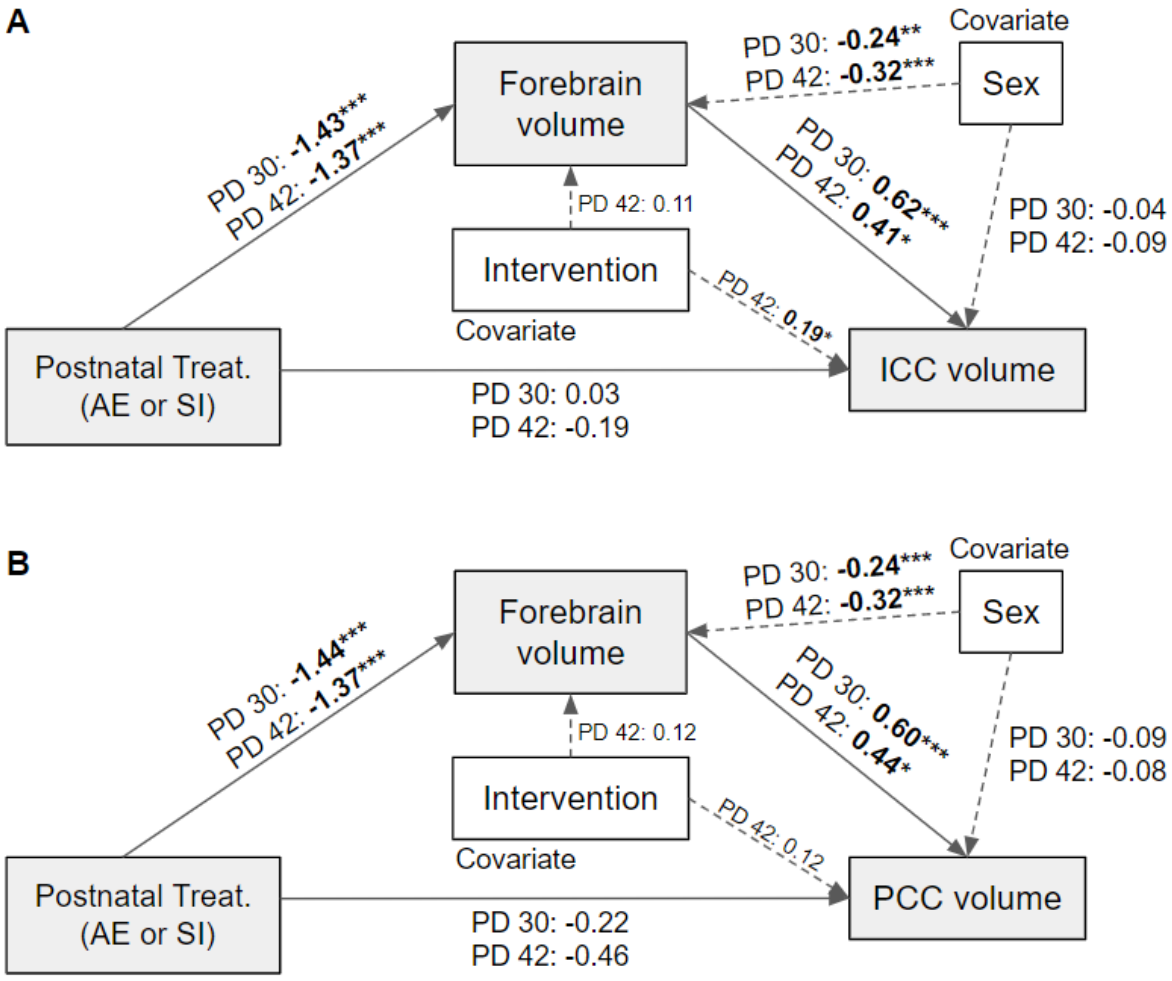

Figure 7. Statistical models for the regression-based mediation analyses which illustrate that postnatal treatment indirectly affects ICC (A) and PCC (B) volumes through lasting alterations to forebrain volume on PD30 and 42. All values represent standardized regression coefficients. Bolded coefficients indicate statistically significant relationships. Factors: postnatal treatment, ICC/PCC volume; Mediator: Forebrain volume; Covariates: sex and intervention group. Published in Milbocker et al. (2022). N = 80, ***$p < .0001$, **$p < .01$, *$p < .05$.

Given the lasting effect of AE during the BGS on developmental weight gain, we then explored the potential relationship between body weight, forebrain volume, and ICC/PCC volume in adolescence. We show through regression-based mediation analysis that weight on PD30 and 42 has an indirect effect on ICC volume as mediated through total forebrain volume in both AE and SI control rats (**Fig. 8A**). However, this relationship is not maintained when comparing the mediation effect of forebrain volume on the body weight-PCC volume relationship. Instead, body weight directly affects PCC volume on PD30 only (**Fig. 8B**). Moreover, postnatal treatment group (covariate) influences weight and forebrain volume, indirectly impacting ICC/PCC volumes on PD30 and 42. Despite the main effects of sex and intervention exposure on the absolute and percent of weight gain across adolescent, sex had no effect on forebrain or ICC/PCC volumes in our model. Intervention exposure had an observed effect on forebrain volume only in this model. Mediation results are further described in **Table 2**. Together, these analyses suggest that adolescent weight gain may contribute to whole and regional brain growth and that postnatal treatment has a significant impact on this mediating relationship. The results described in Sections. 3.1 and 3.2 illustrate a sexual dimorphism in the rate of or percent increase in body weight that occurs in adolescence, likely due to pubertal onset (Juraska et al., 2020), do not affect the observed relationship between weight and forebrain/CC volume. One limitation of this analysis is that it does not comprehensively account for sex or postnatal-treatment specific alterations to the independent variable (body weight).

Furthermore, since we have established that weight on PD nine is positively correlated with weight on PD30 in all rats, it can also be concluded that acute reductions in weight gain resulting from AE during the BGS, prevents age-appropriate

weight gain in adolescence, and ultimately contributes to delayed forebrain and white matter tract growth in adolescence irrespective of sex. It should be noted that while exercise intervention reduced weight gain across adolescence, it did not cause weight loss in our paradigm. Importantly, forebrain volume was increased by exercise intervention exposure. This presents one caveat to the observed mediation effect, likely due to the therapeutic effect that aerobic exercise has on myelinogenesis, and thus brain volume, during juvenile neurodevelopment. Thus, postnatal treatment (AE) and intervention exposure (WR) are shown to have opposite effects on forebrain volume, suggesting a potential restorative effect of adolescent exercise intervention in our rodent model of FASD.

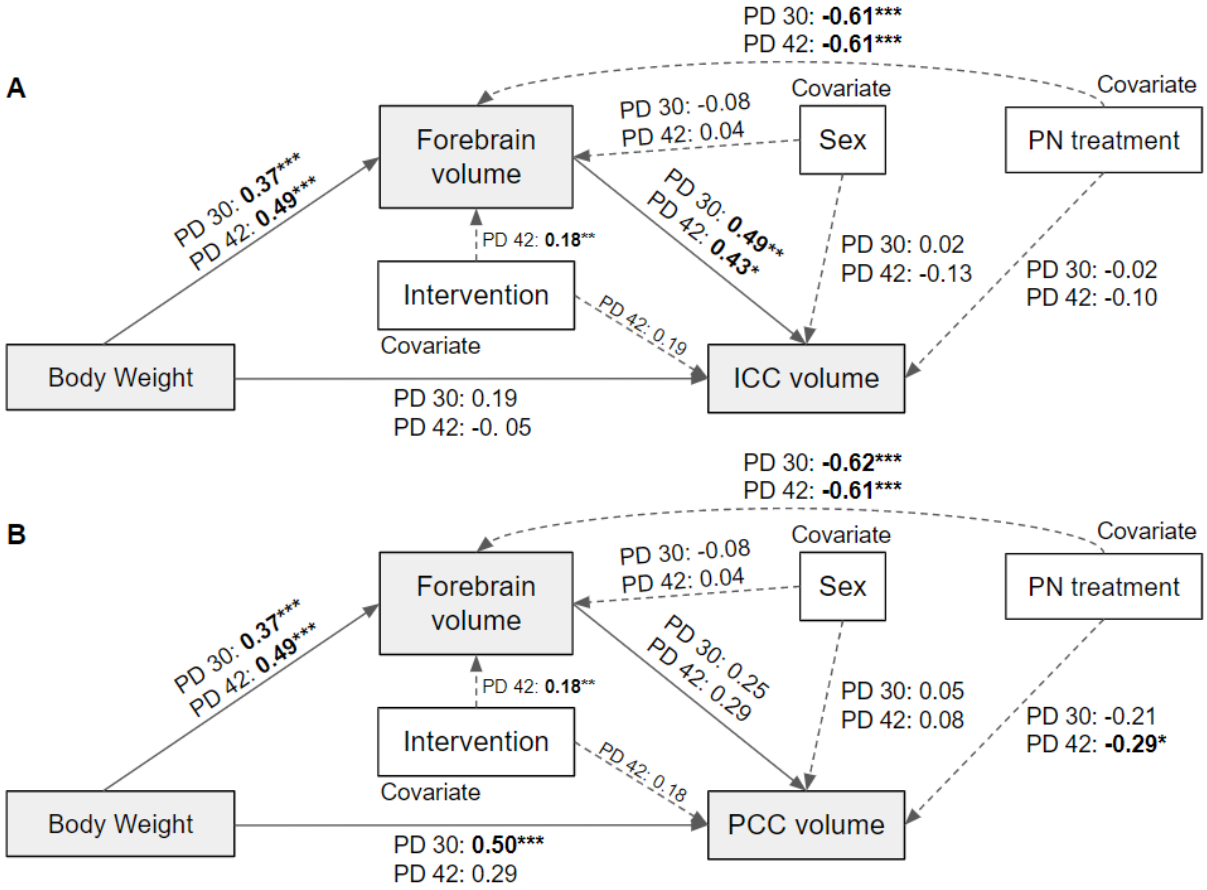

Figure 8. Statistical models for the regression-based mediation analyses which illustrate that body weight indirectly affects ICC (A) but not PCC (B) volumes through alterations to forebrain volume on PD30 and 42. Conversely, body weight influences PCC volume directly on PD30 only. All values represent standardized regression coefficients. Bolded coefficients indicate statistically significant relationships. Factors: body weight, ICC/PCC volume; Mediator: Forebrain volume; Covariates: sex, postnatal treatment group, and intervention group. Published in Milbocker et al. (2022). N = 80, ***$p < .0001$, **$p < .01$, *$p < .05$.

Indirect Path	**Time point**	**Estimated Effect**	**95% CI** **LL** **UL**	**Ratio to total effect**

PN Treatment → Forebrain Volume → ICC Volume	PD 30	-0.90	-1.4	-0.53	87%
PN Treatment → Forebrain Volume → ICC Volume	PD 42	-0.56	-1.02	-0.07	59%
PN Treatment → Forebrain Volume → PCC Volume	PD 30	-0.86	-1.33	-0.43	57%
PN Treatment → Forebrain Volume → PCC Volume	PD 42	-0.61	-1.05	-0.21	38%
Body weight → Forebrain Volume → ICC Volume	PD 30	0.18	0.06	0.37	37%
Body weight → Forebrain Volume → ICC Volume	PD 42	0.01	0.0004	0.0164	>99%
Body weight → Forebrain Volume → PCC Volume	PD 30	0.0059	-0.0005	0.0135	n/a
Body weight → Forebrain Volume → PCC Volume	PD 42	0.0043	-0.0009	0.0105	n/a

Table 2. If a 0 falls within the bounds of the bootstrapping confidence interval (CI), then the proposed relationship does not exist. N = 80, bootstrapping sample size = 500, LL lower limit, UL upper limit, CI confidence interval, all effects are partially standardized. Published in Milbocker et al. (2022).

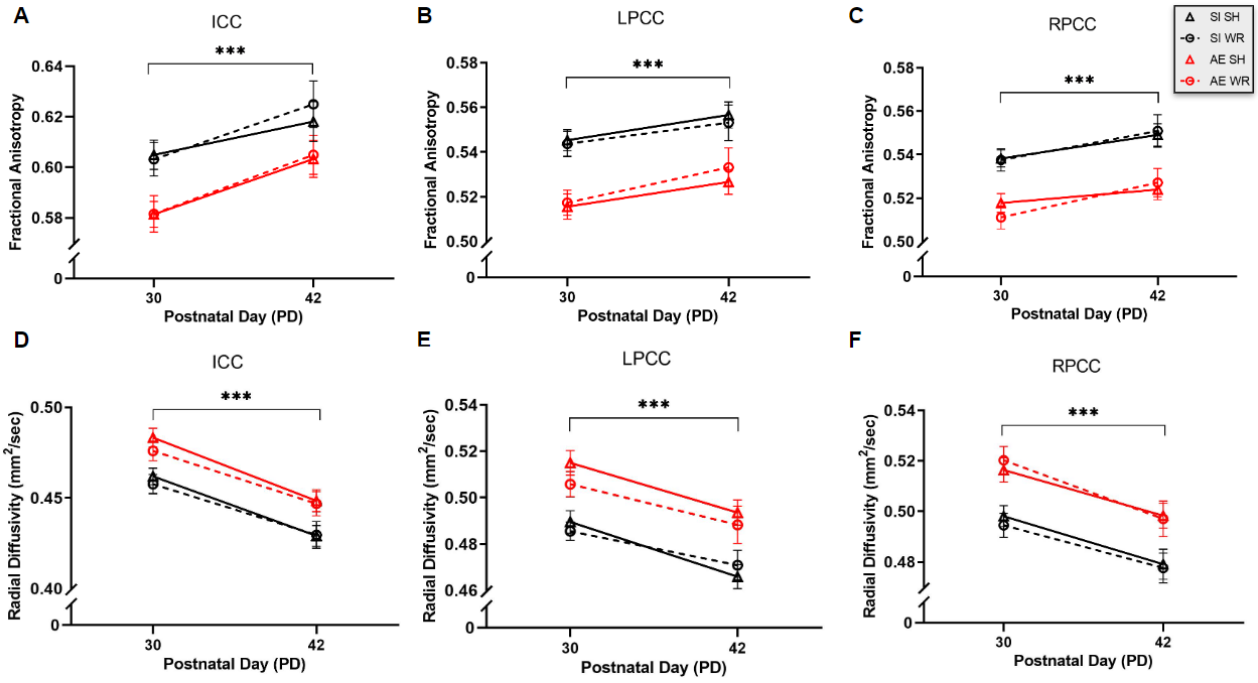

Figure 9. Average within-animal ICC, LPCC, and RPCC fractional anisotropy (A–C, respectively) and radial diffusivity (D–F, respectively) in AE/SH, AE/WR, SI/SH, and SI/WR rats during the intervention period. Mixed repeated measures ANOVA indicates that age-related alterations to fractional anisotropy and radial diffusivity are inversely proportional in all CC sub-regions (***$p < .001$). All data are shown collapsed across sex as the analyses indicate no main or interactive effects of sex on the DTI measures. Published in Milbocker et al. (2022). n = 7–12 per sex/postnatal treatment/intervention group. Error bars are mean ± SEM.

		ICC Fractional Anisotropy		ICC Radial Diffusivity	
		PD30	PD42	PD30	PD42
Main effects	Sex	*n.s.*	n/a	*n.s.*	n/a

	PN Treatment	***** AE < SI**	*** AE < SI**	***** SI < AE**	***** SI < AE**
	Intervention	n/a	*n.s.*	n/a	*n.s.*
Covariates	Sex	n/a	*n.s.*	n/a	*n.s.*
Interactions		*n.s.*	*n.s.*	*n.s.*	*n.s.*

		LPCC Fractional Anisotropy		LPCC Radial Diffusivity	
		PD30	PD42	PD30	PD42
Main effects	Sex	*n.s.*	n/a	*n.s.*	n/a
	PN Treatment	***** AE < SI**	***** AE < SI**	***** SI < AE**	***** SI < AE**
	Intervention	n/a	*n.s.*	n/a	*n.s.*
Covariates	Sex	n/a	*n.s.*	n/a	*n.s.*
Interactions		*n.s.*	*n.s.*	*n.s.*	*n.s.*

		RPCC Fractional Anisotropy		RPCC Radial Diffusivity	
		PD30	PD42	PD30	PD42
Main effects	Sex	*n.s.*	n/a	*n.s.*	n/a
	PN Treatment	***** AE < SI**	***** AE < SI**	***** SI < AE**	**** SI < AE**
	Intervention	n/a	*n.s.*	n/a	*n.s.*
Covariates	Sex	n/a	*n.s.*	n/a	*n.s.*
Interactions		*n.s.*	*n.s.*	*n.s.*	*n.s.*

Table 3. Significant values are in bold. ***$p < .001$, **$p < .01$, *$p < .05$; n/a variable not included in analysis, n.s. non-significant difference. Published in Milbocker et al. (2022).

4.3.3 Alcohol exposure during the BGS delays the myelination of multiple sub-regions of corpus callosum in adolescence

As aforementioned, comparison of fractional anisotropy, axial diffusivity, and radial diffusivity data from DTI scans reveals alterations to brain microstructure. This study harnessed the power of noninvasive neuroimaging to assess the therapeutic potential of an adolescent exercise intervention to restore alcohol- induced changes to fractional anisotropy, axial diffusivity, and radial diffusivity in corpus callosum using a well-established rodent model of FASD. Of note, a paired samples t-test of data collected from left and right PCC indicates that there was a significant difference in DTI data obtained from left and right hemispheres across adolescence, an expected asymmetry that was not identified when evaluating volume data (Kolb et al., 1982).

Analysis using mixed repeated measures ANOVAs (within-subject variable: weight/day; between-subject variables: postnatal treatment and intervention groups; covariate: sex) indicate that fractional anisotropy increases in ICC and left/right PCC across development, as expected (**Fig. 9A–C**). We suspect that this is due to *de novo* axon myelination and/or thickening of existing myelin sheaths which occur naturally during adolescence for circuit refinement (Lebel et al., 2011; Bergles et al., 2016). However, right PCC had lower fractional anisotropy values compared to left PCC on PD30 and 42 in all rats, perhaps due to a difference in the trajectory of maturation of myelin between brain hemispheres. Further, analysis with two and three-way ANOVAs (postnatal treatment × sex; postnatal treatment × intervention exposure with sex as a covariate, respectively) for data collected on PD30 and 42 show that fractional anisotropy values are lower in AE rats compared to SI control rats at both time points,

indicating that water is moving more freely within corpus callosum in AE adolescent rats (**Table 3**).

To explore the potential biomolecular mechanism underlying the observed AE-induced changes to corpus callosum integrity, we examined group differences in axial and radial diffusivity within ICC and PCC. Analysis using mixed repeated measures ANOVAs (within-subject variable: weight/day; between-subject variables: postnatal treatment and intervention groups; covariate: sex) indicates that radial diffusivity decreased in ICC and left/right PCC across adolescence as expected, suggesting that myelination increased in all animals across adolescence (**Fig. 9D–F**). However, analyses using two and three-way ANOVAs (postnatal treatment × sex; postnatal treatment × intervention exposure with sex as a covariate, respectively) for data collected on PD30 or 42 show that radial diffusivity values remained higher in AE compared to SI control rats in all corpus callosum subregions (**Table 3**). Moreover, there were no significant main effects of sex, postnatal treatment group, or intervention group on axial diffusivity values in ICC or left/right PCC across adolescence, confirming that the observed structural alterations did not result from changes to axonal integrity. These data are indicative of either a delay in the trajectory of corpus callosum myelination or of a reduction in the amount of myelination produced in the FASD-affected corpus callosum. Additionally, the observed alterations were not immediately mitigated by adolescent exercise intervention and were not sexually dimorphic. It should be noted that radial diffusivity values were negatively correlated with PCC volume on PD30 ($r = -0.45$, $p < .001$) and 42 ($r = -0.37$, $p < .001$) and with ICC volume on PD30 only ($r = -0.42$, $p < .001$) in all rats. These relationships suggest that alcohol-induced changes to corpus callosum

subregion volume (mediated by growth delay) are correlated with changes to corpus callosum myelination across adolescence. Perhaps the inverse relationships indicate that corpus callosum subregion volumes are smaller when there exists less myelination (increasing radial diffusivity values).

4.4 Discussion

4.4.1 Summary of Findings

This study employed a rodent model of FASD to explore the potential beneficial effect of an aerobic exercise intervention on mitigating alcohol-induced pathology to corpus callosum development and myelination in the adolescent brain. Concordant to findings described in the clinical literature, AE during the BGS was shown to alter age-appropriate weight gain across neonatal and adolescent development (Wozniak et al., 2019). Regression-based mediation analysis shows that the effect of postnatal treatment on body weight indirectly affects corpus callosum growth (volume) through alterations to forebrain volume in adolescence. Importantly, aerobic exercise intervention (voluntary wheel running) in adolescence had the opposite effect on forebrain volume, stimulating forebrain growth in all rats. Additionally, we were the first to show that alcohol-induced alterations to several of these measures across adolescence were discovered to be sexually dimorphic—body weight, forebrain volume, and corpus callosum (ICC and PCC sub-regions) volume. ICC and PCC volumes were largest in SI control female rats exposed to the adolescent exercise intervention. Females were observed to run greater distances on average when exposed to the adolescent intervention compared to their male counterparts which likely contributed to sexual dimorphisms in weight gain and forebrain growth

across adolescence. A follow-up study wherein caloric intake is measured during intervention exposure is needed to confirm this remaining hypothesis.

Analysis of data collected from diffusion tensor imaging scanning demonstrates that corpus callosum myelination in adolescence is reduced by AE during the BGS. Due to the anatomical differences between commissural and projecting corpus callosum fibers, two sub-regions of corpus callosum—ICC and PCC—were examined. Longitudinal analysis confirmed that radial diffusivity values (an indirect measure of myelination) decreased in ICC and PCC between PD30 and 42 in all rats, as expected during this fundamental period of circuit refinement. However, cross-sectional analysis of DTI data collected on PD30 or 42 shows that radial diffusivity values remain higher in the AE ICC and PCC compared to controls, indicative of disrupted or hypomyelination. Adolescent exercise intervention had no immediate effect on radial diffusivity values in adolescence. Future research is needed to investigate if AE leads to a persistent loss of myelination in corpus callosum in adulthood or if the trajectory of this neurodevelopmental process is simply delayed and/or lengthened in cases of FASD.

Further, it was discovered that the progression of PCC myelination was incongruous in the left and right

hemispheres such that myelination was greater in the right PCC in all rats. To our knowledge, this is the first examination of PCC myelination lateralization in rodents. However, much like in the human brain, hemispheric lateralization in the rat brain is a result of genetic predisposition and putative function/early-life experience. Structural and functional brain asymmetries play a key role in individual variations in motor capacity, emotional processing, and language ability in both the rodent and

human brain (Zimmerberg et al., 1989). For example, cortical lateralization is well-described and attributes to paw preference in rodents (Diamond et al., 1981; Hayhow et al., 1962; Lund et al., 1965). We did not find that lateralized PCC development resulted from AE during the BGS (potentially considered an early-life experience in our model of FASD). However, gestational alcohol exposure has been shown to yield limb preference in rodent models of FAS/FASD (Domellof et al., 2009).

4.4.2 Body weight, brain size and white matter volume

As previously described, disruptions to normative growth trajectory in childhood have deleterious effects on brain development. Alcohol teratogenesis is a known risk factor for reaching age-appropriate growth and neurodevelopmental milestones (Riley et al., 1995; King et al., 2019). Moreover, our data link FASD-related changes to body growth with impaired growth of salient white matter tracts in adolescent males. This is demonstrated by a reduction in ICC and PCC volumes in AE male rats on PD30 and 42. Furthermore, the AE corpus callosum in males was comparable in volume to the volume of corpus callosum in both AE and SI female rats. While reductions to corpus callosum subregion volume were mediated by overall reductions to forebrain volume, DTI scanning provided additional evidence that elucidated hypomyelination as another factor contributing to volumetric anomaly. Indeed, PCC volume at each time point was negatively correlated with radial diffusivity values such that the smaller the subregion, the more water was able to move around in the structure. A similar relationship was observed on PD30 in ICC. These relationships suggest that reductions to corpus callosum volume could be attributed, at least in part, to a decrease in tract myelination. Since the smallest corpus callosum were observed in adolescent male rats with previous AE, it is possible to

connect this structural deficit with exposure to alcohol during development. Moreover, it is known that the male brain has a higher susceptibility to structural deficits resulting from alcohol teratogenesis (Inkelis et al., 2020; Ruggiero et al., 2018). Further exploration of this sex-specific vulnerability would benefit from histological analysis of brain tissue from a preclinical model of FASD wherein measures of myelination (i.e. myelin basic protein concentration, oligodendrocyte number, thickness of the myelin sheath) could be quantified.

