IMPACTS OF MATERNAL PRENATAL ENRICHMENT ON OFFSPRING BRAIN MORPHOLOGY AND BEHAVIOUR IN THE LONG-EVANS RAT

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DEDICATION

To Logan Leroux, who has been my rock throughout not one, but now two, theses. Thank you for your unwavering belief in me. I could not have done this without your endless support.

To all of the animals that were involved in this project, thank you for your contributions to science, and the endless laughs and smiles you were responsible for during many long hours in the vivarium. You will always be honoured and remembered.

ABSTRACT

The maternal environment significantly impacts fetal development, and much of the existing research is focussed on outcomes following negative maternal experiences rather than positive experiences like enrichment. Maternal exposure to positive experiences is under-investigated, leaving its potential benefits largely unexplored. Environmental enrichment (EE) is a non-invasive paradigm that utilizes physical, social, and intellectual stimulation to benefit subjects. EE has been found to produce significant changes in behaviour and neuronal morphology. This research explores whether there are any long-term neuroanatomical and behavioural changes in offspring following maternal prenatal enrichment (PE). Dams were housed in distinct conditions, either standard (control dams) or enriched (PE dams) housing, during pregnancy. A total of 37 PE pups and 32 control pups were used for behavioural testing. Offspring underwent behavioural tests during early life (Open Field Test [OFT]), adolescence (Elevated Plus Maze [EPM]), and adulthood (Whishaw Tray Reaching [WTR], Morris Water Task [MWT]). Animals were euthanized, and their brains perfused, extracted, and prepared for Nissl analysis. A pilot sample of 15 male offspring (8 control, 7 PE) were used for volumetric analysis in the mPFC and parietal cortex. No significant differences in OFT, EPM, or MWT were found between PE and control offspring, indicating PE had no significant impact on anxiety-like behaviour, exploratory behaviour, or spatial learning and memory. However, PE offspring were found to have a significantly higher number of total and successful reaches in WTR, despite no differences in accuracy or lateralization, which may indicate increased task engagement or motivation in the PE offspring. No significant differences were found in total volume of both the mPFC and parietal cortex in the PE offspring, suggesting that PE had no impact on the overall volume of these regions.

ETHICS STATEMENT

Work described in this thesis received research ethics approval from the University of Lethbridge Animal Welfare Committee, Project Name “Examining the Impacts of Maternal Enrichment on Brain Morphology of Offspring in the Long-Evans Rat”, Protocol 2303, August 11, 2023.

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LIST OF ABREVIATIONS

ANOVA	Analysis of Variance
EE	Environmental Enrichment
EPM	Elevated Plus Maze
LTD	Long-term Depression
LTP	Long-term Potentiation
MPNE	Maternal Prenatal Enrichment
mPFC	Medial Prefrontal Cortex
MWT	Morris Water Task
NGF	Nerve Growth Factor
OFT	Open Field Test
P/PND	Postnatal Day
PAR	Parietal Cortex
PE	Prenatal Enrichment
PFA	Paraformaldehyde
WTR	Whishaw Tray Reaching

Chapter 1

General Introduction

History of Maternal Experience Research

The maternal environment has long been recognized as a critical determinant of fetal development, influencing both physiological and psychological outcomes in offspring. A substantial portion of the current literature has concentrated on the consequences of adverse maternal experiences, particularly maternal stress, rather than the potential benefits of positive experiences such as environmental enrichment. This focus has created an imbalance in our understanding of the full spectrum of maternal influence. Numerous studies have shown that maternal stress during pregnancy can be detrimental, being associated with a range of negative outcomes in humans—from reduced birth weight to behavioural irregularities in childhood and beyond (Mulder et al., 2002). Stress and depression are among the most prevalent complications encountered during pregnancy, with estimates suggesting that up to 78% of pregnant individuals report experiencing moderate to high levels of stress. This is particularly concerning for socially high-risk populations, such as marginalized or economically disadvantaged groups. For example, in Canada, studies have shown that approximately 30% of socially high-risk pregnant women experience depression during pregnancy.

Given this high prevalence, particularly within vulnerable or underserved populations, there is an urgent and growing need for accessible, effective solutions that support maternal well-being. One such solution lies in promoting a healthy adaptation to pregnancy, which is increasingly being understood as vital not only for the mother's physical and emotional health but also for fostering confidence in parenting and promoting healthier outcomes in children.

Research has found that negative effects of maternal stress may be somewhat mitigated through interventions such as physical exercise (Bowen et al., 2009). These findings point to the potential applicability of intentionally designed environmental enrichment strategies during pregnancy. Despite the promise of such approaches, the concept of maternal exposure to positive experiences, including, but not limited to, increased physical activity and enriched social interaction, remains significantly underexplored, especially in relation to its long-term neuroanatomical and behavioural effects on offspring. As such, the benefits of positive maternal experiences are far from fully understood, leaving a critical gap in our knowledge of maternal-fetal health.

History of Environmental Enrichment

Environmental enrichment (EE) is a widely used, non-invasive research paradigm that incorporates elements of physical, social, and cognitive stimulation to improve the psychological and physiological health of subjects. In laboratory settings, EE is commonly employed to ensure that animals maintain a baseline level of well-being, particularly in contexts where sterile or monotonous environments could otherwise lead to stress or behavioural deficits (Shepherdson et al., 1999). Because EE supports this foundational level of wellness, researchers have increasingly considered whether more intensive or tailored enrichment could further improve well-being or foster specific functional benefits.

Over the years, EE has been repeatedly shown to elicit significant and sometimes profound changes in both behaviour and neuronal structure (Diamond et al., 1972; Greenough et al., 1973; Rosenzweig & Bennett, 1972). It serves as a powerful tool for studying the complex relationships between sensory experiences, brain morphology, and behavioural outcomes. The

concept of EE in scientific inquiry can be traced back to pioneering work by Donald Hebb in the 1940s. In his early studies, Hebb found that animals raised in enriched environments demonstrated superior memory, problem-solving capabilities, and social behaviour compared to those in standard conditions (Hebb, 1947; Clarke et al., 1951). Despite the importance of Hebb's foundational research, widespread use of EE in experimental settings did not gain momentum until the 1980s, when its utility became more widely appreciated (Mellen & Sevenich MacPhee, 2001).

Since then, research on EE has grown steadily, with the number of published studies on the topic continuing to rise, though the focus of EE studies has primarily been neuroanatomy, largely overlooking possible behavioural outcomes. Today, EE is applied across a diverse range of settings and species, including zoos, aquariums, animal shelters, and research laboratories. Remarkably, roughly 80% of published studies report beneficial effects resulting from EE interventions, underscoring the consistency and reliability of its impact (Bachetti et al., 2024). However, despite its widespread application and largely positive results, one area within the EE field remains relatively underdeveloped: the availability of physiological data that can provide objective metrics for measuring the efficiency and mechanisms of EE-based interventions, among other more specific applications of EE beyond daily wellness (Bachetti et al., 2024).

Effects of Environmental Enrichment on Neuroanatomy

To date, environmental enrichment has consistently been shown to produce beneficial neuroanatomical changes in both developing and adult animals. One of the most well-documented effects of EE is its capacity to enhance the complexity and size of dendritic trees and to increase dendritic spine density, indicative of greater synaptic connectivity (Darmopil et

al., 2008). This is particularly significant given that dendritic complexity and spine density are critical markers of neuronal plasticity and overall brain function. Prior research has also demonstrated that EE promotes hippocampal neurogenesis, leading to a notable increase in the number of newborn neurons in the brain (van Praag et al., 2000). For instance, one study in mice revealed that animals exposed to enriched conditions exhibited a significantly higher number of new neurons in the dentate gyrus region of the hippocampus compared to their littermates raised in standard laboratory housing. These enriched animals also showed a larger granule cell layer and approximately 15% more granule cell neurons in the dentate gyrus (Kemperman et al., 1997).

Beyond promoting neurogenesis, EE has been found to elevate the expression of nerve growth factor (NGF), a key neurotrophic factor involved in synaptic development and plasticity. Notably, these neurobiological effects can be observed even when EE is provided in isolation from other potentially confounding factors, such as voluntary running, highlighting the intrinsic power of enriched environments (Birch et al., 2013). While much of the neuroanatomical research on EE has centered on general adult brain function, its application in the context of parent-offspring relationships, particularly prenatal influences, remains relatively underexplored. Nevertheless, some promising findings have emerged. Gibb et al. (2014) investigated the effects of prenatal EE in a rodent model of perinatal cortical injury and found that offspring of enriched mothers exhibited increased cortical thickness at anterior brain planes and enhanced thalamic volume in both anterior and posterior regions. These offspring also showed elevated dendritic spine density, suggesting long-lasting structural benefits. This current project seeks to extend these neuroanatomical findings by examining how prenatal maternal enrichment may influence

overall estimated brain volume in offspring and further extend them by evaluating behaviour throughout the entire offspring lifespan.

Effects of Environmental Enrichment on Behaviour

Perhaps one of the most compelling and well-investigated domains of EE research pertains to its impact on behaviour, particularly behaviour linked to hippocampal function. A growing body of literature has shown that animals exposed to enriched environments tend to exhibit enhanced performance across a variety of cognitive and behavioural domains. For example, when researchers specifically designed a study to isolate EE from other enriching experiences like physical exercise and dietary changes, they found that EE alone led to improved outcomes on multiple behavioural tasks. These included enhanced spatial memory, as assessed by object displacement tasks; improved working memory, as measured by performance in a T-maze; better recognition memory via novel object recognition tasks; and superior contextual fear memory (Clemenson et al., 2014; Birch et al., 2013).

In addition to enhancing neurogenesis in the hippocampus, EE has been shown to improve hippocampus-dependent behaviours more broadly, particularly those related to learning and memory consolidation (van Praag et al., 2000). Despite these promising findings, the application of EE to parent-offspring research remains limited. This represents a key opportunity for future exploration, particularly given the potential of EE to serve as a preventative or therapeutic intervention against the adverse effects of prenatal stress or trauma. Supporting this notion, Gibb et al. (2014) found that prenatal EE not only promoted neuroanatomical recovery in the context of perinatal cortical damage but also enhanced behavioural outcomes in offspring, including improved spatial memory and increased proficiency in skilled reaching tasks. These

findings suggest that maternal enrichment during pregnancy may hold promise as a novel approach for supporting both brain development and cognitive function in offspring.