Finally, we discovered a therapeutic effect of aerobic exercise intervention in adolescence on the rate of forebrain growth in AE and control rats. This relationship exists as a caveat to the observed body weight-forebrain volume relationship wherein exercise-related reductions to weight gain across adolescence were negatively correlated with rate of forebrain growth. Wheel running promoted brain growth in AE and SI rats of both sexes, as expected. Furthermore, it was observed that the volume of the ICC in the female brain (AE and SI control) was significantly increased following intervention exposure. This effect was not observed in the male brain, likely due to the longer distances voluntarily run by female rodents in this study. Several studies indicate that when provided with free access to running wheels, female rodents run more on average than males (Krentzel et al., 2020). It could be predicted that intervention exposure would have the largest effect on changes to myelination in the female brain.

4.4.3 Understanding the observed AE-related changes to corpus callosum structure: does alcohol exposure during the BGS perturb the trajectory of corpus callosum myelination or reduce the amount of myelination produced in the adolescent brain altogether?

The mechanism by which prenatal AE reduces corpus callosum myelination and causes neuroanatomical reorganization across the lifespan is unknown. Identification of these neurobiological processes is critical for evaluating the immediate and long-term effectiveness of behavioral interventions on mitigating impairments to myelination in cases of FASD. Several barriers impede our investigation into the teratogenic impact of AE on white matter tract development in humans at the cellular level. First, brain myelination is a postnatal process that continues over several decades in humans. Longitudinal studies spanning over 30 years have a lower feasibility, and results may be difficult to interpret given that many life experiences encountered during the first 30–40 years of life (i.e. socioeconomic status, malnutrition) may significantly affect the trajectory of myelination in the brain. Several clinical studies assessing the impact of AE during the second to third trimesters on corpus callosum volume and fractional anisotropy values through infancy, childhood, and adolescence describe unique changes to white matter structure across development. Briefly, noninvasive neuroimaging studies demonstrate that changes to corpus callosum volume and fractional anisotropy do not appear until at least two years of age in FASD-affected children (Taylor et al., 2015). During early childhood, from 2 to 7 years of age, FASD- affected children exhibit a reduction in corpus callosum volume with an increase in corpus callosum fractional anisotropy, demonstrating a precocial myelination of white matter (Kar et al., 2021) in early-life in response to alcohol teratogenesis (Darbinian et al., 2021). However, in late childhood/early adolescence, between 7 and 15 years of age, history of prenatal AE

has the opposite effect on fractional anisotropy in corpus callosum, indicating hypomyelination. Corpus callosum remains reduced in volume compared to typically-developing controls, and fractional anisotropy values are reduced in corpus callosum (Treit et al., 2013; Wozniak et al., 2006; Sowell et al., 2008; Nguyen et al., 2017). Corpus callosum volume and fractional anisotropy remain reduced in the brains of FASD-diagnosed subjects throughout adolescence (Inkelis et al., 2020; Wozniak et al., 2009) and adulthood (Ma et al., 2005). More research is needed to understand the underlying anatomical and physiological mechanisms relating to measurable changes in anisotropy and diffusivity across development.

A review of the literature reveals two central hypotheses regarding the effect of prenatal AE on white matter tract development: (1) prenatal AE leads to precocial development of corpus callosum in childhood, and (2) prenatal AE leads to a delay in the trajectory of corpus callosum development in adolescence. It is unclear if these hypotheses are mutually exclusive. Our data support the latter hypothesis only as we did not scan during the neonatal period to confirm that myelination is delayed in early life. Indeed, values of radial diffusivity in AE corpus callosum on PD42 (late adolescence, analogous to neurodevelopment at about 22–25 years of age in humans) are comparable to those recorded in the control brain on PD30 (early adolescence, analogous to neurodevelopment at about 18 years of age in humans).

Preclinical research using animal models of FASD allow for the investigation of myelin-related neuropathology across the lifespan which validates noninvasive neuroimaging findings and is necessary for identifying underlying neurobiological mechanisms associated with alcohol-induced reductions to myelination. Further, such studies are ideal for exploring long-term deficits associated with FASD and for

evaluating the effectiveness of behavioral interventions on circuit refinement in the adolescent brain. Myelination is supported by mature and precursor oligoglia which are largely proliferating during the second and third trimesters of human gestation when they are also most vulnerable to alcohol-induced apoptosis (Creeley et al., 2013). Therefore, resulting corpus callosum neuropathologies vary with the timing and amount of prenatal AE, creating an obstacle for systematic clinical investigation. For example, severe prenatal AE resulting in Fetal Alcohol Syndrome has been shown to lead to full or partial corpus callosum agenesis in school-aged children and adolescents (Goh et al., 2016) while studies examining FASD-affected adolescents report a more moderate effect of prenatal AE on white matter development in corpus callosum (Wozniak et al., 2011).

Several factors contribute to hypomyelination of the corpus callosum. These include but are not limited to a reduction in the number of OPCs (depletion of the progenitor pool) through apoptosis or disrupted genesis during early development, dysregulated differentiation of OPCs to myelinating OLs, apoptosis of myelinating OLs, a thinning of the myelin sheath, and a reduction to the number of axons ensheathed by OL processes. Teratogenic AE leads to immediate apoptosis of mature OLs and shifts the ratio of the total number of OPCs to the total number of mature OLs in favor of precursor cells in the neonatal brain. OPC differentiation is initiated by an epigenetic modification which alters nucleosomal histone deacetylation during the BGS in rodent corpus callosum (Shen et al., 2005). Further, it is postulated that mature oligoapoptosis is mediated by extracellular and internal signaling pathways (Darbinian et al., 2021). Collectively, it is evident that oligoglia are most vulnerable to the teratogenic effects of alcohol during the pre-myelinating stage when MBP is being

produced and oligoglia processes begin to flatten prior to ensheathment (Creeley et al., 2013). An immediate reduction to the number of OPCs and disruption of OPC differentiation depletes the OPC pool, leading to a sustained reduction in OPC population in adolescence and sometimes adulthood that contributes to corpus callosum hypomyelination later in life. Finally, prenatal AE results in neuroimmune system dysfunction (Terasaki et al., 2015, 2016). Microglial and astrocytic proliferation and activity play a significant role in coordinating oligoglia proliferation, differentiation, and signaling to facilitate myelination during later stages of development (Nemes-Baran et al., 2020; Traiffort et al., 2020; Pang et al., 2013). Disruption of these pertinent glial interactions could contribute to the observed deficits in white matter tract development.

4.4.4 Further evaluation of the effects of the proposed adolescent exercise intervention on white matter development in corpus callosum is needed

his study is the first step in a larger investigation into the potential therapeutic effect of an accessible and affordable behavioral intervention on stimulating myelination in the FASD adolescent brain. Further, we show that sexual dimorphisms exist in myelin pathology as a result of alcohol teratogenicity. Our results indicate an enhancing effect of aerobic exercise on forebrain growth and ICC volume growth, particularly in the female brain. However, it is possible that wheel running would have a similar effect on the male brain if male rats ran a comparable distance during the intervention period. At present, our findings do not indicate an immediate effect of aerobic exercise intervention on myelination in the adolescent brain of AE and SI rats. However, other immediate benefits of increased aerobic exercise on myelination may be identifiable at the molecular or ultrastructural levels. Given the propensity for

aerobic exercise to stimulate neuroplasticity, it is expected that alterations to myelin plasticity will be observed at some level. Indeed, it is known that aerobic exercise upregulates OPC proliferation and differentiation as well as thickens the exist- ing myelin sheath (Tomlinson et al., 2016). We predict that exercise intervention might upregulate the proliferation of OPCs in corpus callosum in adolescence to counteract any severe reductions to the OPC pool that may contribute to the hypomyelination observed in the AE brain. Additionally, existing sheaths are expected to be thickened following aerobic exercise intervention.

4.4.5 Limitations and future research

This study is the first to use noninvasive longitudinal neuroimaging to examine the therapeutic effect of an adolescent exercise intervention on corpus callosum myelination in a rodent model of FASD. Our data indicate that increased aerobic exercise via voluntary wheel running did not immediately alter CC myelination when observed using DTI measures. Future studies should continue to collect DTI scans at later stages of adolescence and into adulthood to verify that exercise has no impact on CC myelination and to better conceptualize how the observed alcohol-related deficits to corpus callosum myelination contribute to the trajectory of brain circuit refinement in adolescence. Moreover, interpretation of these data would be enhanced by the analysis of simultaneously acquired magnetic transfer scans (Mackay et al., 1994).

Targeted and early intervention strategies are essential for preventing secondary disabilities and the emergence of maladaptive behaviors in adults diagnosed with FASD. Given the rising prevalence of FASD in the US coupled with the difficulties regarding correct FASD diagnosis (Hoyme et al., 2016) the application of effective behavioral interventions is appealing to clinical professionals and caregivers.

Results from future studies should elucidate: (1) the most effective timing for intervention onset, (2) the shortest intervention period with the greatest benefit for supporting neurodevelopment, and (3) further explore any sexual dimorphisms in intervention effectiveness to additionally tailor therapeutic development for the individual.

4.4.6 Conclusions

Prenatal alcohol exposure resulting in a Fetal Alcohol Spectrum Disorders (FASD)-related diagnosis affects 1 in 20 live births in the United States annually, presenting a growing public health crisis that is greater than the risk for developing other significant neurodevelopmental disorders like Autism Spectrum Disorder or ADHD (Alger et al., 2021; O'Neill et al., 2019) FASD-affected adolescents present with numerous social, cognitive and behavioral deficits associated with executive function that perturb perceptual reasoning, future planning, abstract conceptualization, learning, and memory (Riley et al., 1995). Few therapeutic interventions exist for adolescents with FASD. However, preclinical studies using rodent models of FASD have explored potential pharmaceutical and behavioral interventions that may support late neurodevelopment and cognitive capacity in adulthood. In the study presented, we used non-invasive neuroimaging of a rodent model of FASD for optimal translatability via a longitudinal analysis. Notably, our findings support the theory that FASD delays the trajectory of corpus callosum myelination in adolescence. Further, they suggest that voluntary aerobic exercise in adolescence supports forebrain and corpus callosum growth, particularly benefitting the maturation of commissural fibers that are imperative for abstract and perceptual reasoning. While we did not uncover any immediate impacts of exercise intervention on restoring specific impairments to

corpus callosum myelination, it remains unknown how this intervention alters acute myelination at the cellular level and/or if voluntary exercise yields any long-term benefits to corpus callosum myelination that may enhance adult cognition. In summary, innovative intervention programs which include adolescent interventions like the FAST club (Pritchard et al., 2018) and LEAP program (LaCount et al., 2020) could prove most advantageous for resolving some of the underlying neuropathologies associated with FASD in adolescents and mitigate the resulting cognitive and behavioral deficits. While our DTI findings did not make apparent the mechanism behind the change in corpus callosum growth after aerobic exercise, corpus callosum volume changes were evident and this study contributes substantial evidence that exercise may be a positive effector not only for neuroplasticity, but also for myelin plasticity in a model of FASD.

Chapter 5

HISTOLOGICAL ANALYSIS OF OLIGOGLIA LINEAGE CELL NUMBER AND MYELIN PRODUCTION PRE- AND POST-EXERCISE INTERVENTION IN A RODENT MODEL OF FASD

5.1 Introduction

Reduced oligoglia lineage cell proliferation and maturation during the BGS and early-life have been identified as key mechanisms underlying observed delays in corpus callosum myelination across the lifespan (Treit et al., 2013; Fields & Bukalo, 2020). Apoptosis of mature OLs is induced immediately as a result of the teratogenic effects of alcohol (Creeley et al., 2013; Newville et al., 2017). Moreover, alcohol teratogenesis reduces the number of OLs in white matter resulting from an arrest of OPC maturation linked to alcohol metabolism in neonatal brain parenchyma (Darbinian et al., 2021; Marguet et al., 2022). In addition to changes in cell population, prenatal alcohol exposure alters the function and protein production of myelinating cells through neuroimmune activation and repression of myelin-related genes following aberrant epigenetic modification (Niedzwiedz-Massey et al., 2021; Newville et al., 2022).

This study addresses several gaps in knowledge surrounding the underlying neurobiological changes contributing to hypomyelination of the corpus callosum in FASD-affected youth. First, the effects of alcohol exposure during the BGS on alterations to oligoglia cell proliferation and differentiation in adolescence are controversial. One predominant theory maintains that depletion of the OL pool during

critical stages of early neurodevelopment has lasting consequences on connectome refinement across the lifespan. Indeed, Newville and colleagues demonstrated that male mice exposed to vaporized alcohol during the rodent BGS had a transient reduction in myelinating OLs in infancy and adolescence, which returned to typical levels in adulthood. However, levels of MBP production remained reduced across the lifespan (2017). *In vivo* neuroimaging results described in Chapter 4 confirmed that myelination of corpus callosum is also delayed in rats of both sexes exposed to alcohol during the rat BGS (postnatal first 2 weeks of life). Second, few if any studies using animal models of FASD have investigated the consequences of alcohol teratogenesis on myelination of white matter in the adolescent female brain. While the previous neuroimaging study did not uncover any sexual dimorphisms in the trajectory of corpus callosum myelination in AE or SI control rats, it is well-established that oligoglia cell maturation in the female mammalian brain precedes that in the male brain during infancy and adolescence (Semple et al., 2013; Brenhouse et al., 2011; Juraska et al., 2020; Perrin et al., 2009). Thus, a central goal of Aim 2 was to quantify changes to oligoglia lineage cell number and MBP production in the adolescent male and female corpus callosum following alcohol exposure during the BGS.

In addition, this study explored the potential for a voluntary exercise intervention during adolescence to stimulate corpus callosum myelination in the FASD brain. As aforementioned, environmental stimuli play a key role in optimizing circuit function via alterations to axonal myelination in adolescence. Aerobic exercise is a positive effector of CNS myelination in juvenile mammals as it stimulates the maturation of OLs and increases myelination (Tomlinson et al., 2016, 2018; Xiong et al., 2018; Talukdar et al., 2018). A study conducted in male mice demonstrated that

two weeks of voluntary aerobic activity promoted myelination of axons in the motor cortex in adulthood. Specifically, voluntary wheel running led to an increase in OPC and OL number, increased the production of MBP, and reduced the g-ratio in the motor cortex, suggesting that exercise promoted *de novo* myelination of axons as well as refined existing patterns of ensheathment (Zheng et al., 2019).

The Klintsova lab and others have previously demonstrated that adolescent voluntary exercise intervention via free access to running wheels stimulates hippocampal and cortical neuroplasticity in male and female rats exposed to alcohol during the BGS (Schreiber et al., 2013; Hamilton et al., 2014, 2015; Boschen et al., 2017). However, research regarding the influence of aerobic activity on white matter tract myelination and OL morphology in rodent models of FASD is limited. The results described in Chapter 5 provide seminal evidence that administration of an adolescent intervention targeting the second peak in developmental myelination immediately stimulated corpus callosum myelination. Moreover, sexual dimorphisms in cellular responses to adolescent exercise and alcohol teratogenesis are described.

5.2 Methods

5.2.1 Rat Model of FASD

All rats were acquired, cared for, and methodology was in accordance with the guidelines provided by the University of Delaware's Institutional Animal Care and Use Committee, the NIH Guide for the Care and Use of Laboratory Animals, and comply with the ARRIVE guidelines (https:// arriveguidelines.org). Timed-pregnant dams (Charles River Laboratories, Boston, MA) were acquired, and once pups were born, litters were culled to 10-12 pups each and counterbalanced for sex and postnatal

treatment condition (AE or SI control). On PD 3, each pup received a paw tattoo with India Ink and an ear punch for ease of identification across the animal's lifespan. From PD 4-9 half of each litter received a daily dose of alcohol in milk-substitute at 5.25 g/kg/day (11.9% v/v, divided into two administrations two hours apart) resulting in an average BAC of 300-400 mg/dl. It should be noted that this technique models a binge-like exposure during the BGS, a neurodevelopmental milestone occurring during the third trimester of pregnancy in humans and first two postnatal weeks in rats (Dobbing and Sands, 1979). SI procedural control rats received intragastric intubation without liquid administration to account for the stress of intubation. Notably, this is a well-established model in the field of FASD and it is known that the level of BAC achieved is correlated with region-specific apoptosis of neurons and oligodendrocytes (Bonthius & West, 1991; Bonthius et al., 1988). Following the dosing period, male and female pups were left to mature with a dam. Two cohorts of rats were generated such that one cohort could be sacrificed on PD 30 and the other on PD 45 to compare the immediate effect of an adolescent exercise intervention from PD 30-45 on the cellular features described (Exps. 1 & 2, respectively, see **Fig. 10**).

5.2.2 Adolescent exercise intervention to stimulate myelinogenesis

In experiments 1 and 2 depicted in Figure 10 below, male and female rats from both postnatal treatment groups were weaned on PD 23 and rehoused in same sex groups of 2-3 per cage. On PD 30, rats from each group in experiment 2 were randomly selected to either remain in social housing as sedentary controls or be rehoused into a modified cage with an attached running wheel for voluntary access to the exercise intervention. Rats were sacrificed on PD 45 and brain tissue was collected for immunocytochemical analysis of oligoglia lineage cell number and myelin basic

protein densitometry (n = 7-12). It should be noted that this cohort of rats is the same cohort that underwent longitudinal noninvasive neuroimaging scanning as described in Chapter 4 and there is no evidence to suggest that the scanning protocol had any measurable effect on the cellular features quantified in this study. Additionally, a second cohort of rats was generated and sacrificed on PD 30 to quantify these measures in brain tissue pre-intervention (Exp. 1; n = 10).

5.2.3 Animal euthanasia for tissue collection and fixation

Rats were anesthetized using an intraperitoneal injection of a ketamine-xylazine mixture and inhalation of isoflurane prior to transcardial perfusion with 100mL of heparinized phosphate buffered saline (PBS) and 100mL of 4% paraformaldehyde in PBS at 4°C. Brains were carefully removed and brain tissue was stored in vials containing 4% paraformaldehyde in PBS in a refrigerator. Solutions in the vials were swapped 3 times during post-fixation using a 30% sucrose in 4% paraformaldehyde in PBS solution.

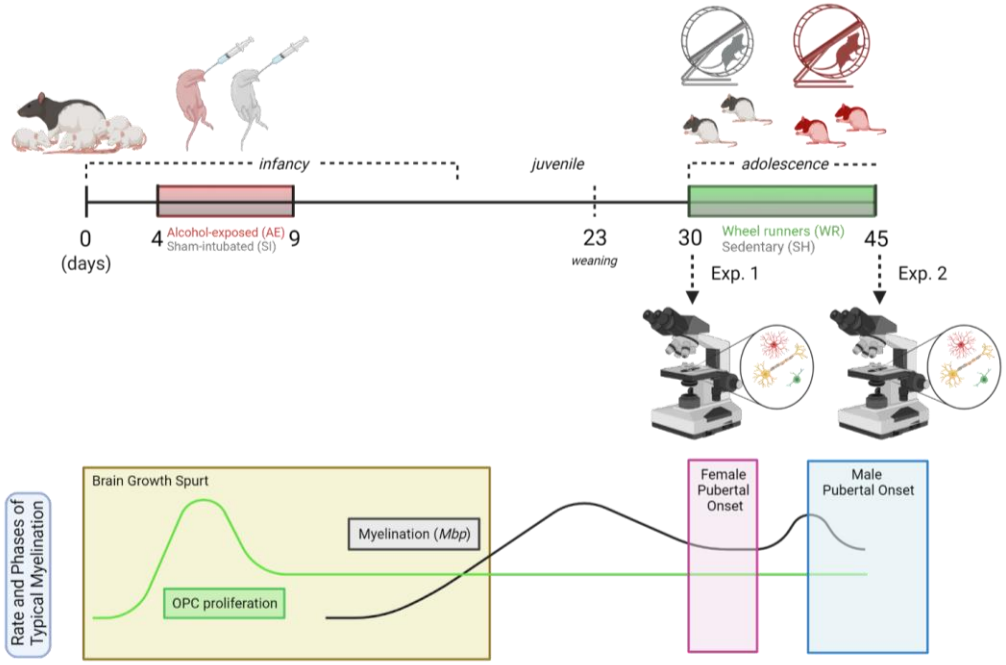

Figure 10. Experimental timeline for Aim 2 with overlay illustrating the rate and phases of typical myelination in the rat brain from infancy to adolescence.

5.2.4 Tissue preparation for oligoglia cell quantification

Once post-fixed with 30% sucrose in 4% paraformaldehyde, brain tissue was sectioned in a coronal plane at 40um on a Leica cryostat, conserving precise anatomical organization and the sequence of the sections. Tissue sections were stored in cryoprotectant at -20℃. Every sixteenth section spanning the corpus callosum was stained using immunofluorescently-tagged antibodies against the CC1 (OL) and PDGFRα (OPC) antigens and counterstained against Hoechst 33342 to accurate identify oligoglia cell somas. Free-floating tissue sections were rinsed with 0.1 M

Tris-Buffered Saline (TBS; pH = 7.40) for 15 minutes and blocked in Blocking Solution [0.1% Triton X-100 (BP-151; ThermoFisher Scientific; Hampton, NH) and 5% Normal Donkey Serum (S30; Merck Millipore; Burlington, MA) in 0.1 M TBS] for 2 hours at room temperature. After blocking, tissue was incubated in Primary Antibody Solution [1:400 mouse anti-APC/CC1 (OP-80; EMD Millipore) and 1:500 rabbit anti-PDGFRα (SAB4502142; Sigma Aldrich) in Blocking Solution] for 12 hours at 4°C. On day two, tissue was rinsed in 0.1 M TBS for 30 minutes and incubated in Secondary Antibody Solution [1:400 goat anti-rabbit biotinylated antibody (30014; Vector) in Blocking Solution] for 2 hours at room temperature to increase visualization of OPCs. Following amplification, tissue was rinsed in 0.1 M TBS for 30 minutes and incubated in Fluorescent Antibody Solution [1:250 donkey anti-mouse antibody with Alexa Fluor 594 (A21203; ThermoFisher Scientific) and 1:750 donkey anti-goat antibody with Alexa Fluor 488 (705-545-003; Jackson ImmunoResearch) in Blocking Solution] for 2 hours at room temperature. Following fluorescent antibody incubation, tissue was rinsed in 0.1 M TBS for 30 minutes followed by 0.1 M PBS for 5 minutes. Tissue was then stained with Hoechst 33342 (62249; ThermoFisher Scientific) for 5 minutes and rinsed with 0.1 M PBS for 15 minutes. Lastly, tissue was slide-mounted in dH2O and air-dried for 1 hour before coverslips were mounted with Gelvatol. Coverslipped slides were stored away from light at 4°C and were allowed to set for at least 12 hours before microscopic analysis (**Fig. 11C**).

5.2.5 Tissue preparation for densitometric analysis of myelin basic protein

Simply quantifying changes to oligoglia cell number is not sufficient to investigate alcohol or exercise-induced alterations to corpus callosum myelination in

this study. Myelin basic protein production was visualized using a non-fluorescent stain and quantified using a densitometric analysis in ImageJ. Every sixteenth section containing the corpus callosum was immunohistochemically stained, conserving rostro-caudal order, with a primary antibody against myelin basic protein (MBP+) for visualization of myelin in corpus callosum. The staining protocol was modified from Milbocker & Klintsova (2020) and Hamilton et al. (2019). Free floating sections were rinsed in a series of 0.1M TBS and quenched in 0.4% Triton X-100 with 0.6% H_2O_2 in TBS for thirty minutes. After additional rinses of TBS wash, the sections were placed in blocking solution [4% Normal Goat Serum (NGS) and 0.4% Triton X-100 in 1X TBS] for one hour. Following the blocking solution, the sections were transferred into non-perforated bubble trays filled with the primary antibody [Rat anti-MBP antibody (MAB386, Millipore) at 1:200 dilution in TBS-NGS blocking solution]. Control sections were placed in TBS-NGS blocking solution with no antibody.