Effects of Maternal Experience on Offspring Development

Fetal brain development occurs within the relatively protected environment of the womb; however, this developmental process is not immune to external maternal influences. A wide range of maternal factors, particularly those related to physical and psychological stress, can disrupt or alter the typical trajectory of brain maturation. Such disruptions during gestation have been associated with increased vulnerability to various cognitive, behavioural, and emotional disorders later in life. Extensive clinical and preclinical research has consistently demonstrated that maternal experiences during pregnancy play a central role in shaping neurodevelopmental outcomes in offspring.

Among these factors, maternal stress during pregnancy has emerged as a particularly influential variable. Research conducted on pregnant women in Quebec who lived through a severe ice storm found that greater levels of stress, measured by exposure to injury, loss, and major life changes, were associated with lower cognitive and language performance in their children at two years of age (LaPlante et al., 2004). Importantly, these developmental delays persisted well into early childhood, with measurable differences still present when the children reached five and a half years old (King & LaPlante, 2005). These findings underscore the lasting impact that acute maternal stress can have on the developing brain.

Broader studies in human populations further support the link between prenatal stress and long-term neurodevelopmental consequences. Exposure to severe or chronic maternal stress during gestation has been associated with increased risk for cognitive impairments and mood-

related disorders in offspring (King & LaPlante, 2005). These effects are thought to arise from altered neuroendocrine function, inflammatory processes, and changes in maternal physiology that directly or indirectly affect fetal development.

Animal models provide additional evidence for the physiological mechanisms by which maternal stress impacts offspring outcomes. In a study by Amugongo and Hlusko (2013), pregnant rats exposed to stress during early gestation exhibited significantly reduced weight gain despite producing the largest average number of pups. This finding was interpreted as a possible indicator of compromised maternal health due to elevated cortisol levels during those gestational periods. Although the study did not collect birth weight data for the offspring, the combination of high pup counts and low maternal weight gain suggests that these offspring may have experienced intrauterine growth restriction, potentially leading to lower birth weights and related developmental risks.

Collectively, findings from both human and animal research highlight the powerful role of maternal experience, particularly stress, in shaping early brain development and behaviour. Despite this growing body of evidence, much of the existing literature remains focused on the negative consequences of prenatal stress. Far less is known about the potential benefits of positive maternal experiences during pregnancy, such as exposure to EE. Investigating the effects of such positive experiences may offer valuable insight into protective or enhancing mechanisms that promote optimal offspring development.

Effects of Maternal Experience on Offspring Neuroanatomy

Emerging evidence from both human and animal research has demonstrated that maternal experiences during pregnancy, particularly stress, can exert lasting effects on offspring

neuroanatomy, with implications for cognitive function, emotional regulation, and behaviour. Experimental models in rodents and non-human primates have provided detailed insight into how prenatal stress affects specific brain structures. For example, studies in rats have shown that exposure to repeated maternal stressors, such as unpredictable handling, saline injections, and novel environments beginning in late gestation, leads to notable changes in the structure of the amygdala, particularly in its connectivity to other regions. These structural differences are thought to increase vulnerability to fear-based behaviours, including heightened anxiety-like responses (Kraszpulski et al., 2006). Given the amygdala's central role in emotional processing, these findings suggest that even moderate prenatal disruptions can result in altered emotional circuitry in the offspring.

Similarly, research conducted in rhesus monkeys has shown that repeated prenatal stress during both early and late gestation, induced by relocation and acoustic startle protocols, can lead to a reduction in hippocampal neurogenesis. Offspring exposed to these conditions exhibited smaller hippocampal volumes, indicating that gestational stress can impair the development of brain regions critical for learning and memory (Coe et al., 2003). The hippocampus is particularly sensitive to glucocorticoid fluctuations, which are often elevated during maternal stress, making it a frequent target of stress-related neurodevelopmental changes.

At the cellular level, prenatal stress has been shown to alter synaptic plasticity mechanisms in the offspring brain. For instance, when pregnant rats were exposed to unpredictable foot shocks during the second half of gestation, their offspring exhibited impaired long-term potentiation (LTP) and enhanced long-term depression (LTD) in the CA1 region of the hippocampus. These changes in synaptic efficiency were accompanied by deficits in spatial learning and memory, as demonstrated in Morris water maze testing (Yang et al., 2006). Such

findings underscore the neurobiological consequences of prenatal stress, linking disrupted cellular processes to observable cognitive impairments.

Taken together, these studies emphasize the vulnerability of the developing brain to maternal experiences during gestation. Structural, volumetric, and functional alterations in key brain regions, including the amygdala and hippocampus, can arise from relatively brief but intense periods of maternal stress. While this body of research has firmly established the risks associated with adverse maternal experiences, far less is known about how positive maternal experiences might support or enhance offspring neurodevelopment.

Volumetric Changes in the Brain Following Experience

Brain volume is a sensitive indicator of neural development and integrity, often correlating with behavioural and cognitive function. Research in animal models has consistently demonstrated that reductions in brain volume are associated with age-related decline, neurological disorders, and behavioural impairments. These structural changes may also reflect early developmental influences, including prenatal environmental factors such as maternal stress or enrichment.

In a longitudinal study examining natural aging in rats, significant volumetric reductions were observed in several key brain regions, including the medial prefrontal cortex (mPFC), hippocampus, and striatum. Older rats (27 months) showed smaller regional volumes compared to younger adults (14 months), and this shrinkage was accompanied by marked deficits in exploratory behaviour and spatial memory performance (Hamezah et al., 2017). These findings suggest that volume loss in these regions is not only a marker of aging but also closely tied to functional outcomes relevant to cognition and locomotion.

Similarly, studies in disease models highlight the relationship between reduced brain volume and behavioural dysfunction. For instance, spontaneously hypertensive rats exhibited a global reduction in brain volume (11–25%) across multiple grey matter structures, including cortical regions. This volumetric decline was also evident in cortical thickness, indicating a widespread pattern of cerebral atrophy associated with physiological stress and systemic disease (Tajima et al., 1993).

Further support comes from transgenic animal models. Casas et al. (2018) reported that HIV-1 transgenic rats displayed smaller total brain volumes compared to wild-type controls, with disproportionately pronounced atrophy in the striatum. This regional volume loss was predictive of neurobehavioural deficits, emphasizing how structural changes in specific brain areas can underlie impairments in motor and cognitive functions.

Taken together, these findings underscore the importance of brain volume as a structural correlate of behavioural capacity. They also provide a compelling rationale for investigating how a positive experience, like maternal prenatal enrichment, rather than a negative one may influence offspring brain volume during development.

Relevance

Despite a growing awareness of the importance of early-life environments on long-term developmental outcomes, there remains a critical gap in the availability of proactive, evidence-based interventions aimed at improving child health and well-being by supporting parents prior to birth. The prenatal period represents a uniquely sensitive window during which maternal health and behaviour can significantly influence fetal development. However, most existing interventions tend to be reactive, addressing problems after they arise, rather than preventative in

nature. This research seeks to address that gap by providing foundational evidence for the development of positive, non-invasive interventions that support maternal well-being during pregnancy and, in doing so, promote healthier outcomes for offspring.

A clear example of the urgent need for such approaches can be seen in socially high-risk populations. In Canada, it has been reported that approximately 30% of pregnant individuals from these groups experience clinical levels of depression during pregnancy, a condition associated with numerous adverse outcomes in both mothers and their children. Notably, some studies have found that this burden of prenatal depression may be reduced through regular physical activity and other forms of lifestyle-based intervention (Bowen et al., 2009). These findings underscore the modifiable nature of the prenatal environment and highlight the potential for targeted supports to mitigate risk.

One key factor that has been consistently linked to improved maternal and offspring outcomes is a mother's ability to adapt healthily to pregnancy. Healthy psychological and behavioural adaptation to the challenges of pregnancy not only contributes to a smoother gestational experience but also builds maternal self-efficacy, an important foundation for effective parenting and positive child development. Research indicates that this adaptive capacity is more often observed in women who intentionally pursue pregnancy and take proactive steps to prepare for it (Teskereci et al., 2023). This concept of intentionality may be extended to structured environmental enrichment during pregnancy, offering a promising avenue for improving both maternal experience and developmental trajectories in offspring.

By exploring maternal prenatal enrichment as a potential tool for enhancing adaptation to pregnancy and create a positive experience for mothers during pregnancy, this project aims to inform the design of future interventions that support maternal mental health and optimize fetal

neurodevelopment. Establishing this foundation could contribute significantly to public health efforts aimed at breaking intergenerational cycles of disadvantage, especially in vulnerable populations and may prove to mitigate experiences like prenatal or parental stress prior to birth or conception (Jenkins et al., 2022).

Research Questions and Hypotheses

This research was conducted on the premise of neuroplasticity which theorizes that the brain can change in response to the environment, thus driving changes in behaviour as a result. This research aims to address two main questions:

1. Are there any neuroanatomical effects of maternal prenatal enrichment on the volume of the parietal and frontal lobes of offspring?
2. Are there any behavioural changes in offspring associated with maternal prenatal enrichment?

Based on these questions, I hypothesized:

1. There will be positive long-term effects from maternal prenatal enrichment observable in the offspring brain.
2. There will be positive long-term effects from maternal prenatal enrichment observable in offspring behaviour.

Specific hypotheses as to the effects of maternal prenatal enrichment on each individual outcome will be discussed in their respective chapter.

Organization of the Thesis

Chapter 2 will describe the general methodology used in this research. This chapter includes an overview of the subjects involved, details of enriched housing and pregnancy timelines, testing paradigms, and a discussion of the statistical analyses used. Each assessment of behaviour or morphology will be dedicated a specific chapter including a brief introduction, methodology, and results, and discussion. These chapters will be as follows: Chapter 3 – Open Field (OFT), Chapter 4 – Elevated Plus Maze (EPM), Chapter 5 – Whishaw Tray Reaching (WTR), Chapter 6 – Morris Water Task (MWT), and Chapter 7 – Volumetric Measurements. Lastly, Chapter 8 will provide a discussion of the main findings of this research, a reflection on experimental design, and an evaluation of how the contributions of this thesis fit into the field of pre-clinical neuroscience research and the field of maternal enrichment.

Chapter 2

General Methodology

Subjects

All procedures were conducted in accordance with the Canadian Council of Animal Care and were approved by the University of Lethbridge Animal Care and Use Committee. This project involved 57 female and 56 male animals, all born in the vivarium at the Canadian Centre for Behavioural Neuroscience.

Two independent cohorts of animals were utilized in this study to evaluate the effects of maternal prenatal enrichment on offspring brain morphology and behaviour. The first cohort consisted of 27 Long-Evans rats forming the parental generation. This included seven female rats assigned to the prenatal enrichment (PE) condition, seven control female rats, ten control/breeder male sires, and two additional males designated as spares. These spare males were available to serve as replacement breeders in the event that any of the initial breeding pairs failed to successfully reproduce. The second cohort included 18 Long-Evans rats in the parental generation. This group was composed of five PE females, five control females, and eight control/breeder males. From the breeding in cohort one, a total of 32 offspring were used for behavioural testing, including 20 PE pups (10 females and 10 males) and 12 control pups (6 females and 6 males). In cohort two, 37 pups were used for behavioural testing, comprising 17 PE pups (7 females and 10 males) and 20 control pups (10 females and 10 males).

Following birth, pups were raised by their biological dam in standard cages until postnatal day (P) 21, at which point they were weaned and subsequently group-housed in standard cages in groups of two to three same-sex littermates per cage. All animals had ad

libitum access to food and water, unless otherwise specified by an experimental protocol. They were maintained under standard laboratory conditions, including a 12:12 hour light/dark cycle and a controlled room temperature of 23°C. To ensure consistent health monitoring, animals were weighed daily throughout the course of the study.

Prenatal Enrichment Paradigm

To investigate the effects of prenatal environmental stimulation, pregnant dams were housed in one of two distinct conditions: standard housing (control group) or enriched housing (PE group) before, during, and after breeding. Control dams were housed in standard laboratory enclosures composed of Plexiglas® units with two levels, and up to three animals were housed together in each unit. These cages had standard bedding material and contained a single play-tube to allow for minimal enrichment.

In contrast, the enriched condition was modeled after the enrichment protocol used in Gibb et al. (2014) and involved housing dams in a large, vertically structured enclosure measuring approximately 2 meters in height. This enriched unit featured three walls constructed of wire mesh and one solid metal wall (see Fig. 1.1). The interior environment was designed to provide multimodal stimulation, including ramps, elevated runways, and a wide assortment of toys that were rotated or replaced weekly during routine pen cleaning (see Fig. 1.2). This enriched setting offered several layers of stimulation; increased social interaction, as up to eight animals were housed in the enclosure at once, enhanced physical activity, facilitated by the large vertical space and climbing structures, and greater cognitive stimulation, due to the diverse and dynamic array of toys.

Together, these elements created a highly stimulating environment that allowed for systematic examination of how complex environmental features, including social, physical, and sensory experiences, interact during gestation to influence maternal and offspring outcomes. The design supports investigation into how prenatal environmental conditions may shape neurodevelopmental processes in offspring via changes in maternal behaviour, sensory exposure, and prenatal physiology.

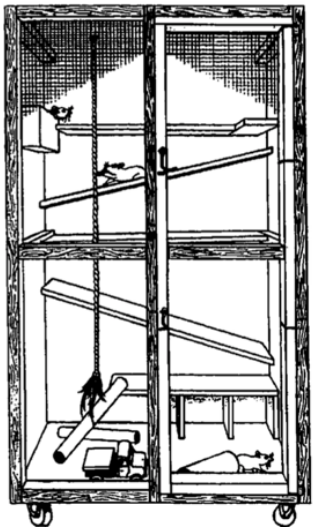


Fig. 2.1. A representation of the condominiums used to provide complex housing for environmental stimulation.

Fig. 2.2. Example of toys used in the enrichment condo.

Breeding Procedure and Housing Timeline

Once female breeder rats reached postnatal day (P) 70, they were assigned to their respective housing conditions, either standard (control) or enriched (PE), for a two-week acclimatization period. This period was implemented to allow the animals sufficient time to adapt to the sensory, social, and physical features of their environment before conception, ensuring that any observed effects could be attributed to prenatal enrichment rather than novelty or stress due to relocation. For PE females, the animals were housed in the enriched enclosure during the light portion of the standard 12:12 hour light/dark cycle and they were returned to their standard cages for the dark phase each night. This routine persisted through acclimatization, breeding, and gestation to reduce stress when returned to standard housing prior to labour.

Following acclimatization, females were bred. Breeding occurred during the dark phase of the standard 12:12 hour light/dark cycle, during which each female was temporarily paired with a male in a standard cage to facilitate mating. After this period, females were returned to their respective housing condition during the light phase of the cycle to minimize environmental disruption. This routine was repeated for one week until pregnancy was confirmed.

Once pregnant, females remained in their assigned housing condition (standard or enriched) during the light cycle for the duration of gestation. All pregnant dams were transferred to individual standard cages shortly before giving birth. Offspring were then raised in the standard housing condition and remained in them for the duration of their lifespan, allowing for a controlled assessment of the prenatal, rather than postnatal, effects of enrichment.

Behavioural Testing

To assess the developmental, psychological, and cognitive effects of prenatal enrichment on offspring, a series of behavioural test batteries were administered across early-life, adolescent, and adult time points.

Early-Life Testing

Before weaning, pups underwent a series of early-life assessments designed to evaluate basic motor function and emotional reactivity. This included:

- *Open Field Testing:* Conducted over multiple days (P10, P11, P12, P13, and P15), this test evaluates exploratory behaviour and anxiety-like responses by recording locomotor activity and time spent in the center vs. periphery of an open arena.

Adolescent Testing

To assess developmental progression of behaviour, animals were retested during adolescence:

- *Open Field Testing (OFT)/Activity Box Testing:* Re-administered on P35 to track changes in exploratory behaviour and activity levels.
- *Elevated Plus Maze (EPM):* Conducted on P36, this task is widely used to assess anxiety-related behaviour, using the animal's willingness to explore open vs. closed arms of an elevated cross-shaped maze.

Adult Testing

In adulthood, a comprehensive behavioural battery was administered to evaluate anxiety, motor coordination, and spatial memory:

- *Whishaw Tray Reaching Task (WTR):* Performed between approximately P70 and P90, this task assesses fine motor skills and skilled forelimb use by measuring the precision and success rate of pellet retrieval from a tray through bars.
- *Open Field Testing (OFT)/Activity Box Testing:* Conducted again at approximately P91, to monitor long-term behavioural changes and anxiety.
- *Elevated Plus Maze (EPM):* Re-administered at approximately P92 to compare adult anxiety levels with those observed during adolescence.
- *Morris Water Task (MWT):* Conducted between P93 and P98, this well-established spatial learning and memory test requires animals to locate a hidden platform in a circular pool using spatial cues.

Negative geotaxis and activity box are excluded from this present thesis due to technological complications.

Perfusion and Tissue Preparation

After the completion of behavioural testing, approximately on P105, all experimental animals were euthanized for histological and anatomical analysis of the brain. Euthanasia was carried out via an intraperitoneal injection of sodium pentobarbital, administered at an overdose level. Following deep anesthesia, animals underwent transcardial perfusion with 200 ml of a 0.9% saline solution to flush the circulatory system.

After perfusion, the brains were extracted, weighed, and post-fixed in paraformaldehyde (PFA). All brains were stored in a light-sensitive environment to prevent tissue degradation. Once adequately fixed, brains were transferred to a 30% sucrose solution and stored for 48 hours, allowing the tissue to cryoprotect and equilibrate before sectioning.

The brains were then frozen and sectioned coronally using a cryostat at a thickness of 50 micrometers (μm). Every fifth section was collected and mounted onto gelatin-coated glass slides in preparation for histological staining. For structural analysis, brain sections underwent Nissl staining following the standard lab protocol established by Gibb and Kolb. This staining technique enables visualization of neuronal cell bodies, allowing for volumetric estimates and gross morphological features relevant to the study's objectives.

Volumetric Measurements

Volumetric measurements of the medial prefrontal cortex (Cg3) and parietal cortex (PAR1) were measured using a microscope and Stereo Investigator. Methods were adapted from previous published articles that measure cortical thickness (Karl et al., 2010; Kolb & Whishaw, 1981; Jenkins et al., 2018). The rat atlas and the stereotaxis atlas (Zilles, 1985) were used to locate and trace the areas.

Volumetric Measurements of the Cg3 Area

Although the cingulate cortex (specifically area Cg3) is frequently examined in rodent neuroanatomical studies, standardized approaches to volumetric measurement remain sparse in the existing literature. A customized protocol was developed by Crump (2023) to enhance the reliability and precision of volume estimation for the Cg3 region.

Volumetric measurements were conducted using Stereo Investigator 10 (MBF Bioscience) in conjunction with a compound light microscope. This protocol was adapted from earlier research focused on cortical structure and thickness (Karl et al., 2010; Kolb & Whishaw, 1981; Jenkins et al., 2018). Cg3 boundaries were delineated based on Paxinos and Watson’s Rat Brain Atlas (1986), with anatomical landmarks verified using Zilles’ stereotaxic atlas (1985).

The Cg3 region anatomically spans from +4.70 mm to +2.20 mm anterior to the bregma, but only the portion between +4.20 mm and +2.70 mm was reliably identifiable across all samples. Within this clearly traceable range, five representative coronal sections were selected from each brain. Each chosen section was outlined manually, using landmarks and reference points such as the corpus callosum and the caudate putamen. These tracings were then imported into Stereo Investigator, where regional volume was computed using the software's integrated analysis tools.

A) **B)**

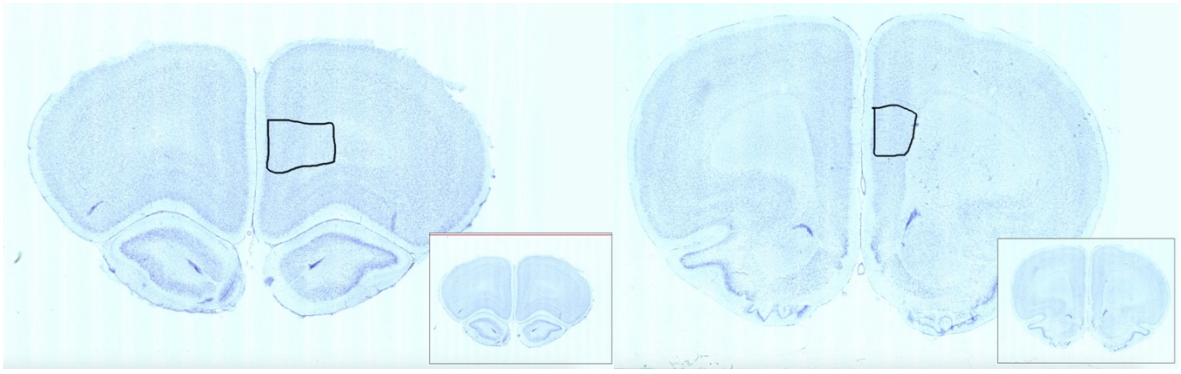


Fig. 2.3. Representative coronal sections indicating the beginning (A) at +4.20 mm and ending (B) at +2.70 mm sites for the Cg3 area in the brain, used for volume measurement and cell counts.