Approximately 48 hours later, the sections were transferred from the non-perforated bubble trays and placed in TBS wash. They were then incubated in the secondary antibody for two hours [Biotinylated goat anti-rat secondary antibody (BA9400, Vector) 1:1000 dilution in blocking solution]. After an additional TBS rinse, the tissue was placed in an avidin-biotin complex amplification solution in blocking solution for 60 minutes. Another TBS rinse was followed by placement of tissue sections in 0.05% 3,30- Diaminobenzidine (DAB)-Peroxidase with nickel enhancement [2ml DAB + 1.39g $NiSO_4(NH_4)_2$ + 200 mL 1X TBS] for an average of 8.83 minutes. Once sufficiently stained, the tissue was placed in a final TBS rinse and mounted onto gelatin-subbed microscope slides. 48 hours later, the slides underwent a series of brief rehydration/dehydration washes in ethanol solutions of increasing

concentrations (30 seconds -1 minute each) before being cover slipped using DPX Mountant.

5.2.6 Unbiased stereological quantification of oligoglia cells

Cells were quantified using a conventional unbiased stereology protocol within the MBF Bioscience Stereoinvestigator software. After acquiring 3D renderings of stained tissue sections (z-stacks) which contained the body of corpus callosum, z-stacks were uploaded into Stereoinvestigator. Using this software, a 3D probe icon was placed over a portion of each section where the corpus callosum was outlined and the cells within the probe borders were quantified resulting in the analysis of a known ratio of the total volume of the body of corpus callosum (Sterio et al., 1984; West et al., 1991). The section sampling ratio was 1/16, the height sampling fraction was 0.625, and the area sampling fraction was .083 (West et al., 1991). Once cell population measurements were collected from a subset of rats in each group, the Coefficient of Error of each measurement was compared to assess the reliability of the parameters used to acquire a cell estimate that is representative of the total cellular distribution through the body of corpus callosum. Parameters were modified to yield optimal Coefficient of Error measurements (Range = 0-0.1, M = .07) for all cell population estimates in these experiments (Glaser & Wilson, 1998). There are two central advantages to using unbiased stereology for cell quantification within a region of interest. First, the implementation of a 3D probe allows users to examine cellular features through the depth of the z-stack to accurately identify cell somas during the quantification step. Second, the software places the probe in systematic yet random areas within the region of interest in a z-stack to limit experimenter bias which would

result from an ability to choose which cells to count in a given tissue section independently.

5.2.7 Densitometric Analysis of MBP

Densitometry was used to quantify the amount of MBP in the ICC, using a protocol modified from Gursky et al. (2020) and Sternberger et al. (1978). Densitometry utilizes a light/dark binary measurement to categorize pixels as either above or below an established brightness threshold. In the case of the DAB-stained tissue, a threshold was established per section to remove the background and leave only MBP+ fibers in corpus callosum. The ICC was traced on the mounted tissue sections at 5x and imaged at 20x magnification using the Zeiss Axioskop2Plus microscope and Stereo Investigator software (MBF Biosciences). Images were acquired in gray scale and white balance was performed. Number of CC tracings per animal ranged from 5-8. Images were saved as TIFF files and converted into jpeg format before being uploaded into ImageJ (Schneider et al., 2012).

Optical densitometry (OD) calibration was performed following the protocol provided by ImageJ. This calibration converts darkness levels ranging across a particular gray scale to arbitrary OD values with a minimum value of 0 and a maximum of 2.6. The ICC was then traced per section per animal using the polymer tool, and with each section, a mean ICC OD value was recorded. Additionally, a mean OD value was recorded per section in a region of the cortex to account for background staining. The background staining mean value was then subtracted from the ICC mean OD value, to give a threshold OD value for each section within-animal. All mean threshold OD values were then averaged per animal, as well as a mean of the caudal

regions of the ICC. Caudal sections were marked based on the appearance of the dorsal hippocampal commissure.

5.3 Results

Analysis of histological data was performed using two-way ANOVA or nonparametric Kruskal Wallis tests when appropriate (IBM SPSS statistical software). Levene's Test to assess homogeneity of variances was run on each dataset *a priori* to determine the appropriate statistical test required to analyze each dataset.

Outliers falling outside of 1.5 times the interquartile range were discarded (max 3/group), and *post hoc* analyses were performed with Bonferroni correction for multiple comparisons. For comparison of cellular parameters measured on PD 30, pre-intervention, sex was included as an independent variable in the analysis. To compare similar parameters on PD 45, post-intervention, the data for each sex were analyzed separately to account for sexual dimorphisms in the data (n = 8-11; Lam et al., 2018).

5.3.1 Quantification of oligoglia and MBP production pre- and post-intervention

We discovered that the estimated number of OPCs remains unaffected by alcohol exposure in the body of corpus callosum pre- and post-intervention irrespective of sex ($p > .05$; **Fig. A, B, D**). However, the number of OPCs was increased following intervention exposure in female SI control rats only ($F_{1, 32} = 5.8$, $p = .02$, $np^2 = .15$; **Fig. 11D**). Since a similar effect of intervention exposure was not identified in alcohol-exposed rats, these data suggest that alcohol exposure during the BGS may limit the capacity for OPC proliferation in response to external stimuli in white matter regions in the adolescent female brain.

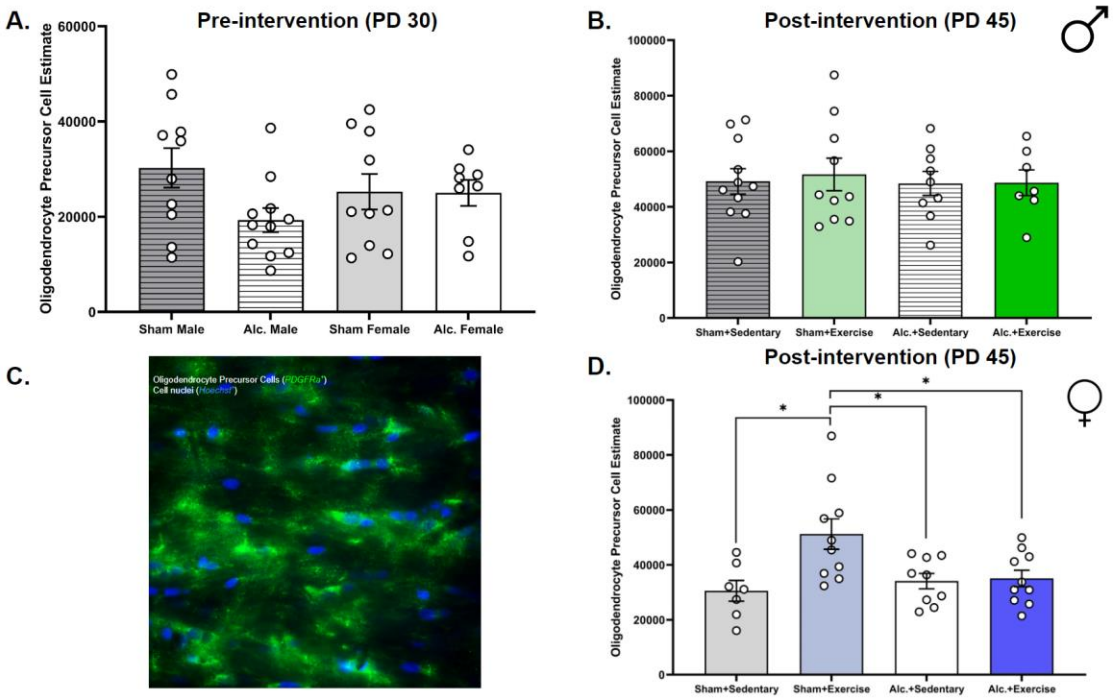

Figure 11. Depicted in the graphs above are the A) estimate of OPCs in the body of corpus callosum pre-intervention, B) estimate of OPCs in the body of corpus callosum post-intervention in male rats, and D) estimate of OPCs in the body of corpus callosum post-intervention in female rats. Each data point represents the mean estimate per rat and error bars represent ± SEM. Panel C shows the immunofluorescent stain used to visualize OPCs for unbiased stereological quantification in the body of corpus callosum. *$p \leq .05$, **$p \leq .01$,***$p \leq .001$; ****$p \leq .0001$

Conversely, there were significant main and interactive effects of the postnatal treatment group on the number of myelinating OLs in the body of corpus callosum pre- and post-intervention. These results were sexually dimorphic. Prior to intervention exposure, female rats from both postnatal treatment groups had the lowest number of OLs in corpus callosum compared to their male SI controls ($F_{1, 36} = 4.5$, $p = .04$, $np^2 = .11$; **Fig. 12A**). Alcohol exposure reduced the number of OLs in the male brain such that it was comparable to the number of myelinating glial cells in the

female brain on PD 30 ($F_{1, 36} = 5.7$, $p = .02$, $np^2 = .14$; **Fig. 12A**). Alcohol-exposed female rats that did not receive exercise intervention exhibited a reduction in the number of OLs on PD 42 ($F_{1,31} = 4.8$, $p = .04$, $np^2 = .14$, **Fig. 12C**). However, when provided with voluntary access to running wheels for twelve consecutive days, the number of OLs rose in the female brain to control levels ($F_{1,31} = 4.4$, $p = .045$, $np^2 = .12$). The highest number of OLs was found in the intervention-exposed sham female brain (**Fig. 12C**). Finally, the number of OLs in male brain did not differ between groups post-intervention (**Fig. 12B**), indicating that alcohol-induced reductions to the OL population were transient. These findings are convergent with those described by Newville and colleagues (2017).

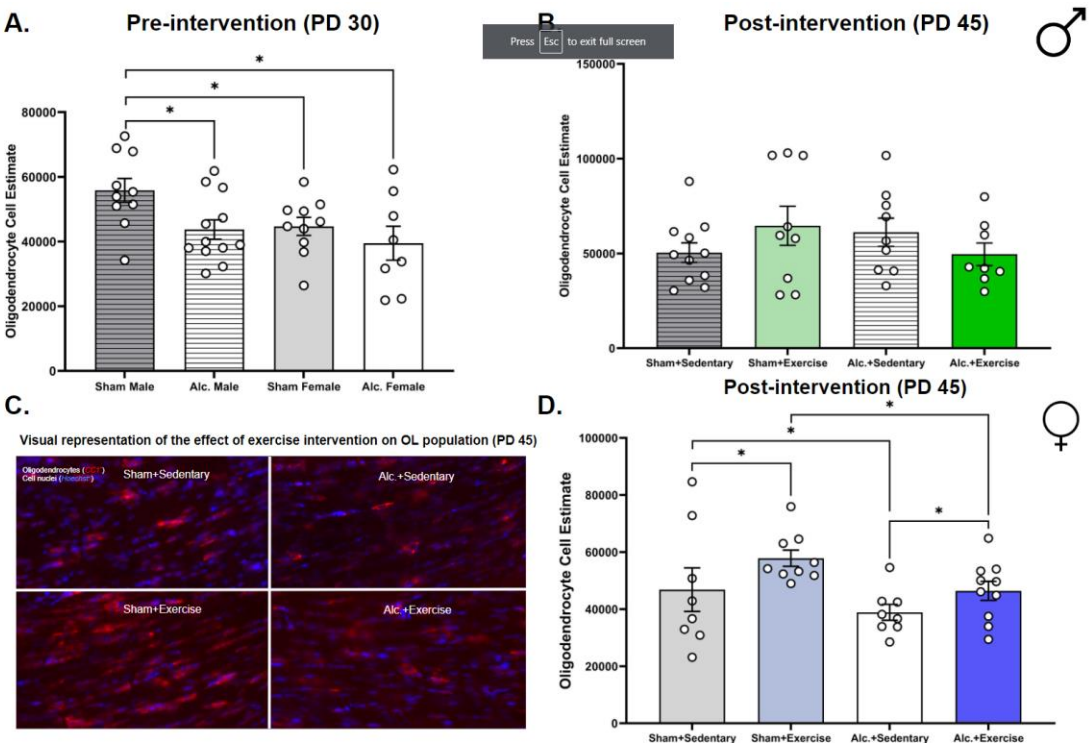

Figure 12: The graphs illustrate the A) estimate of OLs in the body of corpus callosum pre-intervention, B) estimate of OLs in the body of corpus callosum post-intervention in male rats, C) visual representation of the effect of exercise intervention on OL population on PD 45, and D) estimate of OLs in the body of corpus callosum post-intervention in female rats. Each data point represents the mean estimate per rat and error bars represent ± SEM. *$p \leq .05$, **$p \leq .01$,***$p \leq .001$; ****$p \leq .0001$

Densitometric analysis of MBP via comparison of optical density was investigated pre- and post-intervention (**Fig. 13**). Newville et al. (2017, 2021) demonstrated that MBP concentration was reduced in adolescent and adult male mice previously exposed to alcohol during the BGS. The reduction in MBP concentration was linked to deficits in axonal ensheathment by OL lamellae in the splenium subregion of corpus callosum. Thus, examination of MBP density in the whole body of corpus callosum and the splenium subregion was performed. We found that on PD

30, pre-intervention, there is a significant interaction between sex and postnatal treatment group on the optical density of MBP in both subregions (**Fig. 14 A, D**). Alcohol exposure increased MBP density in the female brain while the same exposure led to a reduction in the density of MBP in the male brain ($F_{1,35} = 4.3$, $p = .046$, $np^2 = .11$). MBP production is upregulated following pubertal onset, and these data suggest that alcohol exposure during the BGS may lead to precocial pubertal onset in females. Alterations to the timing of pubertal onset resulting from early-life stress have been shown to alter the trajectory of other neurodevelopmental processes such as synaptic pruning and cortical thinning (Juraska et al., 2020). Following exercise intervention, there is a significant interaction between postnatal treatment and intervention exposures on MBP density in the female body of corpus callosum. MBP density is highest in alcohol-exposed sedentary control rats and is restored to control levels by exercise intervention exposure ($F_{1,30} = 9.23$, $p = .005$, $np^2 = .24$; **Fig. 14C**), suggesting that the maturation of corpus callosum is stimulated by targeted adolescent intervention. Acute benefits of adolescent intervention on myelination of the whole body of corpus callosum are observed in female rats.

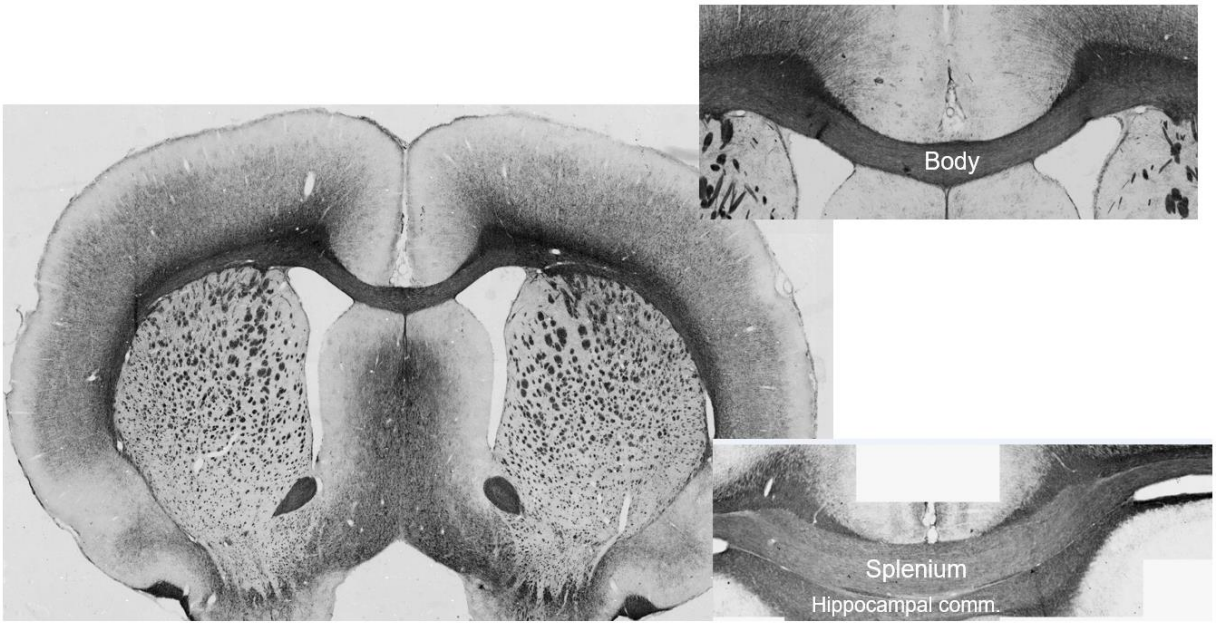

Figure 13. Representative images of the stain for visualizing MBP in the body and splenium of corpus callosum. Images were taken at 5X magnification.

Moreover, there existed a significant treatment by intervention interaction on MBP production in the body of the corpus callosum in male rats post-intervention ($F_{1,36} = 4.1$, $p = .05$, $np^2 = .10$; **Fig. 14B**). Post hoc analysis revealed that MBP density was increased in AE rats that received intervention exposure and was not different between rats from either postnatal treatment condition that did not receive intervention exposure ($p = .01$). Contrary to our original hypothesis, we did not observe any main or interactive effects of postnatal treatment or intervention exposure on MBP density in the splenium subregions of corpus callosum in either sex on PD 45($p > .05$; **Fig. 14 E-F**).

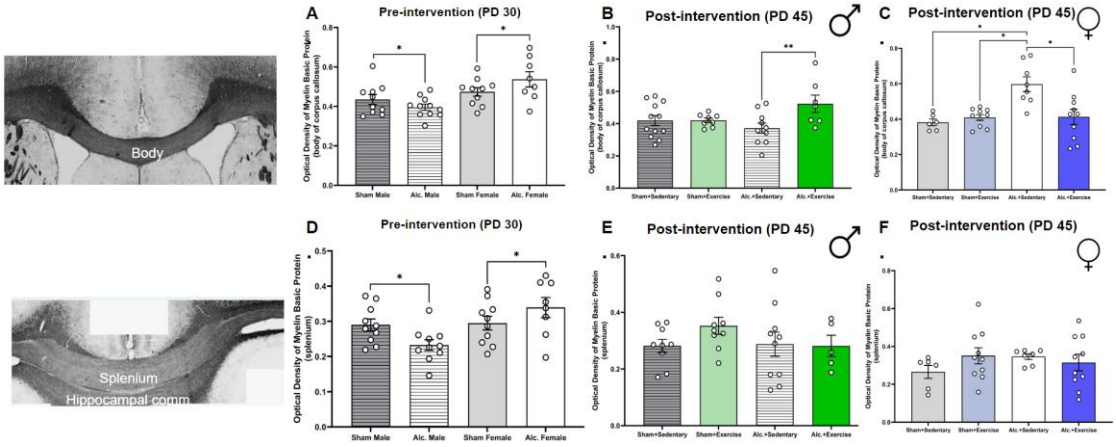

Figure 14. The graphs illustrate a comparison of the mean optical density derived from non-fluorescent labeling of MBP in the body of corpus callosum collected pre- and post-intervention (A-C). Similar comparisons are depicted in graphs D-F where optical density of the splenium was compared. Each data point represents the mean estimate per rat and error bars represent ± SEM. *$p \leq .05$, **$p \leq .01$,***$p \leq .001$; ****$p \leq .0001$

Volumetric analysis of the body of corpus callosum pre- and post-intervention indicates that AE limited the growth of this structure in adolescence. We found that AE rats from both sexes exhibited a smaller body of corpus callosum pre-intervention on PD 30 ($F_{1,36} = 5.4$, $p = .03$, $np^2 = .13$; **Fig. 15A**). There is a significant interaction between postnatal treatment group and intervention exposure on corpus callosum volume on PD 42 in the female brain ($F_{1,34} = 5.2$, $p = .03$, $np^2 = .13$; **Fig. 15C**). Post hoc analysis confirmed that corpus callosum volume increased in female sham rats exposed to the intervention, but not in their AE counterparts. The volume of the body of corpus callosum did not differ between groups in the male brain following intervention exposure (**Fig. 15B**). These results are partially divergent from volumetric analysis of this subregion using noninvasive neuroimaging (described in Chapter 4) and such discrepancies may result from either a lack of resolution in scanning images

or from the robust post-fixation process which leads to tissue shrinkage (Hackett et al., 2011).

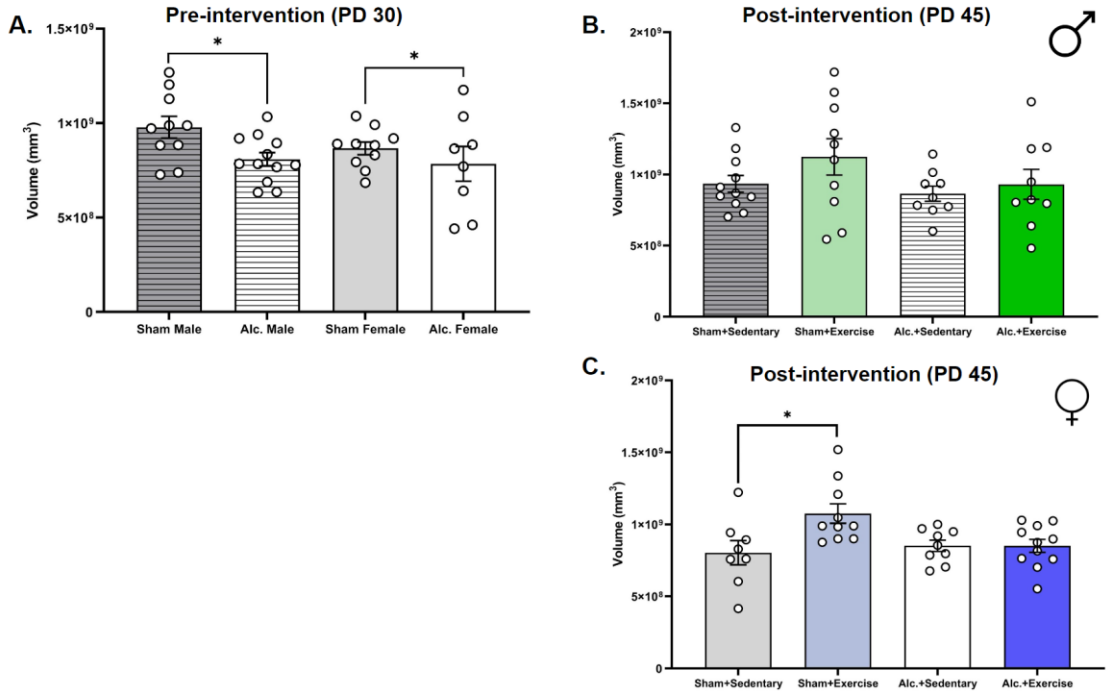

Figure 15. The graphs shown illustrate the A) estimate of the volume of the body of corpus callosum pre-intervention, B) estimate of the volume of the body of corpus callosum post-intervention in male rats, and C) estimate of the volume of the body of corpus callosum post-intervention in female rats. Each data point represents the mean estimate per rat and error bars represent ± SEM. *$p \leq .05$, **$p \leq .01$,***$p \leq .001$; ****$p \leq .0001$

5.3.2 Correlation of myelin basic protein density and radial diffusivity in the body of corpus callosum

One of the main objectives of this dissertation was to validate *in vivo* neuroimaging results with *ex vivo* quantification of cellular features using histology. Radial diffusivity values extracted from diffusion tensor imaging scans describe the

movement of protons perpendicular to white matter bundles. We discovered that alcohol exposure during the rat BGS elevates the radial diffusivity of protons moving perpendicular to corpus callosum fiber bundles when measured in the adolescent brain. Exercise intervention had no immediate effect on radial diffusivity in either sham or alcohol-exposed rats from both sexes. However, aerobic activity is a known effector of myelin cytoarchitecture (Tomlinson et al., 2016, 2018). Thus, we predicted that microscopic alterations to corpus callosum myelination might be observed immediately following intervention, indicating a lack of sensitivity to structural changes in our *in vivo* scanning protocol.