Volumetric Measurements of the PAR1 Area

Volumetric analysis of the parietal cortex (area PAR1) followed the same overall methodology used for the Cg3 region. Identification and delineation of PAR1 were based on anatomical descriptions from Paxinos and Watson (1986), with stereotaxic landmarks verified using Zilles (1985). Located more posteriorly in the brain, PAR1 spans roughly from –2.50 mm to –4.50 mm relative to bregma; however, a consistent and clearly defined range between –2.70 mm and –4.20 mm was selected for analysis across all samples.

Similar to the procedure for Cg3, five coronal sections were selected per brain, evenly distributed within the target range. Manual tracing of the PAR1 region was performed with reference to nearby anatomical features, including the external capsule, hippocampal formation, and secondary somatosensory cortex, depending on the section's level.

The tracings were then analyzed using the volumetric analysis tools in Stereo Investigator, allowing for the calculation of total PAR1 volume within the specified range. Using a parallel approach for both Cg3 and PAR1 ensured consistency in methodology and enabled reliable comparisons between brain regions.

C) **D)**

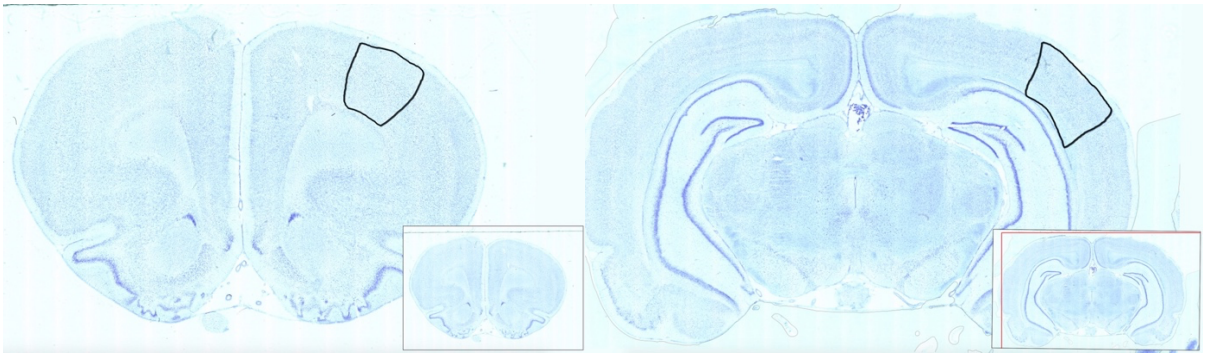


Fig. 2.4. Representative coronal sections indicating the beginning (C) at -2.70 mm and ending (D) at -4.20 mm sites for the PAR1 area in the brain, used for volume measurement and cell counts.

Statistical Analysis

All analyses were conducted using IBM SPSS Statistics 27 software. Graphs and tables were made using Excel 16.25 for Mac. The specific statistical models used in analysis of each neuroanatomical measure are discussed in the appropriate chapter.

Chapter 3

Anxiety-Like Behaviour in Early Life: The Open Field Test and Maternal Prenatal Enrichment

Introduction

The Open Field Test (OFT) is a widely utilized behavioural paradigm in rodent studies to assess both locomotor activity and anxiety-like behaviour through measures such as total distance traveled and time spent in the center of the arena. In the context of developmental neuroscience, the OFT is a valuable tool for evaluating how prenatal environmental conditions, such as maternal exposure to EE, may influence offspring behaviour. Emerging research has pointed to the sex-dependent nature of these effects, with several studies reporting divergent behavioural outcomes in male and female offspring following maternal enrichment during gestation.

A notable example of sex-specific behavioural outcomes comes from the work of Maruoka et al. (2009), who examined the effects of maternal enrichment on hippocampal cell proliferation and open field behaviour in mice. Their study revealed that maternal enrichment significantly influenced offspring behaviour only in females. Specifically, female offspring of enriched dams exhibited increased locomotor activity and spent more time in the center of the open field, which is typically interpreted as reduced anxiety-like behaviour. In contrast, male offspring showed no significant behavioural changes, despite being exposed to the same conditions. These results suggest that maternal enrichment can positively influence both brain development and exploratory behaviour, but that these effects may be selectively expressed in female offspring. These findings are in contrast to much of the existing parental experience

research available, as typically, larger effects are more commonly seen in males (McCreary & Metz, 2016).

Contrasting evidence is presented by Zuena et al. (2016), who also investigated maternal enrichment's influence on offspring behaviour but reported no modification of anxiety by maternal enrichment in both males and females, though enrichment did affect motor activity differently in males and females. Locomotion was found to be slightly increased in male prenatal enrichment animals, while this effect was not observed in females. The contrast between these two studies emphasizes the need to consider sex as a biological variable in the design and interpretation of behavioural neuroscience research, particularly when assessing early environmental influences.

Other studies have added further nuance to this field. For instance, Li, Lund, and Voigt (2016) provided enrichment during the early postnatal period (P1 until weaning) and observed that enriched offspring were more avoidant of the inner zone of the open field, spending less time and making fewer entries into this area compared to controls. These behavioural patterns are commonly interpreted as increased anxiety, which stands in contrast to the anxiolytic-like effects observed in some prenatal enrichment studies. This suggests that the timing of enrichment, prenatal vs. postnatal, may lead to different, even opposing, behavioural outcomes.

Research by Huang et al. (2021) highlights how enrichment can act as a buffer against early life stress. In models of maternal separation, both prenatal and postnatal EE were shown to reduce anxiety-like and depression-like behaviours in the OFT and Elevated Plus Maze. These findings support the idea that enrichment has the potential to ameliorate the negative effects of early adversity, though the developmental window and stress exposure appear to be critical moderators of its effectiveness.

Together, these studies paint a complex picture: environmental enrichment is capable of shaping offspring behaviour, but the direction and magnitude of its effects are highly dependent on sex, timing, and context.

Specific Hypothesis

Drawing on existing research examining behavioural changes in OFT of offspring following prenatal enrichment, I hypothesize that prenatally enriched animals will exhibit more locomotor activity and explore more new areas of the testing apparatus compared to control animals.

Methodology

OFT was employed to assess general locomotor activity and anxiety-like behaviour in offspring. Testing was conducted in a quiet room to minimize external stressors and environmental variability.

Apparatus

The open field apparatus consists of a rectangular arena measuring roughly 30 cm × 15 cm with clear walls 10 cm in height. The floor of the arena is divided into a grid, with the centre grid spaces marked to note the centre of the arena and where to place the animal during testing. The apparatus was thoroughly cleaned with Virkon between trials to eliminate olfactory cues.

Procedure

At P10, 11, 12, 13, and 15, each animal was placed individually into the center of the arena at the start of the trial and allowed to freely explore for a total of 1 minute. Behaviour was recorded using an overhead video camera and analyzed by tracking the following key behavioural variables:

- Number of total squares entered – a measure of general locomotor activity.
- Number of novel squares entered – a measure of exploratory behaviour.

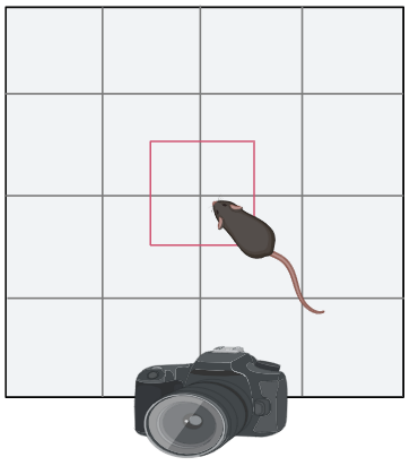
All testing was conducted during the light phase of the light/dark cycle.

Experimental Considerations

Technicians were blinded to group allocation during behavioural scoring to prevent bias.

Figure 3.1

Open Field Test



Note. The above image is a depiction of the OFT procedure and set up.

Statistical Analysis

Statistical analyses were performed using IBM SPSS Statistics 27 software. Both the analyses of total square entries and novel square entries were conducted using a repeated measures ANOVA comparing number of entries by testing day to treatment.

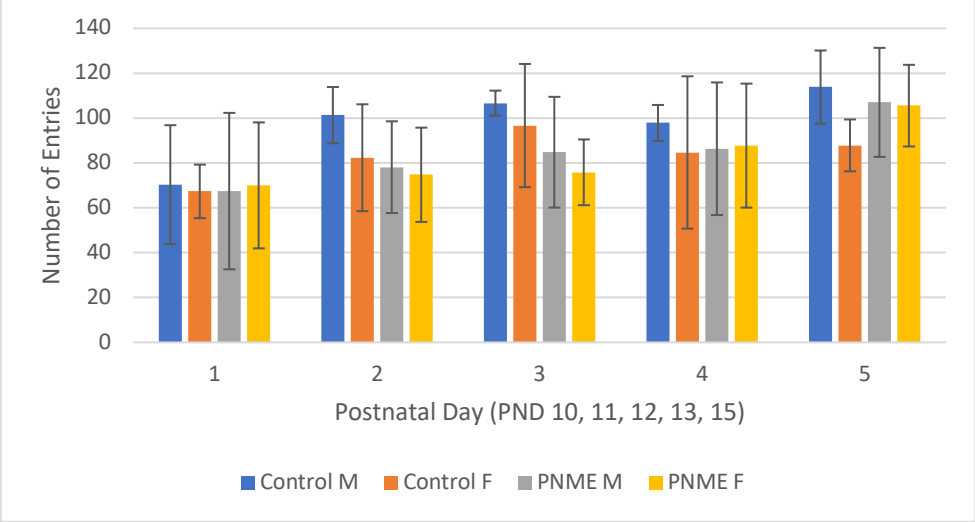
Results

Effects of Prenatal Enrichment on Total Square Entries

There was no significant effect of treatment observed as compared to controls, $F(3,341) = 2.777$, $p = 0.107$ (Fig. 3.2), suggesting that there was no significant effect of maternal prenatal enrichment on total square entries. While there was significance found by postnatal day (PND), $F(3,341) = 9.522$, $p = <0.001$, this is to be expected as the animals habituate to the apparatus and age. There was no significant effect of sex, $F(3,341) = 3.581$, $p = 0.0.069$, though significance trended toward males entering more squares than females. There was no PND by Treatment interaction observed, $F(3,341) = 1.828$, $p = 0.128$. There was no PND by Sex interaction observed, $F(3,341) = 0.413$, $p = 0.799$. There was no Treatment by Sex interaction observed, $F(3,341) = 2.051$, $p = 0.163$. There was no PND by Treatment by Sex interaction observed, $F(3,341) = 0.317$, $p = 0.866$.

Figure 3.2

Total Square Entries in Open Field Test of Animals Exposed to Prenatal Enrichment as Compared to Controls



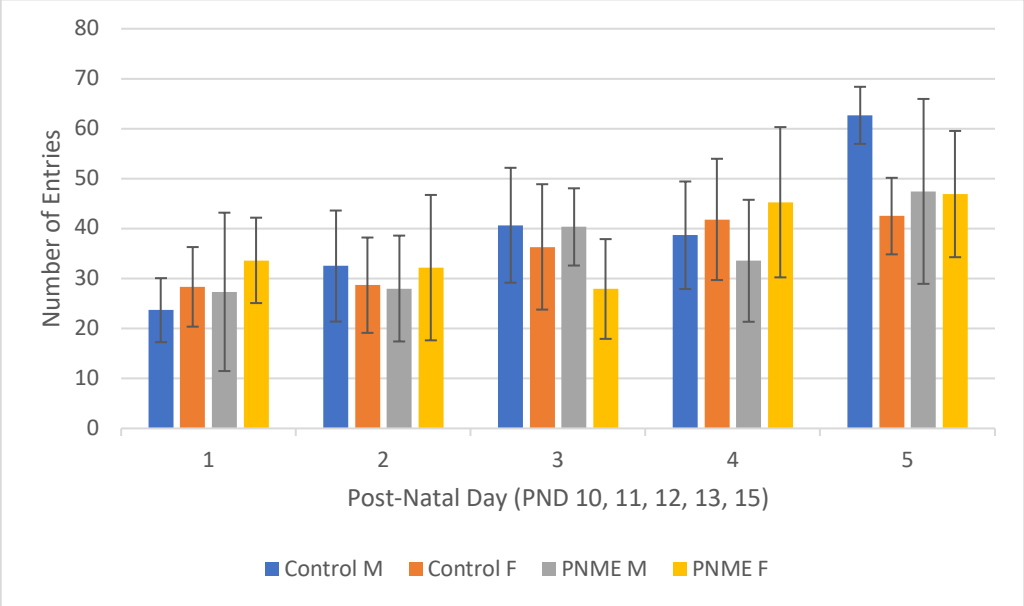
Note. Total square entries of treatment group showed no significant differences as compared to controls, $F(3,341) = 2.777$, $p = 0.107$. Error bars represent the standard error of the mean. Analysis ran via repeated measures ANOVA.

Effects of Prenatal Enrichment on Novel Square Entries

There was no significant effect of treatment observed as compared to controls, $F(3,341) = 0.384$, $p = 0.541$ (Fig. 3.3), suggesting that there was no significant effect of maternal prenatal enrichment on novel square entries. While there was significance found by PND, $F(3,341) = 16.427$, $p = <0.001$, this is to be expected as the animals habituate to the apparatus and age. There was no significant effect of sex, $F(3,341) = 0.277$, $p = 0.603$. There was no PND by treatment interaction observed, $F(3,341) = 0.843$, $p = 0.501$. There was a PND by Sex interaction observed, $F(3,341) = 4.000$, $p = 0.009$, in which males of both treatment groups had more novel square entries compared to females over the duration of testing days. There was no Treatment by Sex interaction observed, $F(3,341) = 1.932$, $p = 0.175$. There was no PND by Treatment by Sex interaction observed, $F(3,341) = 1.438$, $p = 0.226$.

Figure 3.3

Novel Square Entries in Open Field Test of Animals Exposed to Prenatal Enrichment as Compared to Controls



Note. Novel square entries of treatment group showed no significant differences as compared to controls, $F(3,341) = 0.384$, $p = 0.541$. Error bars represent the standard error of the mean. Analysis ran via repeated measures ANOVA.

Discussion

This study investigated the impact of maternal prenatal enrichment on offspring behaviour in the OFT, focusing on locomotor activity and exploratory behaviour. Results showed no significant differences between enriched and control groups in total square entries or novel square entries, suggesting that prenatal enrichment did not significantly alter these behaviours.

These findings contrast with Maruoka et al. (2009), who reported increased locomotor activity and center time in female offspring following maternal enrichment, but no effect in males. Both sexes were included in the present study, and it was determined that males of both control and enriched treatment groups had more exploratory behaviour than females throughout the duration of testing (by PND) in this particular behavioural test. Other research suggests that enrichment may play a more significant role in OFT performance when experienced during early life or with early life stress, as shown by Huang et al.'s (2021) findings of OFT performance in a model of maternal separation coupled with EE and Li et al.'s (2016) results following enrichment administered during early life. In Huang et al.'s (2021) models of maternal separation, both prenatal and postnatal EE were shown to reduce anxiety-like and depression-like behaviours in the OFT, and Li et al.'s (2016) early postnatal period enrichment model observed that enriched offspring were more avoidant of the centre of the open field compared to controls. While no significant behavioural effects were observed, these results highlight the complexity of enrichment outcomes and the need to consider sex, context, and enrichment timing in future studies.

Conclusion

These results demonstrate no significant effect of maternal prenatal enrichment on total or novel square entries in the OFT. These results suggest that maternal prenatal enrichment has no effects on locomotor or exploratory behaviour in the OFT.

Chapter 4

Anxiety-Like Behaviour in Adolescence: The Elevated Plus Maze and Maternal Prenatal Enrichment

Introduction

The Elevated Plus Maze (EPM) is a commonly used test to assess anxiety-like behaviour in rodents, based on their natural tendency to avoid open, exposed areas. The test measures the time spent in the open versus closed arms, with greater time spent in the open arms generally indicating lower anxiety. EE has been widely studied for its beneficial effects on emotional and cognitive development, often showing improvements when introduced later in life. EE, which involves enhanced social, physical, and cognitive stimulation, is thought to model positive life experiences and can promote healthier emotional regulation in rodents (Connors et al., 2015). However, as Connors et al. note, exposure to EE, especially during early development, has also been linked to increased anxiety-like behaviour in some cases.

Studies have found mixed results regarding the effects of maternal prenatal enrichment on offspring anxiety in the EPM. Zuena et al. (2016) observed that male offspring of enriched mothers showed increased anxiety-like behaviour, indicated by reduced exploration of the open arms of the maze. This was coupled with increased locomotor activity in both sexes of offspring from EE dams. On the other hand, female offspring did not show significant changes in anxiety-like behaviour but did exhibit improved learning abilities. These findings suggest that maternal enrichment might produce sex-specific responses, with males showing heightened anxiety and females benefiting in cognitive areas.

Sparling et al. (2018) also found differences in behaviour between the control and enriched groups, though they reported that enriched offspring exhibited less locomotor activity and more self-grooming. Anxiety-like behaviour was also observed in social scenarios. In the social interaction test, maternally enriched pups sniffed their partner more, displayed more self-grooming behaviour, and showed less locomotion; similar to the results seen in EPM.

In contrast, Li et al. (2016) observed no significant impact of postnatal enrichment on EPM behaviour in their study, suggesting that the timing and context of enrichment exposure may influence its effects. This supports the idea that while enrichment may generally impact offspring behaviour, its effectiveness could depend on other factors, such as the developmental stage at which enrichment occurs. Additionally, Huang et al. (2021) showed that maternal enrichment could ameliorate anxiety-like behaviours in mice exposed to early life stress, suggesting that early-life adversity might amplify the positive effects of enrichment on offspring anxiety levels.

This body of literature reveals the complexity of maternal enrichment's influence on offspring behaviour, particularly regarding sex differences and timing of enrichment exposure. Given the mixed findings across studies, the current experiment aims to explore how maternal prenatal enrichment impacts anxiety-like behaviour in the EPM, with a focus on both male and female offspring.

Specific Hypothesis

I hypothesize that maternal enrichment will lead to increased exploratory behaviour and reduced anxiety responses, with potential sex-specific effects, as reported in previous studies.

Methodology

EPM was employed to assess anxiety-like behaviour in offspring by measuring their willingness to explore open, elevated spaces versus enclosed arms. Testing was conducted in a quiet room to minimize external stressors and environmental variability.

Apparatus

The EPM apparatus consists of a plus-shaped maze elevated roughly 50 cm above the ground, with two open arms and two closed arms enclosed by roughly 50 cm high walls. A central platform connects all arms (Fig. 4.1). The entire maze is made of black plastic to minimize visual distractions. The apparatus was thoroughly cleaned with Virkon® between trials to eliminate olfactory cues.

Procedure

At P36 and again at around P90, each animal was placed on the central platform, facing an open arm, at the start of the trial and allowed to explore the maze freely for a total of 5 minutes. Behaviour was recorded using a video camera and analyzed by tracking the following key behavioural variables:

- Time spent in open arms – a measure of reduced anxiety-like behaviour.
- Time spent in closed arms – a measure of increased anxiety-like behaviour.
- Number of entries into open and closed arms – a measure of exploratory behaviour and locomotor activity.

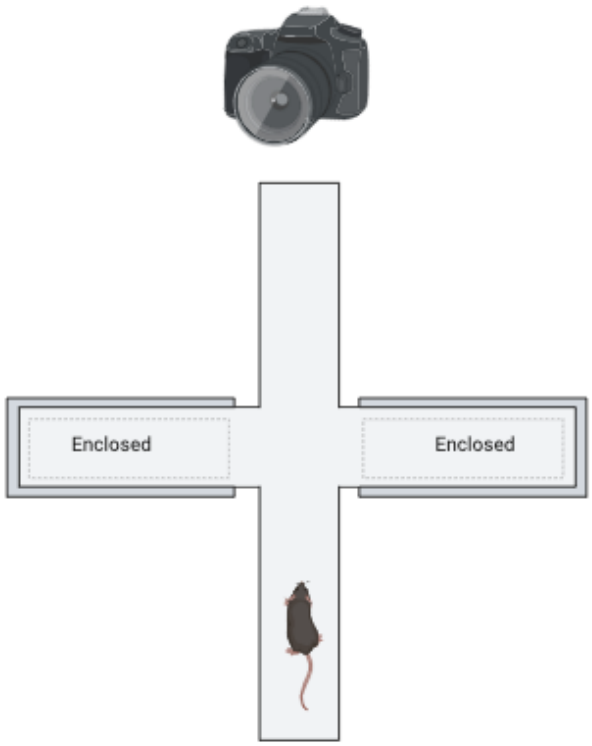
All testing was conducted during the light phase of the light/dark cycle.

Experimental Considerations

Technicians were blinded to group allocation during behavioural scoring to prevent bias.

Figure 4.1

Elevated Plus Maze



Note. The above image is a depiction of the EPM procedure and set up.