We compared values of radial diffusivity to the density of MBP in the body of corpus callosum post-intervention within-rat. Two-tailed partial correlation analysis controlling for treatment and intervention groups revealed that MBP density was not correlated with radial diffusivity in the female brain post-intervention. However, we did discover that these measures were positively correlated in the male brain post-intervention ($R = 0.50$, $p = .02$). This is likely due to the fact that exercise intervention mitigated the impact of alcohol teratogenesis on MBP density in the female adolescent brain, but a similar effect was not identified when comparing radial diffusivity values between groups. Moreover, several cellular features contributing to changes in myelination are changed in the female brain post-intervention. Thus, we would not expect MBP density only to be directly correlated with changes to radial diffusivity in the female brain. Conversely, the positive correlation between MBP density and radial diffusivity in the male brain suggests that changes to MBP density within-rat have corresponding changes to radial diffusivity. It should be noted that MBP density is the only myelin-related measure obtained that was changed following exercise

intervention in the male body of corpus callosum. However, this effect was only demonstrated in the AE brain.

5.4 Discussion

To summarize, we examined several cellular features contributing to myelination to investigate the immediate effect of adolescent exercise intervention on white matter development in a rat model of FASD. In Aim 1 (Chapter 4), analysis of radial diffusivity values from *in vivo* longitudinal neuroimaging illustrated that alcohol exposure during the brain growth delayed corpus callosum myelination in the adolescent male and female brain. In Aim 2 (Chapter 5), histopathological analysis uncovered that adolescent exercise intervention did have some immediate benefits on corpus callosum myelination and these effects were sexually dimorphic.

There is no evidence to suggest that AE during the BGS significantly altered the number of OPCs in the male or female corpus callosum or the number of OLs in the female brain pre-intervention. Despite this, AE reduced the number of OLs, the density of MBP, and the overall volume of the body of corpus callosum in the male brain on PD 30. Unexpectedly, alcohol exposure significantly increased MBP density yet decreased the overall volume of corpus callosum in the female brain pre-intervention.

Several significant effects of exercise intervention were discovered. In the male brain, intervention exposure increased MBP density in the body of the corpus only in AE rats. Conversely, adolescent exercise intervention had a much greater effect on the underlying neurobiology of corpus callosum in the female brain. Voluntary wheel running reversed the sustained AE-produced reduction in OL number. The population of corpus callosum OLs was the highest in SI control rats that

underwent intervention. Moreover, the number of OPCs was significantly increased in SI controls rats with voluntary access to a running wheel. AE appeared to limit OPC proliferation in response to this myelinogenic intervention, suggesting that myelin plasticity is lowered by alcohol teratogenesis. Taken together, these results suggest that exercise intervention had a modest effect on increasing myelination in the alcohol exposed female brain: it stimulated the differentiation of a proportion of OPCs to OLs, but did not upregulate robust OPC proliferation and maturation as is apparent in the SI control brain following exercise intervention. This is further supported by the normalization of MBP density to control levels in AE female rats with access to the aerobic intervention.

5.4.1 Cellular response to adolescent exercise intervention is sexually dimorphic due to underlying differences in the trajectory of myelination in the male and female brain

Refinement of the neural connectome is inextricably linked to pubertal onset in mammals. Synaptic pruning of aberrant connections and the insulation of the remaining pathways optimizes communication between brain regions to support adult cognition. It is well-established that the influx of progesterone production and hormone signaling resulting from pubertal onset alters the landscape of white matter cytoarchitecture and the activity of myelinating glia (Hussain et al., 2011; González-Orozco, 2019). For example, corpus callosum volume is increased in the male and female brain during adolescence and is correlated with increased testosterone production in the human and rat brain (Chavarria et al., 2014; Markham et al., 2007).

The timeline of pubertal onset differs between male and female mammals and partially contributes to sexually dimorphic trajectories for neurodevelopment. Pubertal onset naturally occurs earlier in female mammals and fluctuations in hormone

signaling are regulated by the menstrual cycle (estrus cycle in rodents). However, early-life stress and drug teratogenesis has been shown to affect the timing of pubertal onset, leading to precocial or delayed development of brain regions (Tang et al., 2019; Rao et al., 2010; Honeycutt et al., 2020; Strzelewicz et al., 2019). Kar et al. (2021) demonstrated that prenatal AE leads to precocial development of white matter tracts in late childhood.

We present seminal findings describing the effects of a voluntary adolescent exercise intervention on the trajectory of myelination in the female brain using a rat model of FASD. Collectively, these results show that the effectiveness of behavioral interventions for youth affected by FASD may vary by sex. Pubertal onset occurs in female rats around PD 30 and in male rats around PD 45 (Herting et al., 2017; Juraska et al., 2020). Thus, it was expected that this intervention would affect the trajectory of myelination differently in the female and male rodent brain. Histological analysis of OPC number, OL number, and MBP density pre- and post-intervention supported the findings by Newville et al. (2017): alterations to oligoglia population were transient in the male brain and lasting reductions to MBP density were observed. In addition, these results demonstrated an immediate positive effect of exercise intervention on mitigating alterations to MBP density in AE male rats. It is possible that connectome refinement begins with pubertal onset (after the intervention period) in the male brain, thus examination of the impact of a longer intervention period on corpus callosum myelination could be considered for future studies. In contradiction, the female brain appeared to be more vulnerable to the lasting impact of alcohol teratogenesis on white matter development and was immediately more sensitive to intervention exposure. AE rats exhibited reduced OL population and elevated density of MBP. Both structural

anomalies were rectified with adolescent intervention. Notably, prenatal alcohol exposure has been linked to aberrant synaptic pruning which could account for the elevated levels of MBP in AE rats in the sedentary control condition (Smith et al., 2022; Treit et al., 2014). Thus, our results suggest that adolescent intervention did target the second peak in myelination and was beneficial in supporting the proper development of white matter in the FASD-affected brain. These results may be due, in part, to the greater total distance run by female rats from both postnatal treatment groups compared to their male counterparts. More research is needed to evaluate the lasting impact of adolescent exercise intervention on white matter structure and function in adult females.

5.4.2 MBP density in splenium is transiently affected by alcohol exposure during the brain growth spurt

There is ample evidence from clinical and preclinical studies suggesting that the proper development and function of the splenium subregion of corpus callosum is particularly vulnerable to prenatal AE (Treit et al., 2017; Blaauw et al., 2020). Specifically, Newville and colleagues (2021) demonstrated that MBP production and myelin sheath wrapping were negatively affected in adult mice model of FASD. Nevertheless, separate analysis of MBP density in the splenium of male and female rats in our model of FASD uncovered a transient effect of AE during the BGS on this measure. Future research is needed to assess if there is any evidence of alteration to ensheathment wrapping in the splenium. Such results would illuminate a potential neurobiological mechanism that might contribute to the observed alcohol-related deficits in visuospatial processing and sensory integration.

Chapter 6

EXAMINING THE POTENTIAL THERAPEUTIC EFFECT OF ADOLESCENT EXERCISE INTERVENTION ON THE FUNCTIONAL CONNECTIVITY BETWEEN BRAIN REGIONS CONNECTED BY SPLENIUM AXONS IN ADULTHOOD IN A RODENT MODEL OF FASD

6.1 Introduction

In the previous two chapters, it is shown that AE during the BGS delays the trajectory of myelination in adolescence, affecting late circuit refinement. Histopathological analysis of cellular features relating to myelination revealed that white matter development in the female brain is more vulnerable to alcohol teratogenesis compared to their male counterparts. Furthermore, a targeted exercise intervention in adolescence appeared to immediately support the maturation of OPCs and regulate MBP production in female AE rats. Exercise intervention increased MBP density above control levels in AE male rats, however there was no evidence of a detrimental effect of alcohol on OPC number, OL number, MBP density or volume of the body of corpus callosum in the male brain on PD 45. These results may be due, in part, to the greater physiological effect of running intervention in female rats from both postnatal treatment groups as they consistently ran significantly more compared to their male counterparts.

Collectively, these findings inspired one final research question: what are the lasting benefits of adolescent exercise intervention on corpus callosum structure and function in the female brain? In Experiment 2 of Aim 1, the original experimental timeline was extended to include the period of young adulthood following intervention

exposure. Instead of scanning pre- and post-intervention, only one scanning session in adulthood was required and the scanning protocol was modified to include a resting-state functional MRI scan to assess regional and global changes to functional connectivity. Following *in vivo* MRI analysis, analysis of *ex vivo* brain tissue was completed using electron microscopy to detect changes in myelin sheath wrapping. As in Newville et al. (2021), cross sections of axons in the splenium that connect the brain hemispheres were examined. We predicted that adolescent exercise intervention would promote the maturation and myelination of splenium axons in the female brain.

Examination of the effects of AE on myelin ultrastructure in several mammalian models of FASD has revealed that the g-ratio of axons traveling through white matter tracts is increased in the AE brain. The g-ratio is a comparison of the diameter of the axon to the diameter of the axon plus the myelin sheath. The g-ratio describes the appropriate laminar distribution of myelin sheaths while accounting for changes in axon diameter, both of which contribute to conduction velocity by altering the capacitance or the resistance of the axon, respectively. For example, Newville et al. (2021) discovered that the g-ratio was increased when assessed in the splenium of male mice in a model of FASD. These findings were coupled with a reduction to MBP density in the splenium, suggesting that the myelin sheath was thinner in the FASD brain compared to controls.

Results from electron microscopic analysis of the myelination of axons in the female splenium were compared to global resting-state BOLD patterns between cortical hubs connected by splenium axons in rats from each group. Resting-state functional neuroimaging quantifies regional changes to BOLD signals in the absence of a stimulus. Patterns in activation coupling comprise a global functional connectivity

network that can be organized into a map of hubs. Comparison of the number of connections between specific hubs and the global connectome using graph theory is termed a degree of centrality analysis. We predicted that the degree of centrality between splenium-relevant hubs and the global connectome would be affected by AE and adolescent exercise intervention. Additionally, a separate analysis of local intra-network connectivity was conducted to compare the relative functional connectivity between hubs of one network.

6.2 Methods

6.2.1 Rat Model of FASD

The methodology for modeling FASD in rats and for the administration of voluntary exercise intervention in adolescence is described in detail in Chapters 4 and 5. The timeline below demonstrates that only one set of scans was acquired per rat to assess *in vivo* changes to global and local resting-state functional connectivity in adulthood. There were 8 rats generated per group.

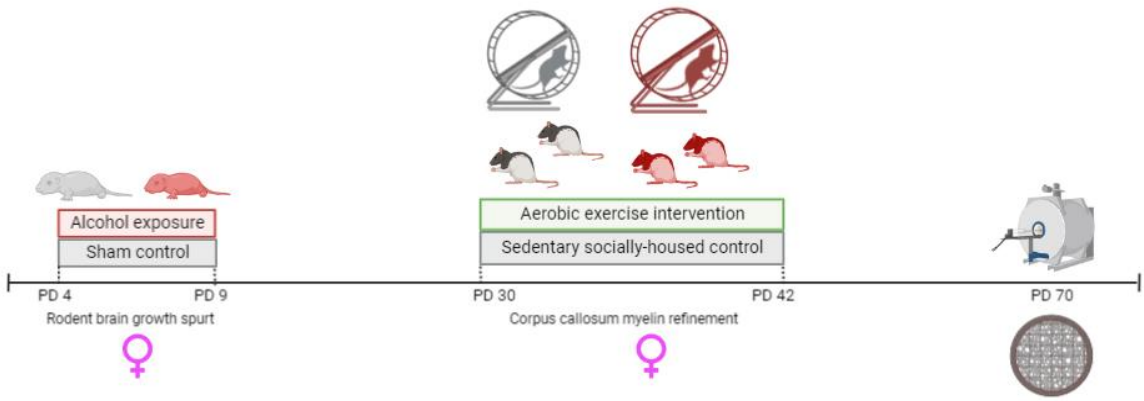

Figure 16. A timeline depicting the periods of alcohol exposure, adolescent intervention, scanning, and tissue extraction in Experiment 2 of Aim 1.

6.2.2 Resting-state functional magnetic resonance imaging

On PD 70, *in vivo* scanning of anesthetized rats was conducted using a 9.4T Bruker Biospec scanner housed in the Center for Biomedical and Brain Imaging at the University of Delaware. During scanning, all rats were anesthetized with continuous ventilation of 1-3% isoflurane in oxygen to maintain an average 50 breaths per minute. When necessary, fluid circulating through tubing in the scanner bed was heated to prevent hypothermia while under anesthesia. Core temperatures were continuously monitored using a rectal thermometer and maintained at 32-36℃. Total scanning time did not exceed 1.5 hours per rat.

The scanning protocol included a diffusion tensor imaging scan and a resting-state functional MRI scan resulting in a total scan time of approximately 30 minutes per rat. The parameters for each scan are described in detail in Kulkarni et al. (2020) at a resolution of 0.312 × 0.312 × 1.2 mm. Lasting changes to functional connectivity with connectomics were assessed in adulthood. The data were preprocessed using AFNI (NIHM, Rockville, MD), FSL (FMRIB, Oxford, UK), DRAMMS (SBIA,

Philadelphia, PA), and MATLAB (Mathworks, Natick, MA) and registered to a 3D MRI rat atlas with 173 segmented brain areas providing site-specific information on BOLD fluctuations between groups. Brain tissue masks were hand-drawn using 3D Slicer (https://www.slicer.org/) and skull-stripping was applied to each dataset.

6.2.3 Electron microscopy

Following scanning, rats were transcardially perfused with 50mL of 0.14M sodium cacodylate buffer solution (pH = 7.3) followed by 250mL of fixative (2% glutaraldehyde-2% paraformaldehyde in 0.14M sodium cacodylate buffer containing 1.5mM magnesium sulfate, pH = 7.3) at room temperature for preparation for electron microscopic analysis. The protocol was modified from that described in Weller et al., 1997 and Briones et al., 2004. Brain tissue was post-fixed in fixative solution and, once fixed, a sagittal block of tissue containing the interhemispheric fibers of the corpus callosum was microdissected from the brain. Blocks were adhered to a chuck using superglue and sectioned at 200μm thickness using a vibratome. Two sections from each rat were collected for embedding into resin.

The splenium with some connected cortex was dissected from the thick section. These tissue pieces were placed in tissue baskets designed for resin embedding and tightly stacked on a metal stand to facilitate transfer of sections between solutions during the embedding process. Once in the baskets, sections were rinsed twice with 0.14M sodium cacodylate buffer for 10 minutes and impregnated in an 2% osmium tetroxide solution in .014M sodium cacodylate buffer for 1 hour at room temperature. Sections were rinsed again with 0.14M sodium cacodylate and then washed on ice for 6 mins with a 0.1N sodium acetate solution, incubated for 30 mins with a 0.5% aqueous uranyl acetate solution, and rinsed again for 6 mins with a 0.1N

sodium acetate solution. Next, sections were dehydrated on ice using solutions with ascending concentrations of ethanol (5 mins 30% ethanol, 5 mins 50% ethanol, 5 mins 70% ethanol, 10 mins 80% ethanol, 10 mins 95% ethanol, and 30 mins in absolute ethanol). Following dehydration, sections were incubated in propylene oxide for 30 mins at room temperature with rotation. All following steps were complete with rotation. Sections were embedded with hard resin made from an Electron Microscopy Sciences embedding kit (cat.14120). First, sections were soaked in a 1:1 solution of resin to propylene oxide overnight. Infiltration continued by soaking the tissue in a 1:3 solution of propylene oxide to resin for 8 hours. Finally, sections were incubated in 100% resin overnight. Sections were carefully placed into plastic embedding molds, the molds were filled with resin and then the molds were placed in an oven at 45℃ to begin the polymerization process. After 24 hours, the oven was turned up to 60℃ for 48 hours.

Once the resin blocks cooled, 1μm sections were collected using a Leica UC7 microtome and stained with toluidine blue to identify the correct location for ultrathin section collection. Once cross-sections of fibers within the splenium were identified, 65nm ultrathin sections were collected onto Formvar carbon film grids and grids were post-stained to visualize myelin laminae. 12μm x 12μm montages were captured at 6,300x magnification using a Zeiss Libra 120 Transmission Electron Microscope and analyzed using MyelTracer software (Kaiser et al., 2021). The diameter of the axon and the fiber were measured.

6.2.4 Statistical analysis

6.2.4.1 fMRI results

The degree of centrality, the number of connections to specific nodes, was compared between groups using two-way ANOVA or nonparametric Kruskal Wallis tests when appropriate (GraphPad, Prism). Post hoc analyses were performed using Tukey's test for multiple comparisons or Dunn's test for multiple comparisons, respectively.

6.2.4.2 Electron microscopy

Analysis of ultrastructural data was performed using two-way ANOVA or nonparametric Kruskal Wallis tests when appropriate (IBM SPSS statistical software). Outliers falling outside of 1.5 times the interquartile range were discarded, and post hoc analyses were performed with Bonferroni correction for multiple comparisons.

6.3 Results

Electron microscopic analysis of axonal ensheathment in splenium revealed that the numbers of typically and atypically myelinated axons do not differ significantly between groups ($p > .05$; **Fig. 17A-B**). However, axonal diameter was reduced in alcohol-exposed adult female rats without adolescent intervention exposure. Aerobic exercise reversed this effect in AE rats such that axon diameter was comparable to that identified in SI control rats ($F_{1,22} = 11.1$, $p = .003$, $np^2 = .34$; **Fig. 17C**). This finding suggests that adolescent exercise intervention supports the maturation of splenium axons in the FASD brain. However, comparison of the weighted g-ratio between groups demonstrated that axonal efficiency was conserved across groups despite the observed difference in axon diameter ($p > .05$; **Fig. 17D**).

This indicates that corresponding alterations to myelin sheath thickness occurred in alcohol-exposed rats that did not receive the intervention. Based on the equation for the g-ratio, we expect that the myelin sheath of splenium axons in these rats is thinner. The weighted g-ratio includes measurements of the axon and fiber diameters from typically and atypically-myelinated axons which are weighted based on the number of each type of myelinated axon in the splenium.

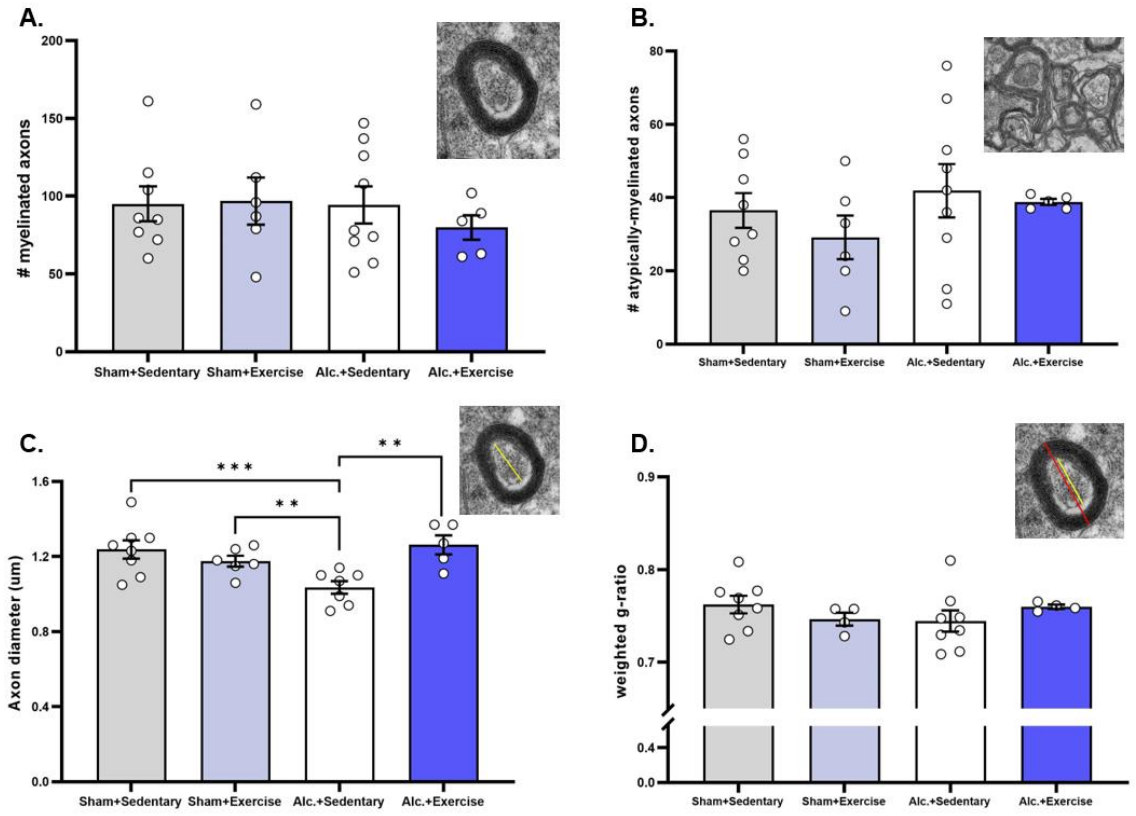

Figure 17. Results from electron microscopic analysis of splenium axons in adult female rats from all treatment and intervention groups. There are no main or interactive effects of postnatal treatment or intervention exposure on the average number of typically myelinated axons (A), the average number of atypically-myelinated axons (B) or the weighted g-ratio of the axons (D). However, there exists a significant interaction between postnatal treatment group and intervention exposure on axon diameter (C). Ultrastructural characteristics (C-D) were measured on 50-200 axons per rat and averaged resulting in one data point per rat as shown. Error bars represent ± SEM. $*p \leq .05$, $**p \leq .01$, $***p \leq .001$; $****p \leq .0001$

Functional data were organized into a global functional connectivity network. Callosal axons facilitate interhemispheric transfer of cortical information, and, thus, cortical hubs were extracted from the global network. In the first set of analyses, the degree of centrality was assessed between salient cortical regions connected via axons

traveling through the splenium (visual, temporal, parietal, and caudal retrosplenial cortices). The degree of centrality describes the number of connections stemming from one particular hub to other hubs within the global (whole brain) network. We found that the degree of centrality of splenium-relevant cortical hubs is not significantly different between groups ($p > .05$; **Fig. 18**).

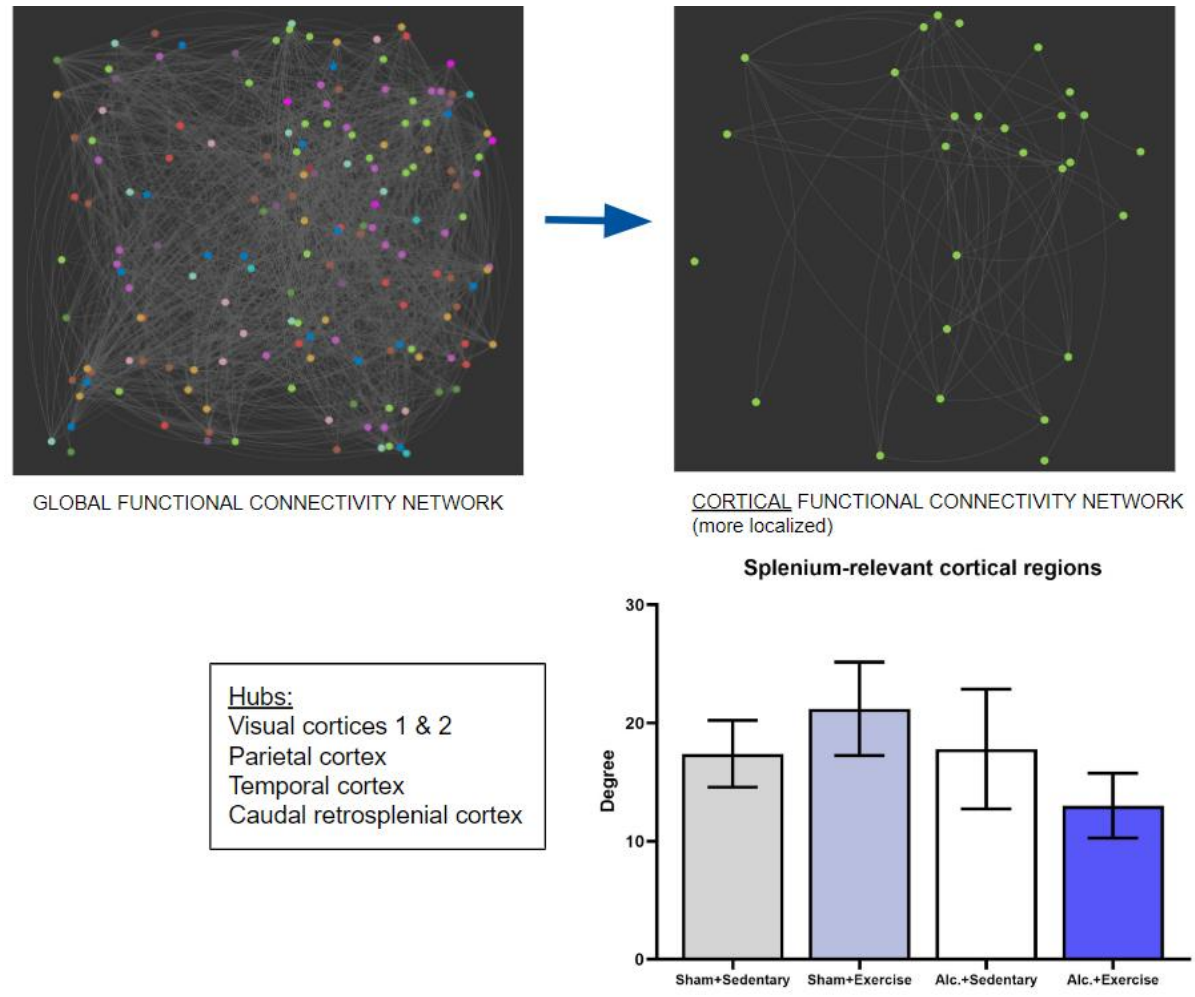

Figure 18. Representation of the global and cortical functional connectivity networks created using Polinode. Each hub is represented by a dot and hubs from similar regions of the brain (i.e., cortex) can be identified by color. The graph depicts the degree of centrality between splenium-relevant cortical hubs.