Statistical Analysis

Statistical analyses were performed using IBM SPSS Statistics 27 software. Both the analyses of duration in a given area of the maze and number of entries into an arm were conducted using a two-way ANOVA comparing treatment and sex.

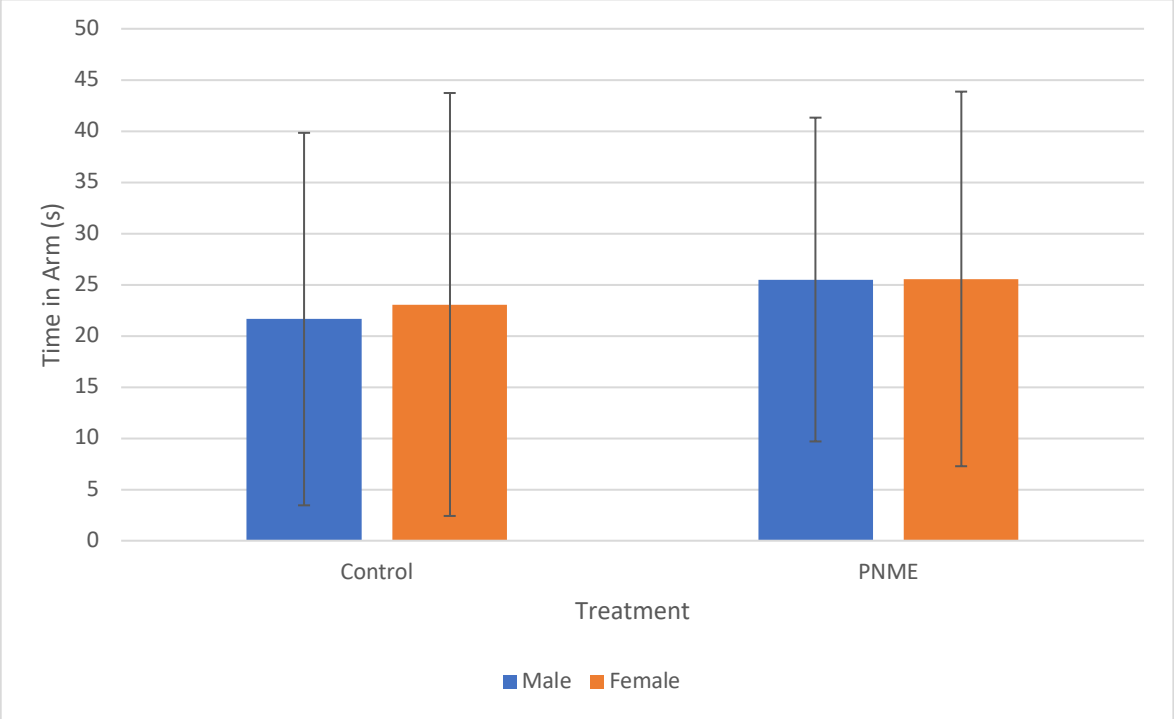
Results

Effects of Prenatal Enrichment on Total Duration Spent in Open Arms of EPM

There was no significant effect of treatment observed, $F(1,67) = 0.499$, $p = 0.482$ (Fig. 4.2), suggesting that there was no significant effect of maternal prenatal enrichment on total duration spent in open arms. There was no effect of sex observed, $F(1,67) = 0.027$, $p = 0.869$. There was no Treatment by Sex interaction observed, $F(1,67) = 0.023$, $p = 0.880$.

Figure 4.2

Total Duration Spent in Open Arms of EPM of Animals Exposed to Prenatal Enrichment as Compared to Controls



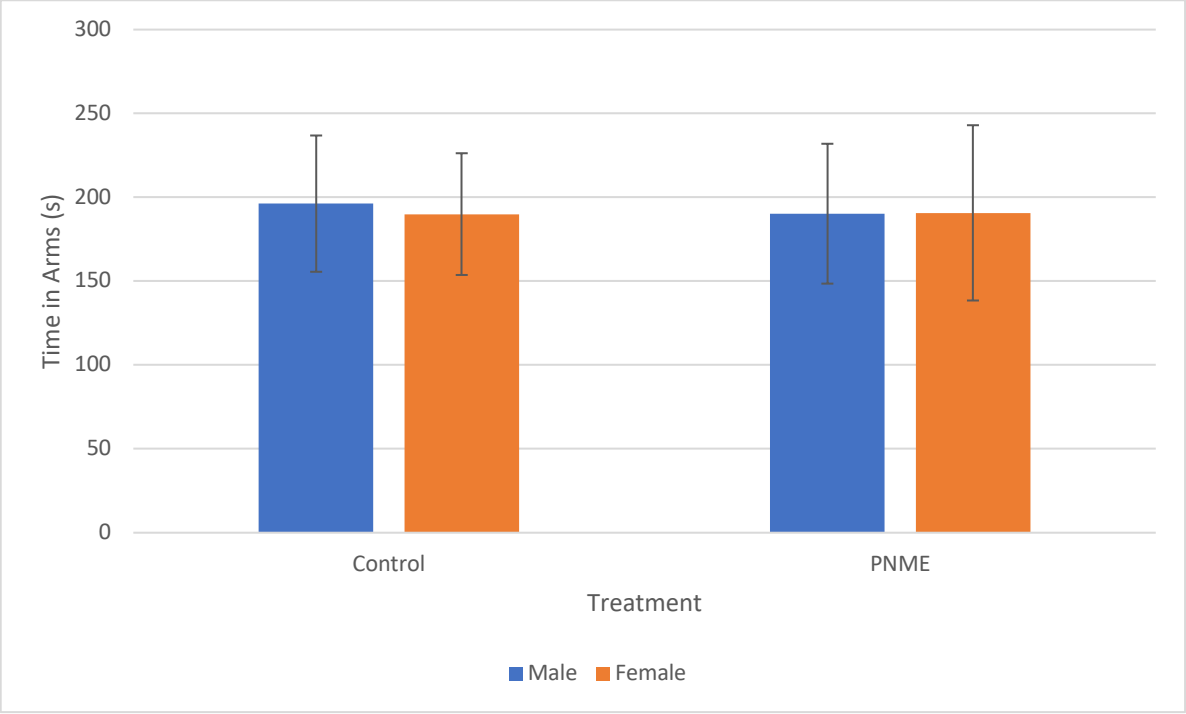
Note. Total duration in open arms by treatment group showed no significant differences as compared to controls, $F(1,67) = 0.499$, $p = 0.482$. Error bars represent the standard error of the mean. Analysis ran via two-way ANOVA.

Effects of Prenatal Enrichment on Total Duration Spent in Closed Arms of EPM

There was no significant effect of Treatment observed as compared to controls, $F(1,67) = 0.065$, $p = 0.800$ (Fig. 4.3), suggesting that there was no significant effect of maternal prenatal enrichment on total duration spent in closed arms. There was no effect of sex observed, $F(1,67) = 0.077$, $p = 0.782$. There was no Treatment by Sex interaction observed, $F(1,67) = 0.106$, $p = 0.746$.

Figure 4.3

Total Duration Spent in Closed Arms of EPM of Animals Exposed to Prenatal Enrichment as Compared to Controls



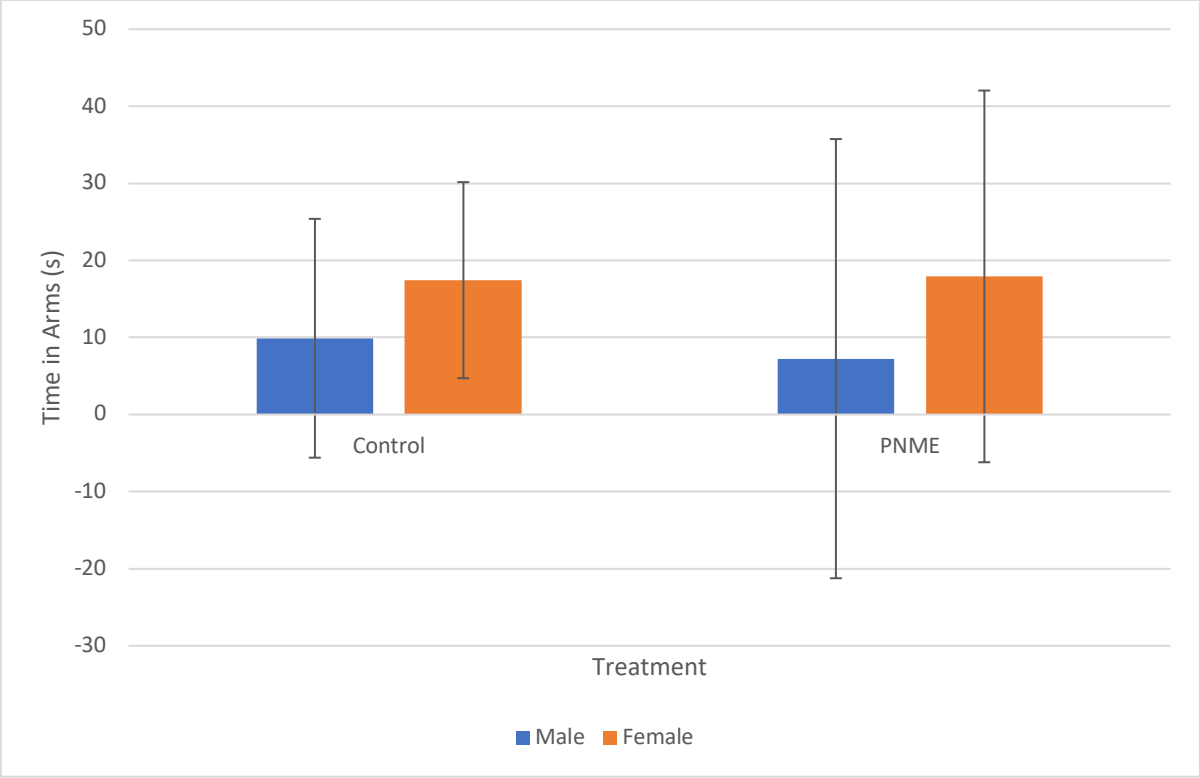
Note. Total duration in closed arms by treatment group showed no significant differences as compared to controls $F(1,67) = 0.065$, $p = 0.800$. Error bars represent the standard error of the mean. Analysis ran via two-way ANOVA.

Effects of Prenatal Enrichment on Total Duration Spent in Outermost Ends of EPM

There was no significant effect of treatment observed, $F(1,67) = 0.499$, $p = 0.482$ (Fig. 4.4), suggesting that there was no significant effect of maternal prenatal enrichment on total duration in outermost ends of open arms. There was no effect of sex observed, $F(1,67) = 3.366$, $p = 0.071$. There was no Treatment by Sex interaction observed, $F(1,67) = 0.100$, $p = 0.753$.

Figure 4.4

Total Duration Spent in Outermost Ends of EPM of Animals Exposed to Prenatal Enrichment as Compared to Controls



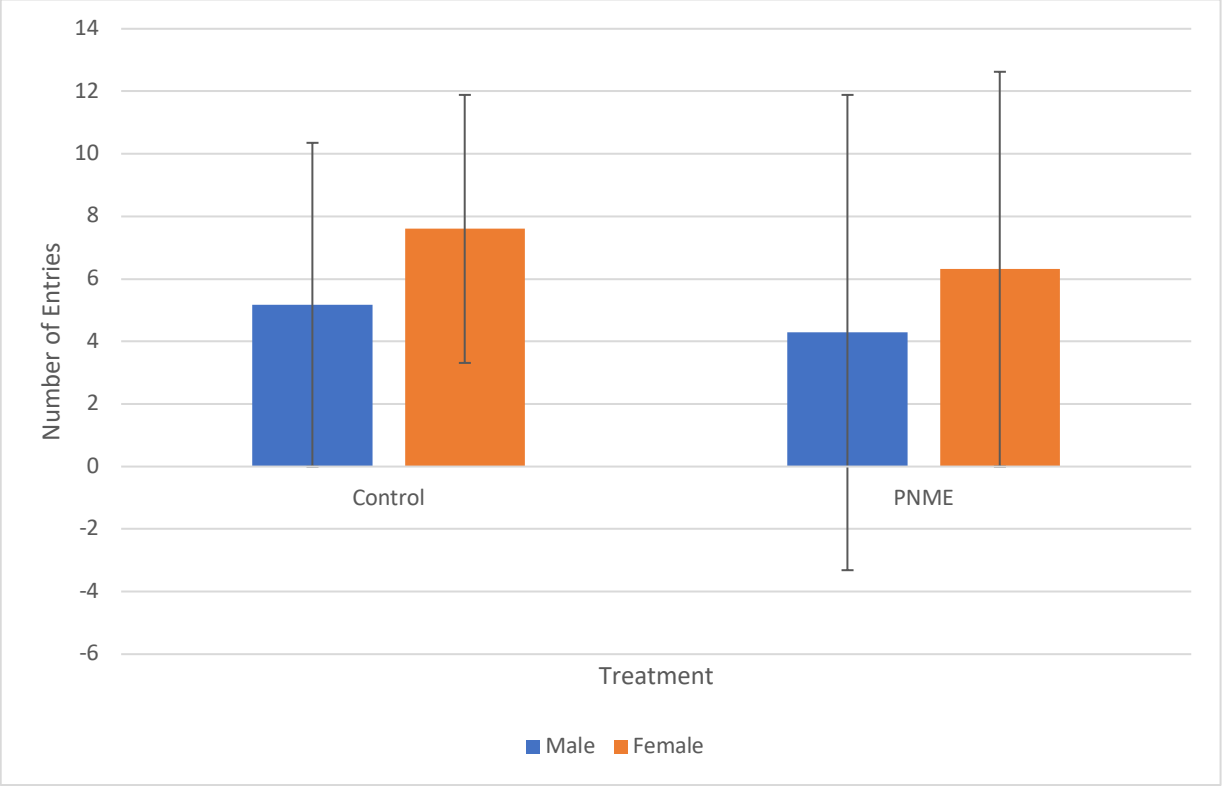
Note. Total duration in outermost ends of open arms by treatment group showed no significant differences as compared to controls, $F(1,67) = 0.499$, $p = 0.482$. Error bars represent the standard error of the mean. Analysis ran via two-way ANOVA.

Effects of Prenatal Enrichment on Open Arm Entries in EPM

There was no significant effect of treatment observed as compared to controls, $F(1,67) = 0.824$, $p = 0.367$ (Fig. 4.5), suggesting that there was no significant effect of maternal prenatal enrichment on number of open arm entries. There was no effect of sex observed, $F(1,67) = 3.441$, $p = 0.068$. There was no Treatment by Sex interaction observed, $F(1,67) = 0.027$, $p = 0.869$.

Figure 4.5

Number of Open Arm Entries in EPM of Animals Exposed to Prenatal Enrichment as Compared to Controls



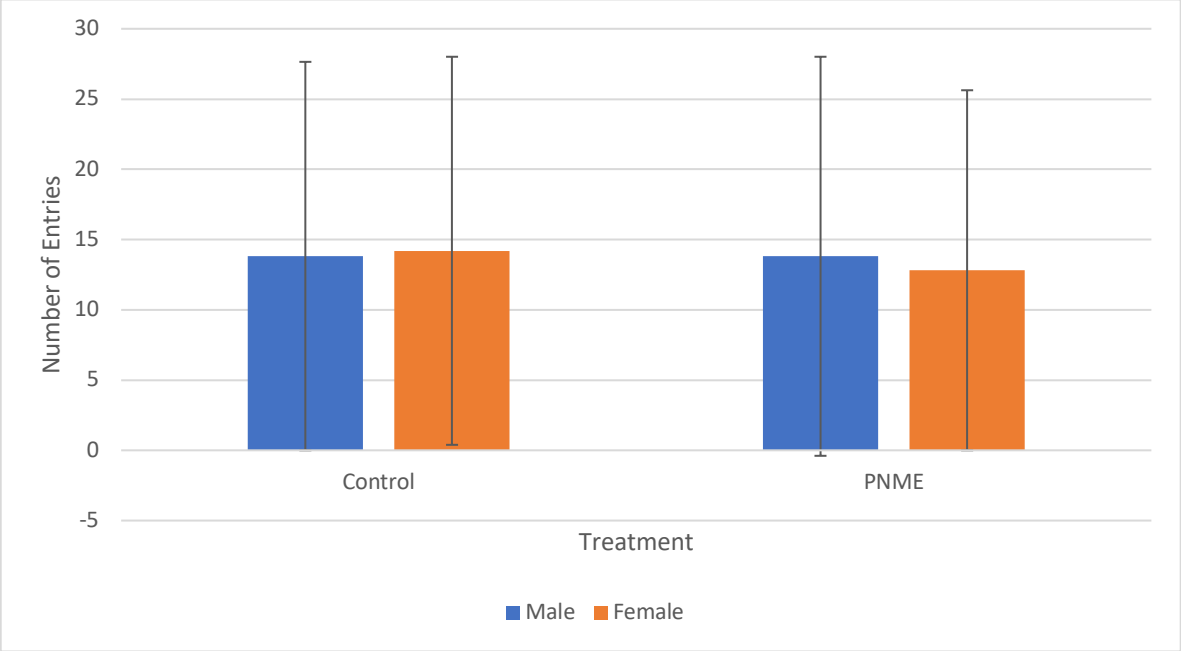
Note. Number of open arm entries of treatment group showed no significant differences as compared to controls, $F(1,67) = 0.824$, $p = 0.367$. Error bars represent the standard error of the mean. Analysis ran via two-way ANOVA.

Effects of Prenatal Enrichment on Closed Arm Entries in EPM

There was no significant effect of treatment observed as compared to controls, $F(1,67) = 0.732$, $p = 0.395$ (Fig. 4.6), suggesting that there was no significant effect of maternal prenatal enrichment on number of closed arm entries. There was no effect of sex observed, $F(1,67) = 0.143$, $p = 0.706$. There was no Treatment by Sex interaction observed, $F(1,67) = 0.703$, $p = 0.405$.

Figure 4.6

Number of Closed Arm Entries in EPM of Animals Exposed to Prenatal Enrichment as Compared to Controls



Note. Number of closed arm entries of treatment group showed no significant differences as compared to controls, $F(1,67) = 0.732$, $p = 0.395$. Error bars represent the standard error of the mean. Analysis ran via two-way ANOVA.

Discussion

The present study aimed to assess the impact of maternal prenatal environmental enrichment on offspring anxiety-like behaviour in EPM. Contrary to expectations based on previous findings, there were no significant differences between prenatally enriched and control

animals across multiple behavioural metrics, including time spent in the open and closed arms, entries into each arm, and time spent in the distal ends of the open arms. These findings suggest that, within the parameters of the current study, maternal enrichment during gestation did not significantly alter anxiety-related responses in the EPM.

This outcome contrasts with the prior studies previously discussed in this chapter that reported notable behavioural differences following prenatal or early-life enrichment. Zuena et al. (2016) highlighted findings that male offspring of enriched mothers exhibited increased anxiety-like behaviour, shown by reduced open arm exploration, while female offspring displayed improved cognitive abilities without significant changes in anxiety-related behaviour. Moreover, their results indicated increased locomotor activity in both sexes, suggesting that enrichment may differentially affect anxiety and activity depending on sex. The absence of such effects in the current study may point to strain, timing, or possibly protocol differences.

Similarly, Sparling et al. (2018) reported differences in enriched offspring, including reduced locomotion and altered grooming behaviours in the EPM and social interaction tests. These effects were particularly notable in male juveniles, indicating potential sex-specific sensitivity to environmental enrichment. It is possible that the behavioural parameters influenced by enrichment in some of the earlier studies—such as social behaviour or grooming—were simply not captured by the present study's behavioural test battery.

The timing of enrichment appears to be critical. Li et al. (2016), who provided enrichment from postnatal day 1 until weaning, found no significant impact of enrichment on EPM behaviour—findings more consistent with the current results. This highlights the possibility that enrichment, particularly when not paired with early-life stressors, may not robustly influence anxiety-like outcomes in all experimental conditions. Huang et al. (2021) showed that

enrichment more clearly ameliorated anxiety in models involving maternal separation stress, suggesting that the beneficial effects of enrichment may be more pronounced when coupled with early adversity.

To summarize, the lack of significant findings in this study adds to the growing body of literature indicating that the behavioural outcomes of maternal prenatal enrichment are not uniformly expressed, and may be highly dependent on sex, developmental timing, enrichment protocols, and interaction with early life stress. While enrichment holds promise as a model for promoting resilience and neural plasticity, these results emphasize the need for more targeted behavioural measures, sex-specific analyses, and potentially multi-modal assessments to fully understand its impact.

Conclusion

These results demonstrate no significant effect of maternal prenatal enrichment on duration in open arms, duration in closed arms, duration in outermost ends of open arms, number of entries in open arms, number of entries in closed arms in EPM. These results suggest that maternal prenatal enrichment has no effect on anxiety-like behaviour in the EPM.