It is possible that the connectivity between splenium-relevant cortical hubs only is affected by AE or intervention. Thus, a local network analysis was performed. Comparison of intra-connectivity reveals that the networks are largely similar between groups as shown ($p > .05$; **Fig. 19**).

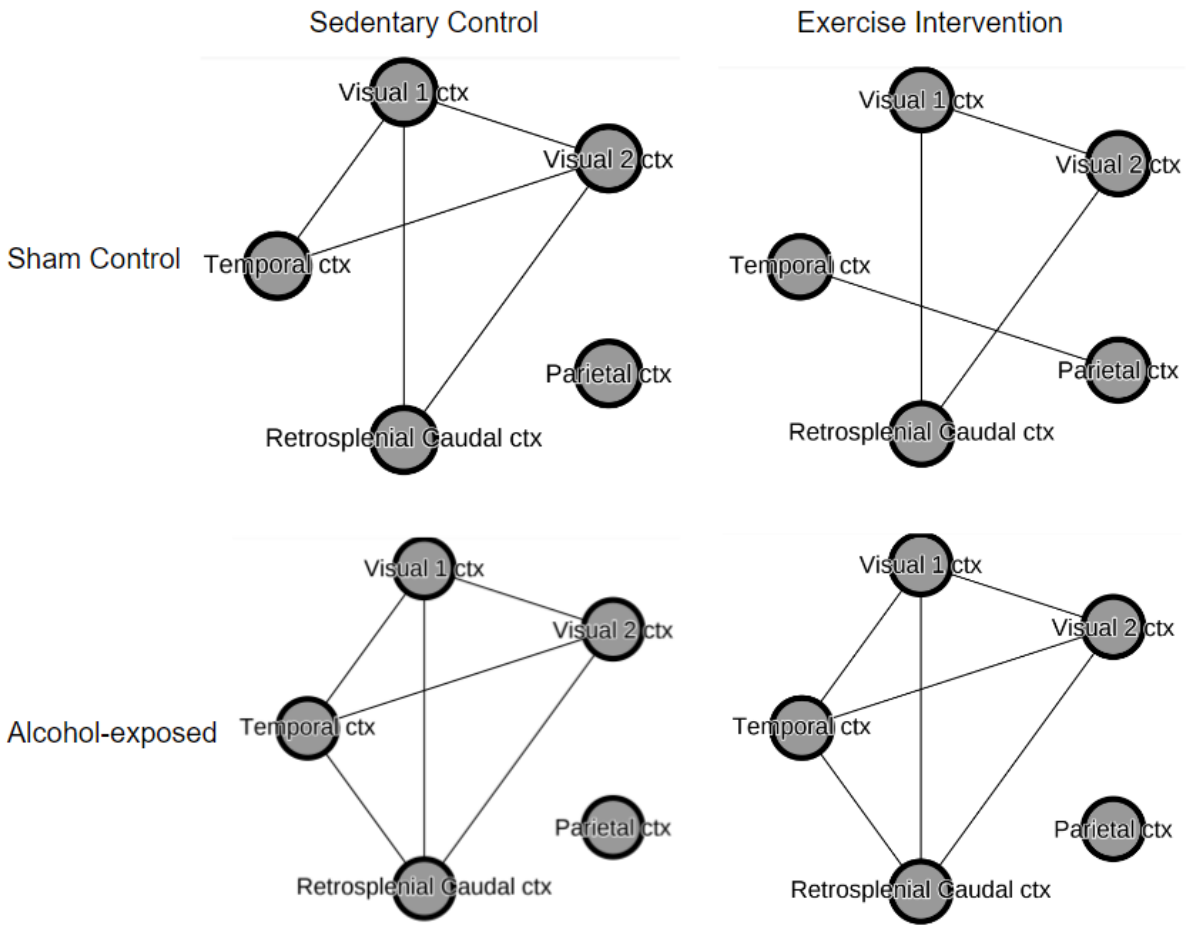

Figure 19. Intra-connectivity networks by group including splenium-relevant hubs.

One central component of visuospatial processing is the capacity to voluntarily direct attention towards a visual stimulus for immediate recall and integration of information pertaining to the stimulus. Children and youth affected by FASD have poor function of the executive control network responsible for directing attention (Paolozza et al., 2014). Ware and colleagues (2021) discovered that children and adolescents with FASD exhibited altered levels of functional connectivity in the executive control network. Longer anterior-posterior pathways showed lower functional connectivity while shorter pathways had higher degrees of functional connectivity. Analysis of the degree of centrality of cortical regions in the executive control network indicates that AE rats without access to the adolescent intervention had a higher overall degree of connectivity compared to controls ($F_{1,32} = 13.77$, $p = .003$; **Fig. 20**). Moreover, adolescent exercise intervention mitigated this relationship in adult females as depicted in the graph below.

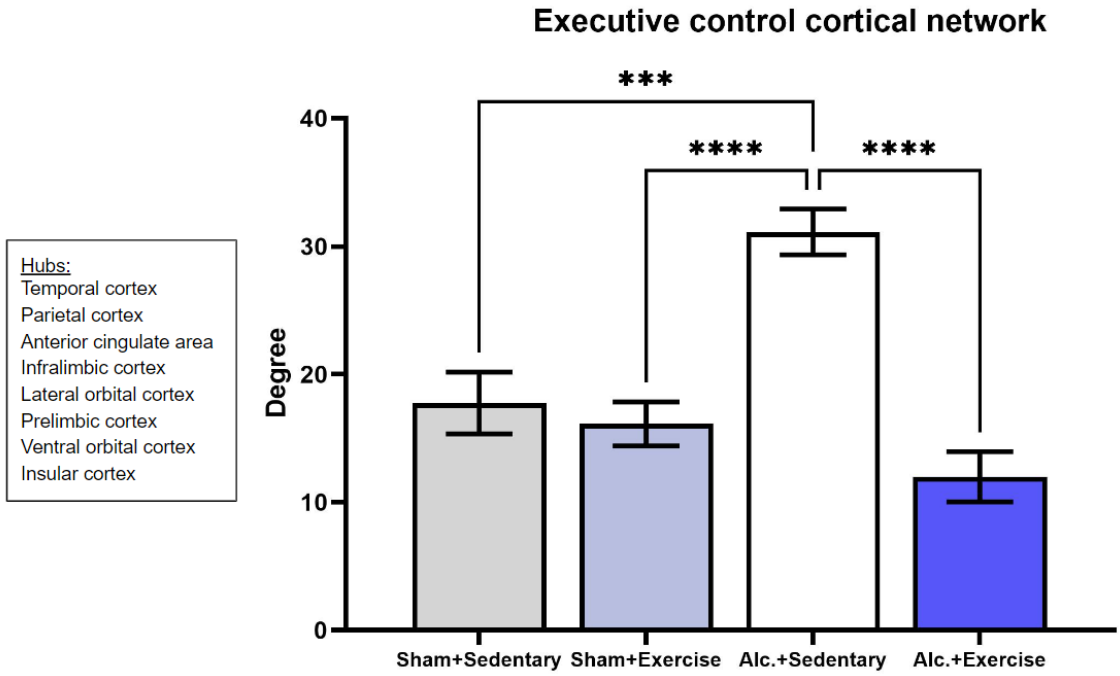

Figure 20. Graph depicting a comparison of the degree of centrality of cortical hubs involved in the executive control network with the global network. *$p \leq$ .05, **$p \leq$.01,***$p \leq$ .001; ****$p \leq$ .0001

Analysis of the intra-connectivity of the cortical hubs of the executive control network reveals that this local network in the AE sedentary control brain is significantly different from that in AE rats that received the adolescent exercise intervention ($F_{1, 32} = 13.77$, $p = .003$; **Fig. 21**). Adult female rats that received alcohol exposure during the brain growth, but did not receive adolescent exercise intervention had the largest degree of local connectivity in the executive control network.

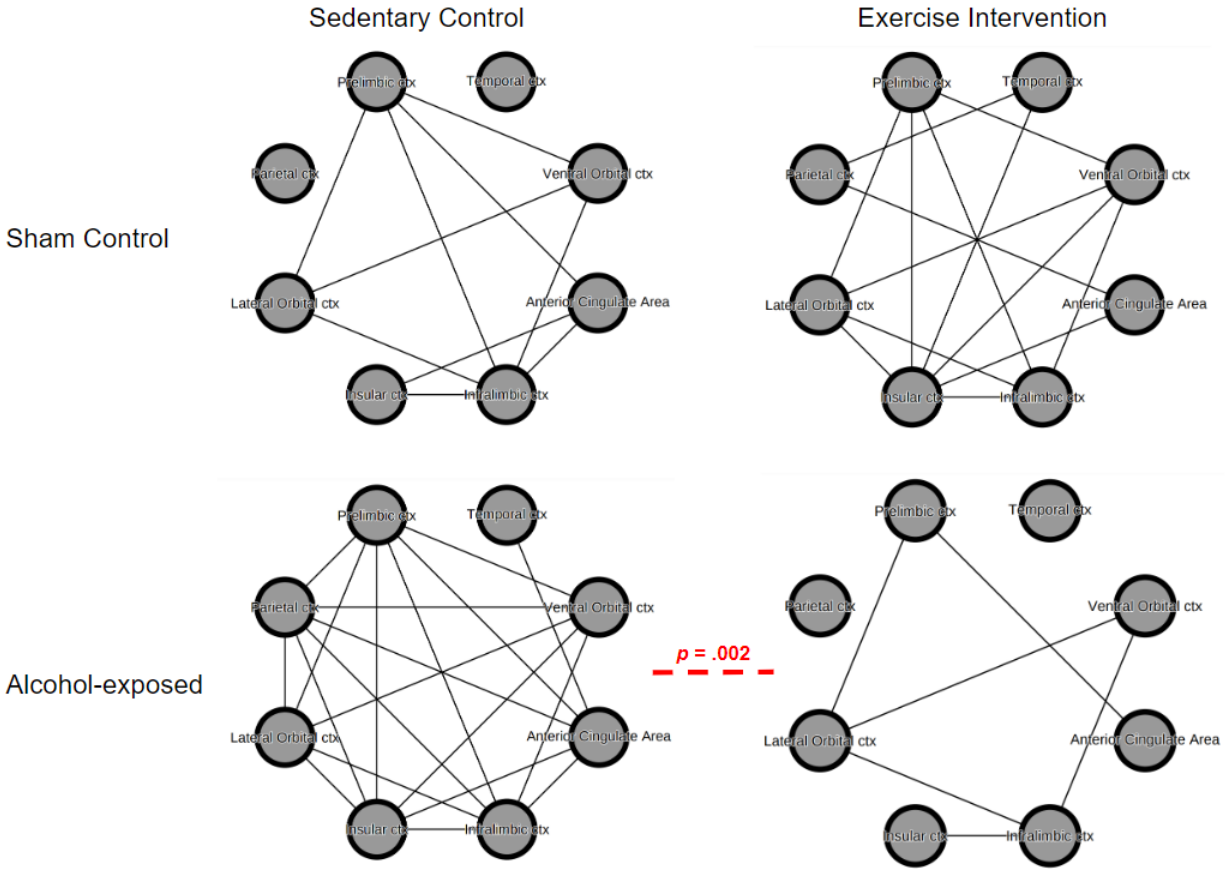

Figure 21. Intra-connectivity networks by group including executive control network cortical hubs.

6.4 Discussion

In the two previous chapters, we have shown that adolescent myelination of the corpus callosum is delayed in a rat model of FASD. Several acute cellular changes resulting from a myelinogenic aerobic exercise intervention administered in adolescence were identified. This study examined the potential lasting benefit of exercise intervention on the structure and function of the splenium, a callosal subregion with high vulnerability to alcohol teratogenesis. Ultrastructural analysis of axonal maturation and ensheathment in the female adult brain demonstrated that the

number of typically and atypically myelinated axons did not differ between groups. Conversely, comparison of the mean axon diameter revealed that AE rats without access to the intervention had significantly smaller axons in adulthood compared to SI controls. This effect was mitigated by adolescent exercise intervention, suggesting that increased aerobic activity stimulated axonal maturation. Moreover, the weighted g-ratio which compares the axon diameter to the diameter of the axon plus the myelin sheath did not differ significantly between groups, indicating that a corresponding reduction to myelin thickness occurred in AE rats without intervention access.

Visuospatial processing is impaired in children and youth with prenatal alcohol exposure (Ware et al., 2021; Willford et al., 2006). Integration of information captured in the occipital, temporal, insular, and parietal cortices is critical for visuospatial processing. The splenium contains axons connecting these cortical regions with a majority of these axons facilitating interhemispheric communication between the visual cortices. Results from analysis of resting-state functional MRI scanning revealed that the global and intra-network functional connectivity of cortical regions connected via axons traversing the splenium is not different between groups. However, it is well-documented that the proper function of attention networks, such as the executive control network, is required to direct an individual's focus towards a specific visual stimulus for optimal visuospatial processing. In this experiment, analysis of resting-state data from this circuit illustrated that the degree of global functional connectivity between the cortical hubs of this network and the rest of the brain was the highest in AE rats without access to the exercise intervention. This effect was prevented by adolescent intervention. Additionally, comparison of the local connectivity between the hubs of the executive control network in each group revealed

that adolescent exercise intervention had the greatest effect on re-shaping these patterns in the AE brain.

Work from the Klintsova lab and others has demonstrated that AE during the BGS increases cortical connectivity in the rat brain (Smith et al., 2022). Aberrant synaptic pruning in late childhood results in greater synaptic connectivity which may impede communication between brain regions in adulthood. Increased "noise" due to hyperconnectivity with redundant synapses reduces circuit efficiency and disrupts functional connectivity (Zhou et al., 2011; Stiles et al., 2020). Thus, increased functional connectivity could be contributing to the visuospatial impairments observed in rats modeling FASD.

6.4.1 Conserved g-ratio despite changes to axon diameter

As the brain grows, axons lengthen and axonal insulation is required for fast conduction of action potentials between discrete brain areas. The modification of myelin sheath organization to optimize signal transmission during development or adaptive periods in adulthood is termed fiber length scaling (Yang et al., 2021). Other features of the axon architecture drive changes in myelination, including alterations to axon diameter or the number of axons recruited within a putative circuit (Xin and Chan, 2020). Assumedly, increasing myelin laminar organization of axons within a discrete circuit would result in the fastest conduction speeds due to a significant corresponding increase in axon capacitance. However, there exist several constraints on myelin plasticity that prevent limitless myelination. First, total brain volume is constrained by the size of the cranium. Minor changes to parenchymal volume have been shown to offset cerebral spinal fluid volume and flow, deleteriously impacting intracranial pressure and the fluid dynamics responsible for waste clearance within the

central nervous system (Maxeiner & Behnke, 2008). Second, Salami and colleagues demonstrated that variations in myelin patterning along individual axons is necessary to tune axon synchrony for coordination of signals to create phasic brain rhythms necessary for complex processing (Salami et al., 2003). Thus, conduction speeds, and therefore myelin patterning, are optimized for the efficiency of coordinated network communication.

In 2009, Chomiak and Hu created a theoretical mathematical model describing the optimal thickness of myelin ensheathment around callosal axons in the adult rodent brain. They predicted that the g-ratio of such axons would be approximately 0.77 in the adult rat brain. We have shown that the average g-ratio of splenium axons in the adult female brain is about 0.77 irrespective of treatment and intervention group. However, we discovered that the diameter of splenium axons remained reduced in adult AE rats without adolescent intervention exposure; this effect was mitigated by adolescent intervention. Taken together, we expect that corresponding changes were made to the myelin sheath to result in similar weighted g-ratios between rats in the four groups. We hypothesize that conduction speed is much slower in the examined splenium circuit in AE rats without adolescent intervention (Firmin et al., 2013).

Chapter 7

CONCLUSIONS, IMPLICATIONS, AND FUTURE DIRECTIONS

The central goal of this dissertation was to investigate the potential of a targeted exercise intervention to stimulate white matter maturation in the FASD brain. As aforementioned, white matter maturation is one of the longest developmental processes in the mammalian brain, spanning from late gestation to 40 years of age in humans (Brenhouse et al., 2011). Thus, the use of an established rat model of FASD was integral for the examination of white matter development across several time points. A multimodal approach consisting of two aims uncovered the immediate and lasting effects of intervention exposure on corpus callosum maturation via measurements of structural and functional integrity.

White matter maturation in adolescence and young adulthood was assessed in a rat model of FASD using *in vivo* longitudinal MRI scanning in Aim 1. Scanning time points were chosen to evaluate the capacity for adolescent exercise intervention to stimulate myelination in the forebrain. Results from Experiment 1 suggested that the trajectory of corpus callosum myelination was delayed in AE rats pre- and post-intervention irrespective of sex. Specifically, radial diffusivity values, obtained with longitudinal Diffusion Tensor Imaging scanning, remained elevated in the corpus callosum of AE male and female rats on PD 30 and 45, suggesting that axons were hypomyelinated. These findings are convergent with results from Newville et al. (2017) who demonstrated that AE during the BGS reduced corpus callosum myelination in adolescent male mice as evidenced by elevated radial diffusivity values

when measured in white matter *in vivo*. Notably, results from Experiment 1 did not uncover an immediate benefit of voluntary exercise intervention on rescuing radial diffusivity values in AE rats.

To validate the results from noninvasive neuroimaging, oligoglia lineage cell populations and MBP density were quantified pre- and post-intervention in Aim 2. Preliminary results from an ongoing study evaluating the immediate effect of AE during the BGS on oligoglia population in the Klintsova lab suggests that OL maturation is limited in infant and juvenile corpus callosum (on PD 10 and 15, respectively) in response to alcohol teratogenesis. Importantly, the number of mature OLs is reduced first in the female FASD brain on PD 10 and in both sexes by PD 15, illustrating a sexual dimorphism in the immediate vulnerability of white matter development to AE. It is hypothesized that differences in the turnover rates of OLs in male and female mammalian white matter (Cerghet et al., 2006) may increase the vulnerability of OLs in the female brain to alcohol-induced apoptosis. Histological analysis of oligoglia cells in adolescence revealed that OL maturation is transiently restricted in the male FASD brain until PD 30, consistent with the findings by Newville and colleagues (2017). There was no persistent reduction to OPCs or OLs in the adolescent female AE brain. However, OPC proliferation was upregulated in SI control female rats following intervention exposure; this effect was blunted in AE female rats. Exercise intervention increased the population of OLs in the corpus callosum of SI and AE female rats. Taken together, we have provided seminal evidence that oligoglia proliferation is difficult to induce in the AE brain in adolescence, and that myelin remodeling is more robust in the brains of SI control rats following adolescent exercise intervention exposure.

Analysis of MBP density pre- and post-intervention revealed several additional sexual dimorphisms in the trajectory of myelin development and plasticity following exercise intervention in this model. MBP density was transiently reduced in the FASD male brain in adolescence. Interestingly, AE rats with intervention access exhibited an elevated density of MBP in the body of corpus callosum compared to their sedentary counterparts. A similar effect of exercise intervention was not observed in male SI controls. Conversely, MBP density was elevated in the FASD female brain across adolescence and the aerobic intervention mitigated this effect by reducing MBP density to control levels by PD 45. Lastly, volumetric analysis of the body of corpus callosum illustrated that corpus callosum growth was transiently reduced (until PD 30) in the FASD brain irrespective of sex. Intervention exposure stimulated corpus callosum growth in SI female rats only, providing more evidence that white matter plasticity is limited in the adolescent AE female brain.

MBP density was assessed in the body and splenium subregions of corpus callosum. The splenium is particularly vulnerable to alcohol teratogenesis (Treit et al., 2017; Qiang et al., 2002), and we illustrated that the density of MBP on PD 30 is reduced in AE male rats and increased in AE female rats compared to their respective SI control groups. Such differences were not observed on PD 45. Further investigation of cellular parameters relating to myelination (i.e. patterns of ensheathment) are needed to comprehensively describe the vulnerability of splenium axons to alcohol teratogenesis across the lifespan.

The MRI protocol developed for Aim 1, Experiment 1 offered the highest signal-to-noise ratio for *in vivo* scanning across development; however, it is clear that interpretation of noninvasive neuroimaging findings is augmented by histological

analysis of underlying white matter cytology. Two limitations to this neuroimaging protocol have emerged from this multimodal study: 1) it is challenging to identify the acute effects of intervention exposure on white matter maturation noninvasively, and 2) sexual dimorphisms in FASD etiology and intervention impact on white matter maturation were not adequately captured with scanning. Indeed, histological analysis of oligoglia populations and MBP density in the body of corpus callosum pre- and post-intervention uncovered several benefits of exercise intervention on white matter refinement and myelin plasticity in the female brain that were not previously identified using *in vivo* scanning. Correspondingly, a significant correlation between radial diffusivity values and MBP density was not discovered in female rats when controlling for postnatal treatment and intervention groups. Interestingly, radial diffusivity values showed a moderate positive correlation ($R = 0.50$) with MBP density in the male corpus callosum. The lack of strong correlation between these values may arise from the similarity in the density of MBP in corpus callosi from AE and SI sedentary male rats –radial diffusivity values from this region were not similar between rats from each postnatal treatment group regardless of intervention condition. As a result, we predicted that noninvasive neuroimaging could be used to detect intervention-related changes to white matter maturation in young adulthood as alterations to axonal ensheathment partially stabilize following development.

In the second half of Aim 1, Experiment 1 was replicated using female rats and resting-state functional MRI scans were acquired once in young adulthood to assess the potential lasting benefits of adolescent exercise intervention on white matter maturation. In addition, brain tissue was collected for electron microscopic analysis of axonal ensheathment patterning. The results from this experiment indicated that axon

diameter and myelin sheath thickness were reduced in the splenium of adult AE rats that did not receive the adolescent intervention. Exercise intervention supported the growth of splenium axons in adult AE rats. Moreover, comparison of resting-state BOLD signal between groups showed that the degree of centrality between cortical regions connected by splenium axons did not differ significantly between groups. However, analysis of the functional connectivity of cortical regions in the executive control network revealed aberrant global and local connectivity in AE rats, and this effect was mitigated by adolescent exercise intervention.

This body of research suggests that behavioral interventions for youth affected by FASD are beneficial for white matter development, particularly in girls. Moreover, these studies provide seminal evidence for sexual dimorphisms in glial vulnerability to teratogenic alcohol exposure. Examination of the effects of both AE and intervention exposure was greatly facilitated with the use of a rodent model of FASD. Finally, the use of several cutting-edge techniques (i.e. neuroimaging, immunocytochemistry, and electron microscopy) allowed for a more comprehensive assessment of how environmental stimuli altered the trajectory of myelination and the capacity for myelin plasticity.

REFERENCES

Acs, P., Kipp, M., Norkute, A., Johann, S., Clarner, T., Braun, A., … Beyer, C. (2009). 17β-Estradiol and progesterone prevent cuprizone provoked demyelination of corpus callosum in male mice. *Glia*, *57*(8), 807–814. https://doi.org/10.1002/glia.20806

Adam, M. P., Jones, K. L., Elliott, A. J., Riley, E. P., Buckley, D., Coles, C. D., … Muetzel, R. L. (2016). Neuro-metabolite profiles of rodent models of psychiatric dysfunctions characterised by MR spectroscopy. *Brain Imaging and Behavior*, *138*(2), 109–116. https://doi.org/10.1111/acer.13021

Ahluwalia, B., Smith, D., Adeyiga, O., Akbasak, B., & Rajguru, S. (1992). Ethanol decreases progesterone synthesis in human placental cells: Mechanism of ethanol effect. *Alcohol*, *9*(5), 395–401. https://doi.org/10.1016/0741-8329(92)90038-C

Alger, J. R., O'Neill, J., O'Connor, M. J., Kalender, G., Ly, R., Ng, A., … Levitt, J. G. (2021). Neuroimaging of Supraventricular Frontal White Matter in Children with Familial Attention-Deficit Hyperactivity Disorder and Attention-Deficit Hyperactivity Disorder Due to Prenatal Alcohol Exposure. *Neurotoxicity Research*. https://doi.org/10.1007/s12640-021-00342-0

Allen, N. J., & Barres, B. A. (2009). Glia - more than just brain glue. *Nature*, *457*(7230), 675–677. https://doi.org/10.1038/457675a

Almeida, R. G., Pan, S., Cole, K. L. H., Williamson, J. M., Early, J. J., Czopka, T., … Lyons, D. A. (2018). Myelination of Neuronal Cell Bodies when Myelin Supply Exceeds Axonal Demand. *Current Biology*, *28*(8), 1296-1305.e5. https://doi.org/10.1016/j.cub.2018.02.068

Bechler, M. E., Byrne, L., & ffrench-Constant, C. (2015). CNS Myelin Sheath Lengths Are an Intrinsic Property of Oligodendrocytes. *Current Biology*, *25*(18), 2411–2416. https://doi.org/10.1016/J.CUB.2015.07.056

Bergles, D. E., & Richardson, W. D. (2016). Oligodendrocyte Development and Plasticity. *Cold Spring Harbor Perspectives in Biology*. https://doi.org/10.1101/cshperspect.a020453

Berman, S., West, K. L., Does, M. D., Yeatman, J. D., & Mezer, A. A. (2018). Evaluating g-ratio weighted changes in the corpus callosum as a function of age and sex. *NeuroImage*, *182*, 304–313. https://doi.org/10.1016/J.NEUROIMAGE.2017.06.076

Blaauw, J., & Meiners, L. C. (2020). The splenium of the corpus callosum: embryology, anatomy, function and imaging with pathophysiological hypothesis. *Neuroradiology*, *62*(5), 563–585. https://doi.org/10.1007/s00234-019-02357-z

Bonthius, D. J., Goodlett, C. R., & West, J. R. (1988). Blood alcohol concentration and severity of microencephaly in neonatal rats depend on the pattern of alcohol administration. *Alcohol*, *5*(3), 209–214. https://doi.org/10.1016/0741-8329(88)90054-7

Bonthius, D. J., & West, J. R. (1990). Alcohol-Induced Neuronal Loss in Developing Rats: Increased Brain Damage with Binge Exposure. *Alcoholism: Clinical and Experimental Research*, *14*(1), 107–118. https://doi.org/10.1111/j.1530-0277.1990.tb00455.x

Boschen, K. E., & Klintsova, A. Y. (2017). *Neurotrophins in the Brain: Interaction With Alcohol Exposure During Development*. *Vitamins and Hormones* (1st ed., Vol. 104). Elsevier Inc. https://doi.org/10.1016/bs.vh.2016.10.008

Boschen, McKeown, S. E., Roth, T. L., & Klintsova, A. Y. (2017). Impact of exercise and a complex environment on hippocampal dendritic morphology, Bdnf gene expression, and DNA methylation in male rat pups neonatally exposed to alcohol. *Developmental Neurobiology*, *77*(6), 708–725. https://doi.org/10.1002/dneu.22448

Bourgeois, J.-P., Goldman-Rakic, P. S., & Rakic, P. (1994). Synaptogenesis in the Prefrontal Cortex of Rhesus Monkeys. *Cerebral Cortex*, *4*(1), 78–96. https://doi.org/10.1093/CERCOR/4.1.78

Boyd-Meredith, J. T., Piet, A. T., Dennis, E. J., El Hady, A., & Brody, C. D. (2022). Stable choice coding in rat frontal orienting fields across model-predicted changes of mind. *Nature Communications*, *13*(1), 1–12. https://doi.org/10.1038/s41467-022-30736-3

Brenhouse, H. C., & Andersen, S. L. (2011). Developmental trajectories during adolescence in males and females: A cross-species understanding of underlying brain changes. *Neuroscience and Biobehavioral Reviews*, *35*(8), 1687–1703. https://doi.org/10.1016/j.neubiorev.2011.04.013

Buchanan, J., Elabbady, L., Collman, F., Jorstad, N. L., Bakken, T. E., Ott, C., … Costa, N. M. da. (2021). Oligodendrocyte precursor cells prune axons in the mouse neocortex. *BioRxiv*, 2021.05.29.446047. https://doi.org/10.1101/2021.05.29.446047

Caldeira, J. C., Wu, Y., Mameli, M., Purdy, R. H., Li, P. K., Akwa, Y., … Valenzuela, C. F. (2004). Fetal alcohol exposure alters neurosteroid levels in the developing rat brain. *Journal of Neurochemistry*, *90*(6), 1530–1539. https://doi.org/10.1111/j.1471-4159.2004.02686.x

Cameron, H. A., Hazel, T. G., & McKay, R. D. G. (1998). Regulation of neurogenesis by growth factors and neurotransmitters. *Journal of Neurobiology*, *36*(2), 287–306. https://doi.org/10.1002/(SICI)1097-4695(199808)36:2<287::AID-NEU13>3.0.CO;2-B

Cerghet, M., Skoff, R. P., Bessert, D., Zhang, Z., Mullins, C., & Ghandour, M. S. (2006). Proliferation and death of oligodendrocytes and myelin proteins are differentially regulated in male and female rodents. *Journal of Neuroscience*, *26*(5), 1439–1447. https://doi.org/10.1523/JNEUROSCI.2219-05.2006

Cerghet, M., Skoff, R. P., Swamydas, M., & Bessert, D. (2009). Sexual dimorphism in the white matter of rodents. *Journal of the Neurological Sciences*, *286*(1–2), 76–80. https://doi.org/10.1016/J.JNS.2009.06.039

Chaddock-Heyman, L., Erickson, K. I., Kienzler, C., Drollette, E. S., Raine, L. B., Kao, S. C., … Kramer, A. F. (2018). Physical Activity Increases White Matter Microstructure in Children. *Frontiers in Neuroscience*, *12*(December), 1–11. https://doi.org/10.3389/fnins.2018.00950

Chechik, G., Meilijson, I., & Ruppin, E. (1999). Neuronal regulation: A mechanism for synaptic pruning during brain maturation. *Neural Computation*, *11*(8), 2061–2080. https://doi.org/10.1162/089976699300016089

Chiappelli, F., Taylor, A. N., De Los Monteros, A. E., & De Vellis, J. (1991). Fetal alcohol delays the developmental expression of myelin basic protein and transferrin in rat primary oligodendrocyte cultures. *International Journal of Developmental Neuroscience*, *9*(1). https://doi.org/10.1016/0736-5748(91)90074-V

Chomiak, T., & Hu, B. (n.d.). What Is the Optimal Value of the g-Ratio for Myelinated Fibers in the Rat CNS? A Theoretical Approach. https://doi.org/10.1371/journal.pone.0007754

Chung, W.-S., Allen, N. J., & Eroglu, C. (2015). Astrocytes Control Synapse Formation, Function, and Elimination. *Cold Spring Harbor Perspectives in Biology*, *7*(9), a020370. https://doi.org/10.1101/CSHPERSPECT.A020370

Colin Carter, R., Jacobson, J. L., Molteno, C. D., Jiang, H., Meintjes, E. M., Jacobson, S. W., & Duggan, C. (2012). Effects of Heavy Prenatal Alcohol Exposure and Iron Deficiency Anemia on Child Growth and Body Composition through Age 9 Years. *Alcoholism: Clinical and Experimental Research*, *36*(11), 1973–1982. https://doi.org/https://doi.org/10.1111/j.1530-0277.2012.01810.x

Conway, G. D., O'Bara, M. A., Vedia, B. H., Pol, S. U., & Sim, F. J. (2012). Histone deacetylase activity is required for human oligodendrocyte progenitor differentiation. *Glia*, *60*(12), 1944–1953. https://doi.org/10.1002/glia.22410

Creeley, C. E., Dikranian, K. T., Johnson, S. A., Farber, N. B., & Olney, J. W. (2013). Alcohol-induced apoptosis of oligodendrocytes in the fetal macaque brain. *Acta Neuropathologica Communications*. https://doi.org/10.1186/2051-5960-1-23

Cudd, T. A. (2005). Animal model systems for the study of alcohol teratology. *Experimental Biology and Medicine*, *230*(6), 389–393. https://doi.org/10.1177/15353702-0323006-06

Cui, Q. L., D'Abate, L., Fang, J., Leong, S. Y., Ludwin, S., Kennedy, T. E., … Almazan, G. (2012). Human fetal oligodendrocyte progenitor cells from different gestational stages exhibit substantially different potential to myelinate. *Stem Cells and Development*, *21*(11), 1831–1837. https://doi.org/10.1089/scd.2011.0494

Darbinian, N., Darbinyan, A., Merabova, N., Bajwa, A., Tatevosian, G., Martirosyan, D., … Goetzl, L. (2021). Ethanol-mediated alterations in oligodendrocyte differentiation in the developing brain. *Neurobiology of Disease*, *148*, 105181. https://doi.org/10.1016/j.nbd.2020.105181

Dean, J. M., Moravec, M. D., Grafe, M., Abend, N., Ren, J., Gong, X., … Back, S. A. (2011). Strain-specific differences in perinatal rodent oligodendrocyte lineage progression and its correlation with human. *Developmental Neuroscience*, *33*(3–4), 251–260. https://doi.org/10.1159/000327242

Denenberg, V. H., Grota, L. J., & Zarrow, M. X. (1963). Maternal behaviour in the rat: analysis of cross-fostering. *Journal of Reproduction and Fertility*, *5*, 133–141. https://doi.org/10.1530/jrf.0.0050133

Denny, C. H., Acero, C. S., Naimi, T. S., & Kim, S. Y. (2019). Consumption of Alcohol Beverages and Binge Drinking Among Pregnant Women Aged 18–44

Years — United States, 2015–2017. *MMWR. Morbidity and Mortality Weekly Report*, *68*(16), 365–368. https://doi.org/10.15585/mmwr.mm6816a1

Deoni, S. C. L., Mercure, E., Blasi, A., Gasston, D., Thomson, A., Johnson, M., … Murphy, D. G. M. (2011). Mapping infant brain myelination with magnetic resonance imaging. *Journal of Neuroscience*, *31*(2), 784–791. https://doi.org/10.1523/JNEUROSCI.2106-10.2011

Diamond, M. C., Dowling, G. A., & Johnson, R. E. (1981). Morphologic cerebral cortical asymmetry in male and female rats. *Experimental Neurology*, *71*(2), 261–268. https://doi.org/10.1016/0014-4886(81)90087-X

Dobbing, J., & Sands, J. (1979). Comparative aspects of the brain growth spurt. *Early Human Development*, *311*, 79–83. https://doi.org/10.1016/0378-3782(79)90022-7

Domellöf, E., Rönnqvist, L., Titran, M., Esseily, R., & Fagard, J. (2009). Atypical functional lateralization in children with fetal alcohol syndrome. *Developmental Psychobiology*, *51*(8), 696–705. https://doi.org/10.1002/dev.20404

Downes, N., & Mullins, P. (2014). The Development of Myelin in the Brain of the Juvenile Rat. *Toxicologic Pathology*, *42*(5), 913–922. https://doi.org/10.1177/0192623313503518

England, L. J., Bennett, C., Denny, C. H., Honein, M. A., Gilboa, S. M., Kim, S. Y., … Boyle, C. A. (2020). Alcohol Use and Co-Use of Other Substances Among Pregnant Females Aged 12–44 Years — United States, 2015–2018. *MMWR. Morbidity and Mortality Weekly Report*, *69*(31), 1009–1014. https://doi.org/10.15585/mmwr.mm6931a1

Farhy-Tselnicker, I., & Allen, N. J. (n.d.). Astrocytes, neurons, synapses: a tripartite view on cortical circuit development. https://doi.org/10.1186/s13064-018-0104-y

Faust, T. E., Gunner, G., & Schafer, D. P. (2021). Mechanisms governing activity-dependent synaptic pruning in the developing mammalian CNS. *Nature Reviews Neuroscience*, *22*(11), 657–673. https://doi.org/10.1038/s41583-021-00507-y

Fields, R. D. (2015). A new mechanism of nervous system plasticity: Activity-dependent myelination. *Nature Reviews Neuroscience*, *16*(12), 756–767. https://doi.org/10.1038/nrn4023

Fields, R. D. (2008). White matter in learning, cognition and psychiatric disorders. *Trends in Neurosciences*, *31*(7), 361–370. https://doi.org/10.1016/j.tins.2008.04.001

Fields, R. D., & Bukalo, O. (2020). Myelin makes memories. *Nature Neuroscience*, *23*(4), 469–470. https://doi.org/10.1038/s41593-020-0606-x

Firmin, L., Field, P., Maier, M. A., Kraskov, A., Kirkwood, P. A., Nakajima, K., … Glickstein, M. (2014). Axon diameters and conduction velocities in the macaque pyramidal tract. *Journal of Neurophysiology*, *112*(6), 1229–1240. https://doi.org/10.1152/jn.00720.2013

Fletcher, J. L., Murray, S. S., & Xiao, J. (2018). Brain-derived neurotrophic factor in central nervous system myelination: A new mechanism to promote myelin plasticity and repair. *International Journal of Molecular Sciences*, *19*(12), 1–16. https://doi.org/10.3390/ijms19124131

Forbes, T. A., & Gallo, V. (2017). All Wrapped Up: Environmental Effects on Myelination. *Trends in Neurosciences*, *40*(9), 572–587. https://doi.org/10.1016/j.tins.2017.06.009

Ganguly, P., & Brenhouse, H. C. (2015). Broken or maladaptive? Altered trajectories in neuroinflammation and behavior after early life adversity. *Developmental Cognitive Neuroscience*, *11*, 18–30. https://doi.org/10.1016/J.DCN.2014.07.001

Gautam, P., Nuñez, S. C., Narr, K. L., Kan, E. C., & Sowell, E. R. (2014). Effects of prenatal alcohol exposure on the development of white matter volume and change in executive function. *NeuroImage: Clinical*, *5*, 19–27. https://doi.org/10.1016/J.NICL.2014.05.010

Geeraert, B. L., Lebel, R. M., Mah, A. C., Deoni, S. C., Alsop, D. C., Varma, G., & Lebel, C. (2018). A comparison of inhomogeneous magnetization transfer, myelin volume fraction, and diffusion tensor imaging measures in healthy children. *NeuroImage*, *182*, 343–350. https://doi.org/10.1016/j.neuroimage.2017.09.019

Ghasemi, Asghar; Sajad, J. K. K. (2021). The laboratory rat: age and body weight matter. *EXCLI Journal 2021;20:1431-1445*, *2005*, 1431–1445.

Ghazi Sherbaf, F., Aarabi, M. H., Hosein Yazdi, M., & Haghshomar, M. (2019). White matter microstructure in fetal alcohol spectrum disorders: A systematic review of diffusion tensor imaging studies. *Human Brain Mapping*, *40*(3), 1017–1036. https://doi.org/10.1002/hbm.24409

Ghoumari, A. M., Ibanez, C., El-Etr, M., Leclerc, P., Eychenne, B., O'Malley, B. W., … Schumacher, M. (2003). Progesterone and its metabolites increase myelin basic protein expression in organotypic slice cultures of rat cerebellum. *Journal*

of Neurochemistry, *86*(4), 848–859. https://doi.org/10.1046/j.1471-4159.2003.01881.x

Gibson, E. M., Purger, D., Mount, C. W., Goldstein, A. K., Lin, G. L., Wood, L. A., … Monje, M. (2014). Neuronal Activity Promotes Oligodendrogenesis and Adaptive Myelination in the Mammalian Brain. *Science*, *344*(6183), 480–481. https://doi.org/10.1126/science.1254446

Giera, S., Luo, R., Ying, Y., Ackerman, S. D., Jeong, S. J., Stoveken, H. M., … Piao, X. (2018). Microglial transglutaminase-2 drives myelination and myelin repair via GPR56/ADGRG1 in oligodendrocyte precursor cells. *ELife*, *7*, 1–25. https://doi.org/10.7554/eLife.33385

Glaser, E. M., & Wilson, P. D. (1998). The coefficient of error of optical fractionator population size estimates: A computer simulation comparing three estimators. *Journal of Microscopy*, *192*(2), 163–171. https://doi.org/10.1046/j.1365-2818.1998.00417.x

Gleichmann, D. C., Pinner, J. F. L., Garcia, C., Hakeem, J. H., Kodituwakku, P., & Stephen, J. M. (2022). A Pilot Study Examining the Effects of Music Training on Attention in Children with Fetal Alcohol Spectrum Disorders (FASD). *Sensors*, *22*(15). https://doi.org/10.3390/s22155642

Goh, P. K., Doyle, L. R., Glass, L., Jones, K. L., Riley, E. P., Coles, C. D., … Mattson, S. N. (2016). A Decision Tree to Identify Children Affected by Prenatal Alcohol Exposure. *The Journal of Pediatrics*, *177*, 121-127.e1. https://doi.org/10.1016/J.JPEDS.2016.06.047

González-Orozco, J. C., & Camacho-Arroyo, I. (2019). Progesterone actions during central nervous system development. *Frontiers in Neuroscience*, *13*, 1–14. https://doi.org/10.3389/fnins.2019.00503

Goodlett, C. R., Kelly, S. J., & West, J. R. (1987). Early postnatal alcohol exposure that produces high blood alcohol levels impairs development of spatial navigation learning. *Psychobiology*, *15*(1), 64–74. https://doi.org/10.3758/BF03327265

Gosdin, L. K., Deputy, N. P., Kim, S. Y., Dang, E. P., & Denny, C. H. (2022). Alcohol Consumption and Binge Drinking During Pregnancy Among Adults Aged 18–49 Years — United States, 2018–2020. *MMWR. Morbidity and Mortality Weekly Report*, *71*(1), 10–13. https://doi.org/10.15585/mmwr.mm7101a2

Gram, M. G., Wogensen, E., Moseholm, K., Mogensen, J., & Malá, H. (2016). Exercise-induced improvement in cognitive performance after fimbria-fornix transection depends on the timing of exercise administration. *Brain Research Bulletin*, *125*, 117–126. https://doi.org/10.1016/J.BRAINRESBULL.2016.06.012

Green, J. G., McLaughlin, K. A., Berglund, P. A., Gruber, M. J., Sampson, N. A., Zaslavsky, A. M., & Kessler, R. C. (2010). Childhood adversities and adult psychiatric disorders in the national comorbidity survey replication I: Associations with first onset of DSM-IV disorders. *Archives of General Psychiatry*, *67*(2), 113–123. https://doi.org/10.1001/archgenpsychiatry.2009.186

Guizzetti, M., Zhang, X., Goeke, C., & Gavin, D. P. (2014). Glia and neurodevelopment: Focus on fetal alcohol spectrum disorders. *Frontiers in Pediatrics*, 1–12. https://doi.org/10.3389/fped.2014.00123

Gursky, Z. H., & Klintsova, A. Y. (2017). Wheel Running and Environmental Complexity as a Therapeutic Intervention in an Animal Model of FASD. *Journal of Visualized Experiments*, (120), 54947. https://doi.org/10.3791/54947

Gursky, Z. H., Johansson, J. R., & Klintsova, A. Y. (2020). Postnatal alcohol exposure and adolescent exercise have opposite effects on cerebellar microglia in rat. *International Journal of Developmental Neuroscience*, *80*(6), 558–571. https://doi.org/10.1002/JDN.10051

Hackett, M. J., McQuillan, J. A., El-Assaad, F., Aitken, J. B., Levina, A., Cohen, D. D., … Lay, P. A. (2011). Chemical alterations to murine brain tissue induced by formalin fixation: Implications for biospectroscopic imaging and mapping studies of disease pathogenesis. *Analyst*, *136*(14), 2941–2952. https://doi.org/10.1039/c0an00269k

Hamilton, G. F., Boschen, K. E., Goodlett, C. R., Greenough, W. T., & Klintsova, A. Y. (2012). Housing in Environmental Complexity Following Wheel Running Augments Survival of Newly Generated Hippocampal Neurons in a Rat Model of Binge Alcohol Exposure During the Third Trimester Equivalent. *Alcoholism: Clinical and Experimental Research*, *36*(7), 1196–1204. https://doi.org/10.1111/j.1530-0277.2011.01726.x

Hamilton, G. F., Criss, K. J., & Klintsova, A. Y. (2015). Voluntary exercise partially reverses neonatal alcohol-induced deficits in mPFC layer II/III dendritic morphology of male adolescent rats. *Synapse*, *69*(8), 405–415. https://doi.org/10.1002/syn.21827

Hamilton, G. F., Jablonski, S. A., Schiffino, F. L., St. Cyr, S. A., Stanton, M. E., & Klintsova, A. Y. (2014). Exercise and environment as an intervention for neonatal alcohol effects on hippocampal adult neurogenesis and learning. *Neuroscience*, *265*, 274–290. https://doi.org/10.1016/J.NEUROSCIENCE.2014.01.061

Hayhow, W. R., Sefton, A., & Webb, C. (1962). Primary optic centers of the rat in relation to the terminal distribution of the crossed and uncrossed optic nerve fibers. *Journal of Comparative Neurology*, *118*(3), 295–321. https://doi.org/10.1002/cne.901180303

Helfer, J. L., Goodlett, C. R., Greenough, W. T., & Klintsova, A. Y. (2009). The effects of exercise on adolescent hippocampal neurogenesis in a rat model of binge alcohol exposure during the brain growth spurt. *Brain Research*, *1294*, 1–11. https://doi.org/10.1016/J.BRAINRES.2009.07.090

Hensch, T. K. (2004). CRITICAL PERIOD REGULATION. *Annual Review of Neuroscience*, *27*(1), 549–579. https://doi.org/10.1146/annurev.neuro.27.070203.144327

Herford, A. S. (2017). Regeneration and Beyond. *Oral and Maxillofacial Surgery Clinics of North America*, *29*(1), ix. https://doi.org/10.1016/j.coms.2016.10.001

Herting, M. M., Kim, R., Uban, K. A., Kan, E., Binley, A., & Sowell, E. R. (2017). Longitudinal changes in pubertal maturation and white matter microstructure. *Psychoneuroendocrinology*, *81*, 70–79. https://doi.org/10.1016/J.PSYNEUEN.2017.03.017

Honeycutt, J. A., Demaestri, C., Peterzell, S., Silveri, M. M., Cai, X., Kulkarni, P., … Brenhouse, H. C. (2020). Altered corticolimbic connectivity reveals sex-specific adolescent outcomes in a rat model of early life adversity. *ELife*, *9*. https://doi.org/10.7554/ELIFE.52651

Hoyme, H. E., Kalberg, W. O., Elliott, A. J., Blankenship, J., Buckley, D., Marais, A.-S., … May, P. A. (2016). Updated Clinical Guidelines for Diagnosing Fetal Alcohol Spectrum Disorders. *Pediatrics*, *138*(2), 1–18. https://doi.org/10.1542/peds.2015-4256

Hoyme, H. E., May, P. A., Kalberg, W. O., Kodituwakku, P., Gossage, J. P., Trujillo, P. M., … Robinson, L. K. (2005). A practical clinical approach to diagnosis of fetal alcohol spectrum disorders: Clarification of the 1996 institute of medicine criteria. *Pediatrics*, *115*(1), 39–47. https://doi.org/10.1542/peds.2004-0259

Hubel, D. H., & Wiesel, T. (1962). Receptive fields, binocular interaction and functional architecture in the cat's visual cortex. *J. Physio.*, 106–154.

Hughes, A. N., & Appel, B. (2020). Microglia phagocytose myelin sheaths to modify developmental myelination. *Nature Neuroscience*, *23*(9), 1055–1066. https://doi.org/10.1038/s41593-020-0654-2

Hughes, E. G., Orthmann-Murphy, J. L., Langseth, A. J., & Bergles, D. E. (2018). Myelin remodeling through experience-dependent oligodendrogenesis in the adult somatosensory cortex. *Nature Neuroscience*, *21*(5), 696–706. https://doi.org/10.1038/s41593-018-0121-5

Hussain, R., El-Etr, M., Gaci, O., Rakotomamonjy, J., Macklin, W. B., Kumar, N., … Ghoumari, A. M. (2011). Progesterone and nestorone facilitate axon remyelination: A role for progesterone receptors. *Endocrinology*, *152*(10), 3820–3831. https://doi.org/10.1210/en.2011-1219

Huttenlocher, P. R., & Dabholkar, A. S. (1997). Regional Differences in Synaptogenesis in Human Cerebral Cortex. *J. Comp. Neurol*, *387*, 167–178. https://doi.org/10.1002/(SICI)1096-9861(19971020)387:2

Ieraci, A., & Herrera, D. G. (2020). Early Postnatal Ethanol Exposure in Mice Induces Sex-Dependent Memory Impairment and Reduction of Hippocampal NMDA-R2B Expression in Adulthood. *Neuroscience*, *427*, 105–115. https://doi.org/10.1016/J.NEUROSCIENCE.2019.11.045

Ikonomidou, C., Bittigau, P., Koch, C., Genz, K., Hoerster, F., Felderhoff-Mueser, U., … Olney, J. W. (2001). Neurotransmitters and apoptosis in the developing brain. *Biochemical Pharmacology*, *62*(4), 401–405. https://doi.org/10.1016/S0006-2952(01)00696-7

Inkelis, S. M., Moore, E. M., Bischoff-Grethe, A., & Riley, E. P. (2020). Neurodevelopment in adolescents and adults with fetal alcohol spectrum disorders (FASD): A magnetic resonance region of interest analysis. *Brain Research*, *1732*(January), 146654. https://doi.org/10.1016/j.brainres.2020.146654

Jakovcevski, I., Filipovic, R., Mo, Z., Rakic, S., & Zecevic, N. (2009). Oligodendrocyte development and the onset of myelination in the human fetal brain. *Frontiers in Neuroanatomy*, 1–15. https://doi.org/10.3389/neuro.05.005.2009

Johe, K. K., Hazel, T. G., Muller, T., Dugich-Djordjevic, M. M., & McKay, R. D. G. (1996). Single factors direct the differentiation of stem cells from the fetal and

adult central nervous system. *Genes and Development*, *10*(24), 3129–3140. https://doi.org/10.1101/gad.10.24.3129

Juraska, J. M., & Drzewiecki, C. M. (2020). Cortical reorganization during adolescence: What the rat can tell us about the cellular basis. *Developmental Cognitive Neuroscience*, *45*, 100857. https://doi.org/10.1016/j.dcn.2020.100857

Kaiser, T., Allen, H. M., Kwon, O., Barak, B., Wang, J., He, Z., … Feng, G. (2021). Myeltracer: A semi-automated software for myelin g-ratio quantification. *ENeuro*, *8*(4), 1–9. https://doi.org/10.1523/ENEURO.0558-20.2021

Kar, P., Reynolds, J. E., Grohs, M. N., Gibbard, W. Ben, McMorris, C., Tortorelli, C., & Lebel, C. (2021). White Matter Alterations in Young Children with Prenatal Alcohol Exposure. *Developmental Neurobiology*. https://doi.org/10.1002/dneu.22821

Kilanowski-Press, L. (2011). Alcohol Exposure. *Encyclopedia of Child Behavior and Development*, 76–78. https://doi.org/10.1007/978-0-387-79061-9_93

King, K. E., Rudser, K. D., Nestrasil, I., Kovac, V., Delaney, K. A., Wozniak, J. R., … Shapiro, E. G. (2019). Attention and corpus callosum volumes in individuals with mucopolysaccharidosis type I. *Neurology*, *92*(20), e2321 LP-e2328. https://doi.org/10.1212/WNL.0000000000007496

Klintsova, A. Y., Hamilton, G. F., & Boschen, K. E. (2013). *Long-term consequences of developmental alcohol exposure on brain structure and function: Therapeutic benefits of physical activity*. *Brain Sciences* (Vol. 3). https://doi.org/10.3390/brainsci3010001

Klintsova, A. Y., Helfer, J. L., Calizo, L. H., Dong, W. K., Goodlett, C. R., & Greenough, W. T. (2007). Persistent impairment of hippocampal neurogenesis in young adult rats following early postnatal alcohol exposure. *Alcoholism: Clinical and Experimental Research*, *31*(12), 2073–2082. https://doi.org/10.1111/j.1530-0277.2007.00528.x

Klintsova, A. Y., Scamra, C., Hoffman, M., Napper, R. M. A., Goodlett, C. R., & Greenough, W. T. (2002). Therapeutic effects of complex motor training on motor performance deficits induced by neonatal binge-like alcohol exposure in rats:: II. A quantitative stereological study of synaptic plasticity in female rat cerebellum. *Brain Research*, *937*(1–2), 83–93. https://doi.org/10.1016/S0006-8993(02)02492-7

Knudsen, E. I. (2004). Sensitive periods in the development of the brain and behavior. *Journal of Cognitive Neuroscience*, *16*(8), 1412–1425. https://doi.org/10.1162/0898929042304796

Knyazeva, M. G. (2013). Splenium of corpus callosum: Patterns of interhemispheric interaction in children and adults. *Neural Plasticity*, *2013*. https://doi.org/10.1155/2013/639430

Kolb, B., Sutherland, R. J., Nonneman, A. J., & Whishaw, I. Q. (1982). Asymmetry in the cerebral hemispheres of the rat, mouse, rabbit, and cat: The right hemisphere is larger. *Experimental Neurology*, *78*(2), 348–359. https://doi.org/10.1016/0014-4886(82)90053-X

Kravitz, D. J., Saleem, K. S., Baker, C. I., & Mishkin, M. (2011). A new neural framework for visuospatial processing. *Nature Reviews Neuroscience*, *12*(4), 217–230. https://doi.org/10.1038/nrn3008

Krentzel, A. A., Proaño, S., Patisaul, H. B., & Meitzen, J. (2020). Temporal and bidirectional influences of estradiol on voluntary wheel running in adult female and male rats. *Hormones and Behavior*, *120*. https://doi.org/10.1016/j.yhbeh.2020.104694.Temporal

Kwon, D., Pfefferbaum, A., Sullivan, E. V., & Pohl, K. M. (2020). Regional growth trajectories of cortical myelination in adolescents and young adults: longitudinal validation and functional correlates. *Brain Imaging and Behavior*, *14*(1), 242–266. https://doi.org/10.1007/s11682-018-9980-3

LaCount, P. A., Kuhn, M., Trần, N., Hsu, A., Tandon, P. S., Sasser, T., … Gonzalez, E. S. (2020). 5.16 Lifestyle Enhancement for Adhd Program (Leap): Health-Focused Parent Training Intervention Effects on Physical Activity and Screen Time Behaviors Among Children With Adhd. *Journal of the American Academy of Child & Adolescent Psychiatry*, *59*(10), S154. https://doi.org/10.1016/j.jaac.2020.08.077

Lam, V. Y. Y., Raineki, C., Takeuchi, L. E., Ellis, L., Woodward, T. S., & Weinberg, J. (2018). Chronic stress alters behavior in the forced swim test and underlying neural activity in animals exposed to alcohol prenatally: Sex- and time-dependent effects. *Frontiers in Behavioral Neuroscience*, *12*(March). https://doi.org/10.3389/fnbeh.2018.00042

Lebel, C., & Beaulieu, C. (2011). Longitudinal development of human brain wiring continues from childhood into adulthood. *Journal of Neuroscience*, *31*(30), 10937–10947. https://doi.org/10.1523/JNEUROSCI.5302-10.2011

Lebel, C., & Deoni, S. (2018). The development of brain white matter microstructure. *NeuroImage*, *182*, 207–218. https://doi.org/10.1016/j.neuroimage.2017.12.097

Lebel, C., Rasmussen, C., Wyper, K., Andrew, G., & Beaulieu, C. (2010). Brain Microstructure Is Related to Math Ability in Children With Fetal Alcohol Spectrum Disorder. *Alcoholism: Clinical and Experimental Research*, *34*(2), 354–363. https://doi.org/10.1111/j.1530-0277.2009.01097.x

Lee, J. C., Mayer-Proschel, M., & Rao, M. S. (2000). Gliogenesis in the central nervous system. *Glia*, *30*(2), 105–121. https://doi.org/10.1002/(SICI)1098-1136(200004)30:2<105::AID-GLIA1>3.0.CO;2-H

Lehmann, N., Villringer, A., & Taubert, M. (2020). Colocalized white matter plasticity and increased cerebral blood flow mediate the beneficial effect of cardiovascular exercise on long-term motor learning. *Journal of Neuroscience*, *40*(12), 2416–2429. https://doi.org/10.1523/JNEUROSCI.2310-19.2020

Lemoine, P. (1968). Children of Alcoholic Parents, Anomalies observed in 127 cases. *Ouest Med.*, *21*, 476–482. Retrieved from https://cir.nii.ac.jp/crid/1573950400660362752.bib?lang=en

Levison, S. W., Chuang, C., Abramson, B. J., & Goldman, J. E. (1993). The migrational patterns and developmental fates of glial precursors in the rat subventricular zone are temporally regulated. *Development*, *119*(3), 611–622. https://doi.org/10.1242/dev.119.3.611

Liu, J., Dietz, K., Deloyht, J. M., Pedre, X., Kelkar, D., Kaur, J., … Casaccia, P. (2012). Impaired adult myelination in the prefrontal cortex of socially isolated mice. *Nature Neuroscience*, *15*(12), 1621–1623. https://doi.org/10.1038/nn.3263

Long, K. L. P., Breton, J. M., Barraza, M. K., Perloff, O. S., & Kaufer, D. (2021). Hormonal regulation of oligodendrogenesis i: Effects across the lifespan. *Biomolecules*, *11*(2), 1–36. https://doi.org/10.3390/biom11020283

Lu, Q. R., Yuk, D. I., Alberta, J. A., Zhu, Z., Pawlitzky, I., Chan, J., … Rowitch, D. H. (2000). Sonic Hedgehog–Regulated Oligodendrocyte Lineage Genes Encoding bHLH Proteins in the Mammalian Central Nervous System. *Neuron*, *25*(2), 317–329. https://doi.org/10.1016/S0896-6273(00)80897-1

Lund, R. D. (1965). Uncrossed Visual Pathways of Hooded and Albino Rats. *Science*, *149*(3691), 1506–1507.

Ma, X., Coles, C. D., Lynch, M. E., LaConte, S. M., Zurkiya, O., Wang, D., & Hu, X. (2005). Evaluation of corpus callosum anisotropy in young adults with fetal alcohol syndrome according to diffusion tensor imaging. *Alcoholism: Clinical and Experimental Research*, *29*(7), 1214–1222. https://doi.org/10.1097/01.ALC.0000171934.22755.6D

Mackay, A., Whittall, K., Adler, J., Li, D., Paty, D., & Graeb, D. (1994). In vivo visualization of myelin water in brain by magnetic resonance. *Magnetic Resonance in Medicine*, *31*(6), 673–677. https://doi.org/10.1002/mrm.1910310614

Madden, J. T., Thompson, S. M., Magcalas, C. M., Wagner, J. L., Hamilton, D. A., Savage, D. D., … Pentkowski, N. S. (2020). Moderate prenatal alcohol exposure reduces parvalbumin expressing GABAergic interneurons in the dorsal hippocampus of adult male and female rat offspring. *Neuroscience Letters*, *718*, 134700. https://doi.org/10.1016/J.NEULET.2019.134700

Makinodan, M., Rosen, K. M., Ito, S., & Corfas, G. (2012). A critical period for social experience-dependent oligodendrocyte maturation and myelination. *Science*, *337*(6100), 1357–1360. https://doi.org/10.1126/science.1220845

Marguet, F., Brosolo, M., Friocourt, G., Sauvestre, F., Marcorelles, P., Lesueur, C., … Laquerrière, A. (2022). Oligodendrocyte lineage is severely affected in human alcohol-exposed foetuses. *Acta Neuropathologica Communications*, *10*(1), 1–14. https://doi.org/10.1186/s40478-022-01378-9

Marin-Husstege, M., Muggironi, M., Raban, D., Skoff, R. P., & Casaccia-Bonnefil, P. (2004). Oligodendrocyte progenitor proliferation and maturation is differentially regulated by male and female sex steroid hormones. *Developmental Neuroscience*, *26*(2–4), 245–254. https://doi.org/10.1159/000082141

Mauch, D. H., Nägier, K., Schumacher, S., Göritz, C., Müller, E. C., Otto, A., & Pfrieger, F. W. (2001). CNS synaptogenesis promoted by glia-derived cholesterol. *Science*, *294*(5545), 1354–1357. https://doi.org/10.1126/science.294.5545.1354

May, P. A., Blankenship, J., Marais, A.-S., Gossage, J. P., Kalberg, W. O., Joubert, B., … Seedat, S. (2013). Maternal alcohol consumption producing fetal alcohol spectrum disorders (FASD): Quantity, frequency, and timing of drinking. *Drug and Alcohol Dependence*, *133*(2), 502–512. https://doi.org/10.1016/J.DRUGALCDEP.2013.07.013

May, P. A., Keaster, C., Bozeman, R., Goodover, J., Blankenship, J., Kalberg, W. O., … Hoyme, H. E. (2015). Prevalence and characteristics of fetal alcohol syndrome and partial fetal alcohol syndrome in a Rocky Mountain Region City. *Drug and Alcohol Dependence*, *155*, 118–127. https://doi.org/10.1016/j.drugalcdep.2015.08.006

McKenzie, I. A., Ohayon, D., Li, H., De Faria, J. P., Emery, B., Tohyama, K., & Richardson, W. D. (2014). Motor skill learning requires active central myelination. *Science*, *346*(6207), 318–322. https://doi.org/10.1126/science.1254960

McKinnon, R. D., Waldron, S., & Kiel, M. E. (2005). PDGF α-receptor signal strength controls an RTK rheostat that integrates phosphoinositol 3′-kinase and phospholipase Cγ pathways during oligodendrocyte maturation. *Journal of Neuroscience*, *25*(14), 3499–3508. https://doi.org/10.1523/JNEUROSCI.5049-04.2005

McLachlan, K., Flannigan, K., Temple, V., Unsworth, K., & Cook, J. L. (2020). Difficulties in Daily Living Experienced by Adolescents, Transition-Aged Youth, and Adults With Fetal Alcohol Spectrum Disorder. *Alcoholism: Clinical and Experimental Research*, *44*(8), 1609–1624. https://doi.org/10.1111/acer.14385

Medaglia, J. D., & Assistant Professor, R. (n.d.). Graph Theoretic Analysis of Resting State fMRI. https://doi.org/10.1016/j.nic.2017.06.008

Milbocker, K. A., Campbell, T. S., Collins, N., Kim, S., Smith, I. F., Roth, T. L., & Klintsova, A. Y. (2021). Glia-Driven Brain Circuit Refinement Is Altered by Early-Life Adversity: Behavioral Outcomes. *Frontiers in Behavioral Neuroscience*, 1–21. https://doi.org/10.3389/fnbeh.2021.786234

Milbocker, K. A., LeBlanc, G. L., Brengel, E. K., Hekmatyar, K. S., Kulkarni, P., Ferris, C. F., & Klintsova, A. Y. (2022). Reduced and delayed myelination and volume of corpus callosum in an animal model of Fetal Alcohol Spectrum Disorders partially benefit from voluntary exercise. *Scientific Reports*, *12*(1), 1–17. https://doi.org/10.1038/s41598-022-14752-3

Milbocker, K. A., & Klintsova, A. Y. (2020). Examination of cortically projecting cholinergic neurons following exercise and environmental intervention in a rodent model of fetal alcohol spectrum disorders. *Birth Defects Research*, 1–15. https://doi.org/10.1002/bdr2.1839

Murao, N., Noguchi, H., & Nakashima, K. (2016). Epigenetic regulation of neural stem cell property from embryo to adult. *Neuroepigenetics*, *5*, 1–10. https://doi.org/10.1016/j.nepig.2016.01.001

Nägler, K., Mauch, D. H., & Pfrieger, F. W. (2001). Glia-derived signals induce synapse formation in neurones of the rat central nervous system. *Journal of Physiology*, *533*(3), 665–679. https://doi.org/10.1111/j.1469-7793.2001.00665.x

Nelson III, C. A., & Gabard-durnam, L. J. (2020). Early Adversity and Critical Periods : Neurodevelopmental Consequences of Violating the Expectable Environment. *Trends in Neurosciences*, *43*(3), 133–143. https://doi.org/10.1016/j.tins.2020.01.002

Nemes-Baran, A. D., White, D. R., & DeSilva, T. M. (2020). Fractalkine-Dependent Microglial Pruning of Viable Oligodendrocyte Progenitor Cells Regulates Myelination. *Cell Reports*, *32*(7), 108047. https://doi.org/10.1016/j.celrep.2020.108047

Nery, S., Wichterle, H., & Fishell, G. (2001). Sonic hedgehog contributes to oligodendrocyte specification in the mammalian forebrain. *Development*, *128*(4), 527–540. https://doi.org/10.1242/dev.128.4.527

Newville, J., Howard, T. A., Chavez, G. J., Valenzuela, C. F., & Cunningham, L. A. (2021). Persistent myelin abnormalities in a third trimester-equivalent mouse model of fetal alcohol spectrum disorder. *Alcoholism: Clinical and Experimental Research*, 1–10. https://doi.org/10.1111/acer.14752

Newville, J., Valenzuela, C. F., Li, L., Jantzie, L. L., & Cunningham, L. A. (2017). Acute oligodendrocyte loss with persistent white matter injury in a third trimester equivalent mouse model of fetal alcohol spectrum disorder. *Glia*, *65*(8), 1317–1332. https://doi.org/10.1002/glia.23164

Nguyen, V. T., Chong, S., Tieng, Q. M., Mardon, K., Galloway, G. J., & Kurniawan, N. D. (2017). Radiological studies of fetal alcohol spectrum disorders in humans and animal models: An updated comprehensive review. *Magnetic Resonance Imaging*, *43*, 10–26. https://doi.org/10.1016/J.MRI.2017.06.012

Noori, R., Park, D., Griffiths, J. D., Bells, S., Frankland, P. W., Mabbott, D., & Lefebvre, J. (2020). Activity-dependent myelination: A glial mechanism of oscillatory self-organization in large-scale brain networks. *Proceedings of the National Academy of Sciences*, *117*(24), 13227–13237. https://doi.org/10.1073/PNAS.1916646117

Olney, J. W., Wozniak, D. F., Jevtovic-Todorovic, V., Farber, N. B., Bittigau, P., & Ikonomidou, C. (2006). Drug-induced Apoptotic Neurodegeneration in the Developing Brain. *Brain Pathology*, *12*(4), 488–498. https://doi.org/10.1111/j.1750-3639.2002.tb00467.x

O'Neill, J., O'Connor, M. J., Yee, V., Ly, R., Narr, K., Alger, J. R., & Levitt, J. G. (2019). Differential neuroimaging indices in prefrontal white matter in prenatal alcohol-associated ADHD versus idiopathic ADHD. *Birth Defects Research*, *111*(12), 797–811. https://doi.org/10.1002/bdr2.1460

Pahl, P. J. (1969). Growth curves for body weight of the laboratory rat. *Australian Journal of Biological Sciences*, *22*(4), 1077–1080. https://doi.org/10.1071/BI9691077

Palliser, H. K., Yates, D. M., & Hirst, J. J. (2012). Progesterone receptor isoform expression in response to in utero growth restriction in the fetal guinea pig brain. *Neuroendocrinology*, *96*(1), 60–67. https://doi.org/10.1159/000335138

Pang, Y., Fan, L. W., Tien, L. T., Dai, X., Zheng, B., Cai, Z., … Bhatt, A. (2013). Differential roles of astrocyte and microglia in supporting oligodendrocyte development and myelination in vitro. *Brain and Behavior*, *3*(5), 503–514. https://doi.org/10.1002/brb3.152

Paolicelli, R. C., & Ferretti, M. T. (2017). Function and dysfunction of microglia during brain development: Consequences for synapses and neural circuits. *Frontiers in Synaptic Neuroscience*, 1–17. https://doi.org/10.3389/fnsyn.2017.00009

Passetti, F., Chudasama, Y., & Robbins, T. W. (2002). The frontal cortex of the rat and visual attentional performance: Dissociable functions of distinct medial prefrontal subregions. *Cerebral Cortex*, *12*(12), 1254–1268. https://doi.org/10.1093/cercor/12.12.1254

Patten, A. R., Fontaine, C. J., & Christie, B. R. (2014). A comparison of the different animal models of fetal alcohol spectrum disorders and their use in studying complex behaviors. *Frontiers in Pediatrics*, *2*(SEP), 1–19. https://doi.org/10.3389/FPED.2014.00093/ABSTRACT

Perrin, J. S., Leonard, G., Perron, M., Pike, G. B., Pitiot, A., Richer, L., … Paus, T. (2009). Sex differences in the growth of white matter during adolescence. *NeuroImage*, *45*(4), 1055–1066. https://doi.org/10.1016/J.NEUROIMAGE.2009.01.023

Pfeiffer, S. E., Warrington, A. E., & Bansal, R. (1993). The oligodendrocyte and its many cellular processes. *Trends in Cell Biology*, *3*(6), 191–197. https://doi.org/10.1016/0962-8924(93)90213-K

Phillips, N. L. H., & Roth, T. L. (2019). Animal models and their contribution to our understanding of the relationship between environments, epigenetic modifications, and behavior. *Genes*, *10*(1). https://doi.org/10.3390/genes10010047

Popova, S., Lange, S., Probst, C., Gmel, G., & Rehm, J. (2018). Global prevalence of alcohol use and binge drinking during pregnancy, and fetal alcohol spectrum disorder. *Biochemistry and Cell Biology*, *96*(2), 237–240. https://doi.org/10.1139/bcb-2017-0077

Pritchard Orr, A. B., Keiver, K., Bertram, C. P., & Clarren, S. (2018). FAST club: The impact of a physical activity intervention on executive function in children with fetal alcohol spectrum disorder. *Adapted Physical Activity Quarterly*, *35*(4), 403–423. https://doi.org/10.1123/apaq.2017-0137

Pujol, J., Vendrell, P., Junqué, C., Martí-Vilalta, J. L., & Capdevila, A. (1993). When does human brain development end? Evidence of corpus callosum growth up to adulthood. *Annals of Neurology*, *34*(1), 71–75. https://doi.org/10.1002/ana.410340113

Qiang, M., Wang, M. W., & Elberger, A. J. (2002). Second trimester prenatal alcohol exposure alters development of rat corpus callosum. *Neurotoxicology and Teratology*, *24*, 719–732. https://doi.org/10.1016/S0892-0362(02)00267-2

Raff, M. C., Lillien, L. E., Richardson, W. D., Burne, J. F., & Noble, M. D. (1988). Platelet-derived growth factor from astrocytes drives the clock that times oligodendrocyte development in culture. *Nature*, *333*(6173), 562–565. https://doi.org/10.1038/333562a0

Rao, U., Chen, L. A., Bidesi, A. S., Shad, M. U., Thomas, M. A., & Hammen, C. L. (2010). Hippocampal Changes Associated with Early-Life Adversity and Vulnerability to Depression. *Biological Psychiatry*, *67*(4), 357–364. https://doi.org/10.1016/j.biopsych.2009.10.017

Reemst, K., Noctor, S. C., Lucassen, P. J., & Hol, E. M. (2016). The indispensable roles of microglia and astrocytes during brain development. *Frontiers in Human Neuroscience*, *10*(NOV2016), 1–28. https://doi.org/10.3389/fnhum.2016.00566

Richardson, W. D., Pringle, N., Mosley, M. J., Westermark, B., & Dubois-Dalcg, M. (1988). A role for platelet-derived growth factor in normal gliogenesis in the central nervous system. *Cell*, *53*(2), 309–319. https://doi.org/10.1016/0092-8674(88)90392-3

Riley, E. P., Mattson, S. N., Sowell, E. R., Jernigan, T. L., Sobel, D. F., & Jones, K. L. (1995). Abnormalities of the Corpus Callosum in Children Prenatally Exposed to Alcohol. *Alcoholism: Clinical and Experimental Research*, *19*(5), 1198–1202. https://doi.org/10.1111/j.1530-0277.1995.tb01600.x

Riley, E. P., Wozniak, J. R., Kable, J. A., Sowell, E. R., Boys, C. J., Mueller, B. A., … Lim, K. O. (2016). Functional connectivity abnormalities and associated cognitive deficits in fetal alcohol Spectrum disorders (FASD). *Brain Imaging and Behavior*, *11*(5), 1432–1445. https://doi.org/10.1007/s11682-016-9624-4

Roth, T. L., Lubin, F. D., Funk, A. J., & Sweatt, J. D. (2009). Lasting Epigenetic Influence of Early-Life Adversity on the BDNF Gene. *Biological Psychiatry*, *65*(9), 760–769. https://doi.org/10.1016/j.biopsych.2008.11.028

Ruggiero, M. J., Boschen, K. E., Roth, T. L., & Klintsova, A. Y. (2018). Sex Differences in Early Postnatal Microglial Colonization of the Developing Rat Hippocampus Following a Single-Day Alcohol Exposure. *Journal of Neuroimmune Pharmacology*, *13*(2), 189–203. https://doi.org/10.1007/s11481-017-9774-1

Ruotsalainen, I., Tammelin, T. H., Karvanen, J., & Parviainen, T. (2020). Developmental Cognitive Neuroscience Physical activity, aerobic fitness , and brain white matter : Their role for executive functions in adolescence. *Developmental Cognitive Neuroscience*, *42*. https://doi.org/10.1016/j.dcn.2020.100765

Salami, M., Itami, C., Tsumoto, T., & Kimura, F. (2003). Change of conduction velocity by regional myelination yields constant latency irrespective of distance between thalamus and cortex. *Proceedings of the National Academy of Sciences of the United States of America*, *100*(10), 6174–6179. https://doi.org/10.1073/pnas.0937380100

Sauvageot, C. M., & Stiles, C. D. (2002). Molecular mechanisms controlling cortical gliogenesis. *Current Opinion in Neurobiology*, *12*(3), 244–249. https://doi.org/10.1016/S0959-4388(02)00322-7

Schneider, C. A., Rasband, W. S., & Eliceiri, K. W. (2012). NIH Image to ImageJ: 25 years of image analysis. *Nature Methods*, *9*(7), 671–675. https://doi.org/10.1038/nmeth.2089

Schreiber, W. B., St. Cyr, S. A., Jablonski, S. A., Hunt, P. S., Klintsova, A. Y., & Stanton, M. E. (2013). Effects of exercise and environmental complexity on deficits in trace and contextual fear conditioning produced by neonatal alcohol exposure in rats. *Developmental Psychobiology*, *55*(5), 483–495. https://doi.org/10.1002/dev.21052

Schumacher, M., Liere, P., & Ghoumari, A. (2020). Progesterone and fetal-neonatal neuroprotection. *Best Practice & Research Clinical Obstetrics & Gynaecology*, *69*, 50–61. https://doi.org/10.1016/J.BPOBGYN.2020.09.001

Semple, B. D., Blomgren, K., Gimlin, K., Ferriero, D. M., & Noble-Haeusslein, L. J. (2013). Brain development in rodents and humans: Identifying benchmarks of maturation and vulnerability to injury across species. *Progress in Neurobiology*, *106–107*, 1–16. https://doi.org/10.1016/J.PNEUROBIO.2013.04.001

Shaw, G. A., Dupree, J. L., & Neigh, G. N. (2020). Adolescent maturation of the prefrontal cortex: Role of stress and sex in shaping adult risk for compromise. *Genes, Brain and Behavior*, *19*(3), 1–9. https://doi.org/10.1111/gbb.12626

Shen, S., Li, J., & Casaccia-Bonnefil, P. (2005). Histone modifications affect timing of oligodendrocyte progenitor differentiation in the developing rat brain. *Journal of Cell Biology*, *169*(4), 577–589. https://doi.org/10.1083/jcb.200412101

Simons, M., & Nave, K.-A. (2016). Oligodendrocytes: Myelination and Axonal Support. *Cold Spring Harbor Perspectives in Biology*, *8*(1), a020479. https://doi.org/10.1101/CSHPERSPECT.A020479

Smith, I. F., Gursky, Z. H., & Klintsova, A. Y. (2022). Representation of prefrontal axonal efferents in the thalamic nucleus reuniens in a rodent model of fetal alcohol exposure during third trimester. *Frontiers in Behavioral Neuroscience*, *16*(September), 1–11. https://doi.org/10.3389/fnbeh.2022.993601

Sommer, L. (2006). Growth Factors Regulating Neural Crest Cell Fate Decisions. In J.-P. Saint-Jeannet (Ed.), *Neural Crest Induction and Differentiation* (pp. 197–205). Boston, MA: Springer US. Retrieved from https://doi.org/10.1007/978-0-387-46954-6_12

Sowell, E. R., Johnson, A., Kan, E., Lu, L. H., Van Horn, J. D., Toga, A. W., … Bookheimer, S. Y. (2008). Mapping white matter integrity and neurobehavioral

correlates in children with fetal alcohol spectrum disorders. *Journal of Neuroscience*, *28*(6), 1313–1319. https://doi.org/10.1523/JNEUROSCI.5067-07.2008

Steadman, P. E., Xia, F., Ahmed, M., Mocle, A. J., Penning, A. R. A., Geraghty, A. C., … Frankland, P. W. (2020). Disruption of Oligodendrogenesis Impairs Memory Consolidation in Adult Mice. *Neuron*, *105*(1), 150-164.e6. https://doi.org/10.1016/j.neuron.2019.10.013

Sternberger, N. H., Itoyama, Y., Kies, M. W., & Webster DeF., H. (1978). Myelin basic protein demonstrated immunocytochemically in oligodendroglia prior to myelin sheath formation. *Proceedings of the National Academy of Sciences of the United States of America*, *75*(5), 2521–2524. https://doi.org/10.1073/pnas.75.5.2521

Stikov, N., Campbell, J. S. W., Stroh, T., Lavelée, M., Frey, S., Novek, J., … Pike, G. B. (2015). In vivo histology of the myelin g-ratio with magnetic resonance imaging. *NeuroImage*, *118*, 397–405. https://doi.org/10.1016/j.neuroimage.2015.05.023

Stiles, J., & Jernigan, T. L. (n.d.). The Basics of Brain Development. https://doi.org/10.1007/s11065-010-9148-4

Straathof, M., Sinke, M. R. T., Roelofs, T. J. M., Blezer, E. L. A., Sarabdjitsingh, R. A., van der Toorn, A., … Dijkhuizen, R. M. (2020). Distinct structure-function relationships across cortical regions and connectivity scales in the rat brain. *Scientific Reports*, *10*(1), 1–14. https://doi.org/10.1038/s41598-019-56834-9

Swamydas, M., Bessert, D., & Skoff, R. (2009). Sexual dimorphism of oligodendrocytes is mediated by differential regulation of signaling pathways. *Journal of Neuroscience Research*, *87*(15), 3306–3319. https://doi.org/https://doi.org/10.1002/jnr.21943

Takao, T., Flint, N., Lee, L., Ying, X., Merrill, J., & Chandross, K. J. (2004). 17Beta-Estradiol Protects Oligodendrocytes From Cytotoxicity Induced Cell Death. *Journal of Neurochemistry*, *89*(3), 660–673. https://doi.org/10.1111/j.1471-4159.2004.02370.x

Talukdar, T., Nikolaidis, A., Zwilling, C. E., Paul, E. J., Hillman, C. H., Cohen, N. J., … Barbey, A. K. (2018). Aerobic Fitness Explains Individual Differences in the Functional Brain Connectome of Healthy Young Adults. *Cerebral Cortex*, *28*, 3600–3609. https://doi.org/10.1093/cercor/bhx232

Tang, S., Xu, S., Waddell, J., Zhu, W., Gullapalli, R. P., & Mooney, S. M. (2019). Functional Connectivity and Metabolic Alterations in Medial Prefrontal Cortex in a Rat Model of Fetal Alcohol Spectrum Disorder: A Resting-State Functional Magnetic Resonance Imaging and in vivo Proton Magnetic Resonance Spectroscopy Study. *Developmental Neuroscience*, *28081*, 1–12. https://doi.org/10.1159/000499183

Taylor, P. A., Jacobson, S. W., van der Kouwe, A., Molteno, C. D., Chen, G., Wintermark, P., … Meintjes, E. M. (2015). A DTI-based tractography study of effects on brain structure associated with prenatal alcohol exposure in newborns. *Human Brain Mapping*, *36*(1), 170–186. https://doi.org/10.1002/hbm.22620

Terasaki, L. S., Blades, A. L., & Schwarz, J. M. (2015). Moderate in utero alcohol exposure results in sex-dependent inflammation in the developing rat brain. *Brain, Behavior, and Immunity*, *49*, e5. https://doi.org/10.1016/J.BBI.2015.06.040

Terasaki, L. S., Gomez, J., & Schwarz, J. M. (2016). An examination of sex differences in the effects of early-life opiate and alcohol exposure. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *371*(1688). https://doi.org/10.1098/rstb.2015.0123

Terasaki, L. S., & Schwarz, J. M. (2016). Effects of Moderate Prenatal Alcohol Exposure during Early Gestation in Rats on Inflammation across the Maternal-Fetal-Immune Interface and Later-Life Immune Function in the Offspring. *Journal of Neuroimmune Pharmacology*, *11*(4), 680–692. https://doi.org/10.1007/s11481-016-9691-8

Tomlinson, D., Wilce, P., & Bedi, K. . (1998). Spatial Learning Ability of Rats Following Differing Levels of Exposure to Alcohol During Early Postnatal Life. *Physiology & Behavior*, *63*(2), 205–211. https://doi.org/10.1016/S0031-9384(97)00424-1

Tomlinson, L., Huang, P. H., & Colognato, H. (2018). Prefrontal cortex NG2 glia undergo a developmental switch in their responsiveness to exercise. *Developmental Neurobiology*, *78*(7), 687–700. https://doi.org/10.1002/dneu.22590

Tomlinson, L., Leiton, C. V., & Colognato, H. (2016). Behavioral experiences as drivers of oligodendrocyte lineage dynamics and myelin plasticity. *Neuropharmacology*, *110*, 548–562. https://doi.org/10.1016/J.NEUROPHARM.2015.09.016

Traiffort, E., Kassoussi, A., Zahaf, A., & Laouarem, Y. (2020). Astrocytes and Microglia as Major Players of Myelin Production in Normal and Pathological Conditions. *Frontiers in Cellular Neuroscience*, *14*(April), 1–21. https://doi.org/10.3389/fncel.2020.00079

Traiffort, E., Zakaria, M., Laouarem, Y., & Ferent, J. (2016). Hedgehog: A key signaling in the development of the oligodendrocyte lineage. *Journal of Developmental Biology*, *4*(3), 1–20. https://doi.org/10.3390/jdb4030028

Treit, S., Chen, Z., Zhou, D., Baugh, L., Rasmussen, C., Andrew, G., … Beaulieu, C. (2017). Sexual dimorphism of volume reduction but not cognitive deficit in fetal alcohol spectrum disorders: A combined diffusion tensor imaging, cortical thickness and brain volume study. *NeuroImage: Clinical*, *15*, 284–297. https://doi.org/10.1016/j.nicl.2017.05.006

Treit, S., Lebel, C., Baugh, L., Rasmussen, C., Andrew, G., & Beaulieu, C. (2013). Longitudinal MRI Reveals Altered Trajectory of Brain Development during Childhood and Adolescence in Fetal Alcohol Spectrum Disorders. *The Journal of Neuroscience*, *33*(24), 10098–10109. https://doi.org/10.1523/JNEUROSCI.5004-12.2013

Treit, S., Zhou, D., Lebel, C., Rasmussen, C., Andrew, G., & Beaulieu, C. (2014). Longitudinal MRI reveals impaired cortical thinning in children and adolescents prenatally exposed to alcohol. *Human Brain Mapping*, *35*(9), 4892–4903. https://doi.org/https://doi.org/10.1002/hbm.22520

Uban, K. A., Herting, M. M., Wozniak, J. R., & Sowell, E. R. (2017). Sex differences in associations between white matter microstructure and gonadal hormones in children and adolescents with prenatal alcohol exposure. *Psychoneuroendocrinology*, *83*(May), 111–121. https://doi.org/10.1016/j.psyneuen.2017.05.019

Umer, A., Lilly, C., Hamilton, C., Baldwin, A., Breyel, J., Tolliver, A., … Maxwell, S. (2020). Prevalence of alcohol use in late pregnancy. *Pediatric Research*, *88*(2), 312–319. https://doi.org/10.1038/s41390-019-0731-y

van Tilborg, E., de Theije, C. G. M., van Hal, M., Wagenaar, N., de Vries, L. S., Benders, M. J., … Nijboer, C. H. (2018). Origin and dynamics of oligodendrocytes in the developing brain: Implications for perinatal white matter injury. *Glia*, *66*(2), 221–238. https://doi.org/10.1002/glia.23256

Wang, L. C., & Almazan, G. (2016). Role of Sonic Hedgehog Signaling in Oligodendrocyte Differentiation. *Neurochemical Research*, *41*(12), 3289–3299. https://doi.org/10.1007/s11064-016-2061-3

Ware, A. L., Long, X., & Lebel, C. (2021). Functional connectivity of the attention networks is altered and relates to neuropsychological outcomes in children with prenatal alcohol exposure. *Developmental Cognitive Neuroscience*, *48*, 100951. https://doi.org/10.1016/j.dcn.2021.100951

West, M. J., Slomianka, L., & Gundersen, H. J. G. (1991). Unbiased stereological estimation of the total number of neurons in the subdivisions of the rat hippocampus using the optical fractionator. *The Anatomical Record*, *231*(4), 482–497. https://doi.org/10.1002/ar.1092310411

Whitworth, A. L., Mann, N. H., & Larkum, A. W. D. (2006). This article is protected by copyright. All rights 1 reserved. *Ultrasound Obstet Gynecol.*, *50*(6), 776–780. https://doi.org/10.1111/acer.14928

Willford, J. A., Richardson, G. A., Leech, S. L., & Day, N. L. (2004). Verbal and Visuospatial Learning and Memory Function in Children With Moderate Prenatal Alcohol Exposure. *Alcoholism: Clinical and Experimental Research*, *28*(3), 497–507. https://doi.org/https://doi.org/10.1097/01.ALC.0000117868.97486.2D

Williamson, A. V, Compston, D. A. S., & Randall, A. D. (1997). Analysis of the Ion Channel Complement of the Rat Oligodendrocyte Progenitor in a Commonly Studied In vitro Preparation. *European Journal of Neuroscience*, *9*(4), 706–720. https://doi.org/https://doi.org/10.1111/j.1460-9568.1997.tb01419.x

Williamson, J. M., & Lyons, D. A. (2018). Myelin dynamics throughout life: An ever-changing landscape? *Frontiers in Cellular Neuroscience*, *12*(November), 1–8. https://doi.org/10.3389/fncel.2018.00424

Wilson, J. H., Kelly, S. J., & Wilson, M. A. (1996). Early postnatal alcohol exposure in rats: Maternal behavior and estradiol levels. *Physiology and Behavior*, *59*(2), 287–293. https://doi.org/10.1016/0031-9384(95)02094-2

Wlodarczyk, A., Holtman, I. R., Krueger, M., Yogev, N., Bruttger, J., Khorooshi, R., … Owens, T. (2017). A novel microglial subset plays a key role in myelinogenesis in developing brain. *The EMBO Journal*, *36*(22), 3292–3308. https://doi.org/10.15252/embj.201696056

Wozniak, J. R., Mueller, B. A., Chang, P. N., Muetzel, R. L., Caros, L., & Lim, K. O. (2006). Diffusion tensor imaging in children with fetal alcohol spectrum

disorders. *Alcoholism: Clinical and Experimental Research*, *30*(10), 1799–1806. https://doi.org/10.1111/j.1530-0277.2006.00213.x

Wozniak, J. R., & Muetzel, R. L. (2011). What does diffusion tensor imaging reveal about the brain and cognition in fetal alcohol spectrum disorders? *Neuropsychology Review*, *21*(2), 133–147. https://doi.org/10.1007/s11065-011-9162-1

Wozniak, J. R., Muetzel, R. L., Mueller, B. A., McGee, C. L., Freerks, M. A., Ward, E. E., … Lim, K. O. (2009). Microstructural corpus callosum anomalies in children with prenatal alcohol exposure: An extension of previous diffusion tensor imaging findings. *Alcoholism: Clinical and Experimental Research*, *33*(10), 1825–1835. https://doi.org/10.1111/j.1530-0277.2009.01021.x

Wozniak, J. R., Riley, E. P., & Charness, M. C. (2019). Diagnosis, epidemiology, assessment, pathophysiology, and management of fetal alcohol spectrum disorders. *Lancet Neurology*, 760–770. https://doi.org/10.1016/S1474-4422(19)30150-4.

Wozniak, J. R., Riley, E. P., & Charness, M. E. (2019). Clinical presentation, diagnosis, and management of fetal alcohol spectrum disorder. *The Lancet Neurology*, *18*(8), 760–770. https://doi.org/10.1016/S1474-4422(19)30150-4

Xin, W., & Chan, J. R. (2020). Myelin plasticity: sculpting circuits in learning and memory. *Nature Reviews Neuroscience*, *21*(12), 682–694. https://doi.org/10.1038/s41583-020-00379-8

Xiong, X., Zhu, L.-N., Dong, X.-X., Wang, W., Yan, J., & Chen, A.-G. (2018). Aerobic Exercise Intervention Alters Executive Function and White Matter Integrity in Deaf Children: A Randomized Controlled Study. *Neural Plasticity*, *2018*. https://doi.org/10.1155/2018/3735208

Xue, R., Van Zijl, P. C. M., Crain, B. J., Solaiyappan, M., & Mori, S. (1999). In vivo three-dimensional reconstruction of rat brain axonal projections by diffusion tensor imaging. *Magnetic Resonance in Medicine*, *42*(6), 1123–1127. https://doi.org/10.1002/(SICI)1522-2594(199912)42:6<1123::AID-MRM17>3.0.CO;2-H

Yang, L., Zhao, C., Xiong, Y., Zhong, S., Wu, D., & Peng, S. (2021). Callosal fiber length scales with brain size according to functional lateralization , evolution , and development. *BioRxiv*, 1–19. https://doi.org/https://doi.org/10.1101/2021.04.01.437788

Ye, F., Chen, Y., Hoang, T., Montgomery, R. L., Zhao, X. H., Bu, H., … Lu, Q. R. (2009). HDAC1 and HDAC2 regulate oligodendrocyte differentiation by disrupting the B-catenin-TCF interaction. *Nature Neuroscience*, *12*(7), 829–838. https://doi.org/10.1038/nn.2333

Zerlin, M., Levison, S. W., & Goldman, J. E. (1995). Early patterns of migration, morphogenesis, and intermediate filament expression of subventricular zone cells in the postnatal rat forebrain. *Journal of Neuroscience*, *15*(11), 7238–7249. https://doi.org/10.1523/jneurosci.15-11-07238.1995

Zhang, C., Paolozza, A., Tseng, P. H., Reynolds, J. N., Munoz, D. P., & Itti, L. (2019). Detection of children/youth with fetal alcohol spectrum disorder through eye movement, psychometric, and neuroimaging data. *Frontiers in Neurology*, *10*(FEB), 1–15. https://doi.org/10.3389/fneur.2019.00080

Zheng, J., Sun, X., Ma, C., Li, B. M., & Luo, F. (2019). Voluntary wheel running promotes myelination in the motor cortex through Wnt signaling in mice. *Molecular Brain*, *12*(1), 1–10. https://doi.org/10.1186/s13041-019-0506-8

Zheng, S., Chan, W. S., Leung, S. H., & Xue, Q. (2007). Broadband Butler matrix with flat coupling. *Electronics Letters*, *43*(10), 576–577. https://doi.org/10.1049/el:20070274

Zhou, Q., Wang, S., & Anderson, D. J. (2000). Identification of a Novel Family of Oligodendrocyte Lineage-Specific Basic Helix–Loop–Helix Transcription Factors. *Neuron*, *25*(2), 331–343. https://doi.org/10.1016/S0896-6273(00)80898-3

Zimmerberg, B., & Reuter, J. M. (1989). Sexually dimorphic behavioral and brain asymmetries in neonatal rats: effects of prenatal alcohol exposure. *Developmental Brain Research*, *46*(2), 281–290. https://doi.org/10.1016/0165-3806(89)90291-5

Zimmerberg, B.& Scalzi, L. (1989). Commissural size in neonatal rats: effects of sex and prenatal alcohol exposure, *Int. J. Devl. Neuroscience*. *7*(1).

Appendix A

INSTITUTIONAL ANIMAL CORE AND USE COMMITTEE (IACUC) APPROVAL

University of Delaware
Institutional Animal Care and Use Committee
Application to Use Animals in Research
(New and 3-Yr submission)

Title of Protocol: Therapeutic Motor Training and Fetal Alcohol Effects	
AUP Number: 1134-2022-0	**← (4 digits only — if new, leave blank)**
Principal Investigator: Anna Klintsova	
Common Name (Strain/Breed if Appropriate): Rat, Long Evans **Genus Species:** Rattus norvegicus	
Date of Submission: 04/17/2022	

Official Use Only

IACUC Approval Signature: Jean Tallon, DVM

Date of Approval: 7.1.2022

Appendix B

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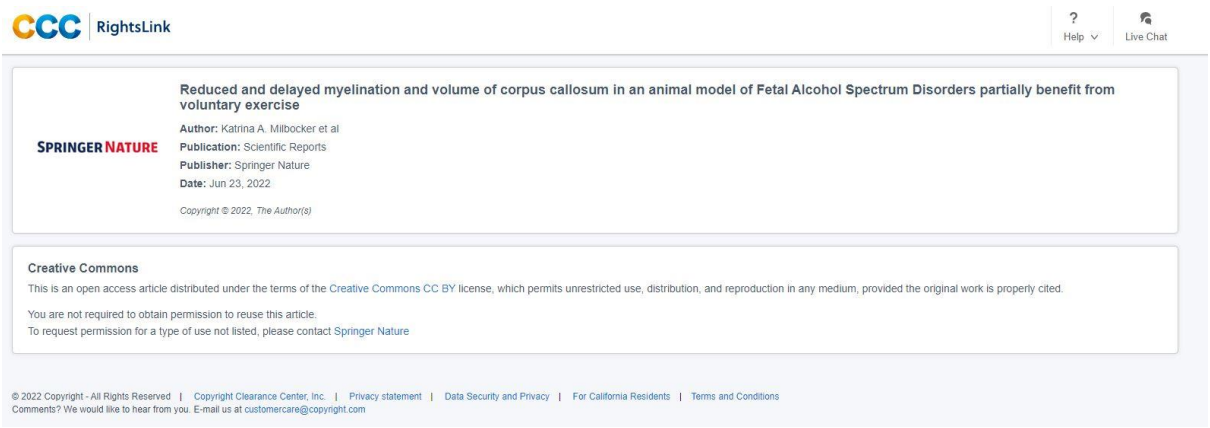

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Reduced and delayed myelination and volume of corpus callosum in an animal model of Fetal Alcohol Spectrum Disorders partially benefit from voluntary exercise

Author: Katrina A. Milbocker et al
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