Chapter 5

Motor Control and Sensorimotor Coordination in Adulthood: The Whishaw Tray Reaching Task and Maternal Prenatal Enrichment

Introduction

Skilled reaching tasks, such as the Whishaw Tray Reaching (WTR) test, are commonly used behavioural paradigms to assess fine motor control, sensorimotor coordination, and goal-directed limb use in rodents. These behaviours rely on the precise movement and coordination of the forelimb and are considered sensitive indicators of neurodevelopmental status. Due to this sensitivity, reaching behaviours are frequently used in experimental models to detect the impact of early life experiences, injuries, or interventions on motor system development. In this context, environmental factors experienced during gestation, such as maternal prenatal EE, are of particular interest, as they may have long-lasting effects on offspring behaviour and brain plasticity.

The influence of maternal EE during pregnancy has been associated with altered neuroanatomical development, modulation of stress responses, and behavioural changes in offspring, ranging from emotional regulation to cognitive and motor outcomes. Of specific interest to the present chapter is the extent to which maternal EE may enhance or impair the development of skilled motor behaviours, as measured by the WTR task.

With respect to motor learning and skilled reaching performance specifically, Ulupinar et al. (2015) reported sex-dependent effects of enrichment. In their study, female offspring demonstrated improved reaching ability following prenatal enrichment, suggesting a possible enhancing effect on fine motor development in females. However, the same study also found that

overall motor learning was negatively impacted in some EE-exposed groups, raising the possibility that enrichment may not uniformly benefit all aspects of motor function. These findings suggest that EE may selectively influence certain motor behaviours, depending on biological sex, developmental timing, and the specific task demands of the behaviour being measured.

Beyond general enrichment, task-specific training has been proposed as a more effective approach for promoting motor recovery and skill acquisition. In particular, pairing environmental enrichment with structured, goal-directed motor training, such as daily reach rehabilitation, has been shown to yield more robust improvements in skilled reaching. Previous research has found that rats exposed to an enriched rehabilitation paradigm, which combined EE with daily reach training following neonatal hypoxia-ischemia, committed fewer reaching errors and retrieved more food pellets than non-rehabilitated animals (Schuch et al., 2016). These results indicate that while EE may prime the brain for plasticity, repeated task-specific practice is likely necessary to drive functional improvements in motor skill, particularly during periods of rapid development or recovery.

Taken together, these findings suggest that maternal prenatal enrichment may influence motor system development, with potentially beneficial effects on skilled reaching performance, especially in females. However, the literature also points to variability in outcomes, underscoring the importance of sex differences, task specificity, and intervention timing. The current chapter investigates whether maternal prenatal enrichment alone, absent postnatal task-specific training, is sufficient to enhance skilled reaching ability in offspring, using WTR as a sensitive measure of motor coordination and limb use.

Specific Hypothesis

I hypothesize that maternal enrichment will lead to increased reaching and improved accuracy in WTR, with potential sex-specific effects, as reported in previous studies.

Methodology

The WTR test is used to assess skilled forelimb use and fine motor control in offspring. This task evaluates the ability of rodents to retrieve food pellets using their forepaws through narrow vertical bars, requiring precise motor coordination and sensorimotor integration. The procedure is adapted from established protocols (Whishaw et al., 2008) for measuring skilled reaching in rats.

Apparatus

The reaching chamber consists of a clear Plexiglas® box (approximately 45 cm × 15 cm × 20 cm) with vertical metal bars as the front wall and metal mesh grid on the floor for any excrement to fall out of reach of the animal. The box is composed of three separate chambers, in which one animal is placed in each chamber. A shallow tray is mounted just outside the front wall bars, positioned so that the rat can reach through the bars to retrieve food pellets placed on the tray (approximately 5 cm wide). The spacing between the bars permits only the extension of a single forelimb at a time, encouraging lateralised reaching behaviour (Fig. 5.1). The apparatus is cleaned thoroughly with Virkon® between trials to eliminate olfactory cues.

Procedure

During training, animals are mildly food restricted to 90% of their free-feeding body weight to increase motivation to retrieve pellets. Training and testing are conducted during the light phase of the light/dark cycle in a quiet room to minimize stress and external distractions.

Prior to testing, animals undergo habituation and testing. During habituation (approximately P70-75), animals are placed in the chamber once per day for 30 min. In training phase one (approximately P76-82), animals begin food restriction and learn to reach through the bars for pellets once per day for 20 min. Training phase two (approximately P83-86) continues food restriction and reaching training while the duration drops to 15 min. The final training phase three (approximately P87-90) continues food restriction and reaching training while the duration lowers to 10 min. Each rat was always placed in the same chamber to avoid any environmental distractions on testing day.

On testing day (approximately P91) groups of rats are placed into their reaching chamber and given a five min. testing session. Trials were recorded using a front-facing camera for later scoring.

Behavioural Measures

The following variables were analysed:

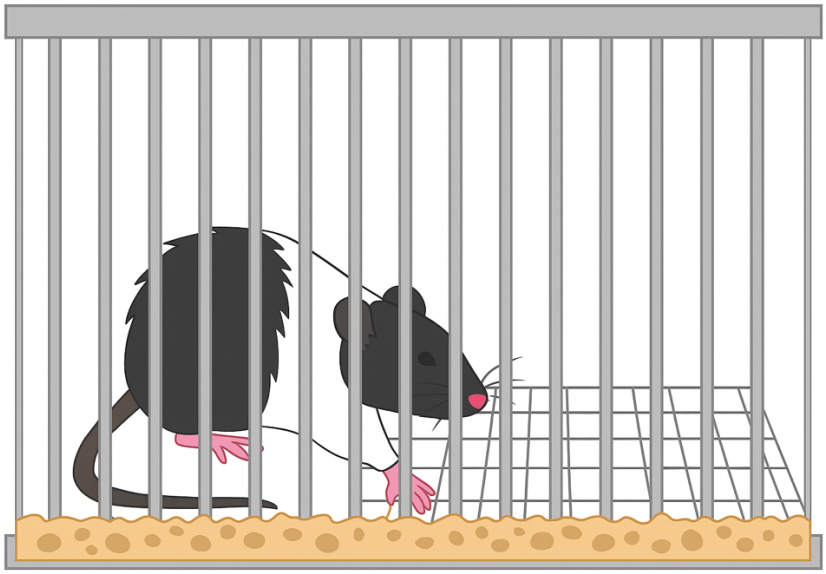
- Number of successful reaches – defined as forelimb extensions that successfully grasped and retrieved a pellet.
- Total number of reach attempts – all forelimb extensions directed toward a pellet, including unsuccessful attempts.
- Left paw/right paw/both paw reach attempts – forelimb extensions from a given side, including unsuccessful attempts.
- Reach success rate – calculated as the proportion of successful reaches relative to total attempts.

Experimental Considerations

All behavioural scoring was completed by experimenters who were blinded to the experimental group assignment in order to minimize bias.

Figure 5.1

Whishaw Tray Reaching



Note. The above image is a depiction of the WTR procedure and apparatus.

Statistical Analysis

Statistical analyses were performed using IBM SPSS Statistics 27 software. Both the analyses of quantity of a given reach and success rate were conducted using a two-way ANOVA comparing treatment and sex.

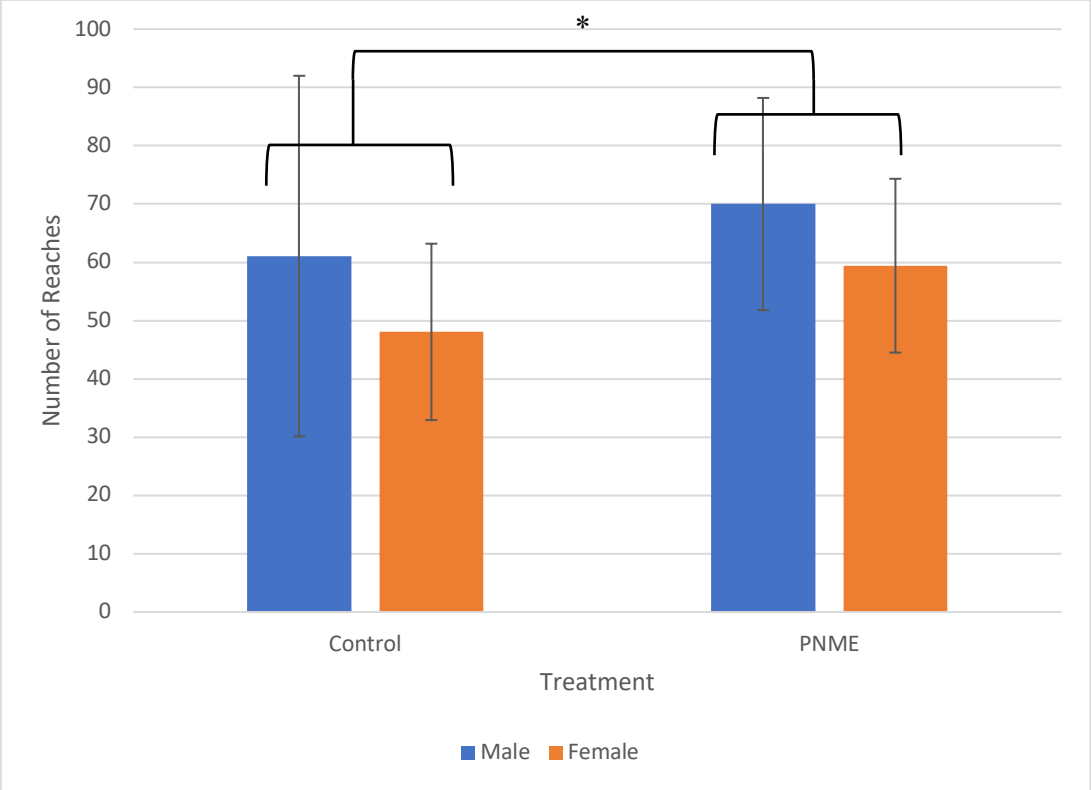
Results

Effects of Prenatal Enrichment on Total Reaches in WTR

There was a significant effect of treatment observed as compared to controls, $F(1,67) = 4.092$, $p = 0.047$ (Fig. 5.2), suggesting that there was a significant effect of maternal prenatal enrichment on total number of reaches in WTR, in which enriched animals reached more than controls. There was an effect of sex observed, $F(1,67) = 5.527$, $p = 0.022$, where males reached more than females. There was no Treatment by Sex interaction observed, $F(1,67) = 0.058$, $p = 0.810$.

Figure 5.2

Total Reaches in WTR of Animals Exposed to Prenatal Enrichment as Compared to Controls



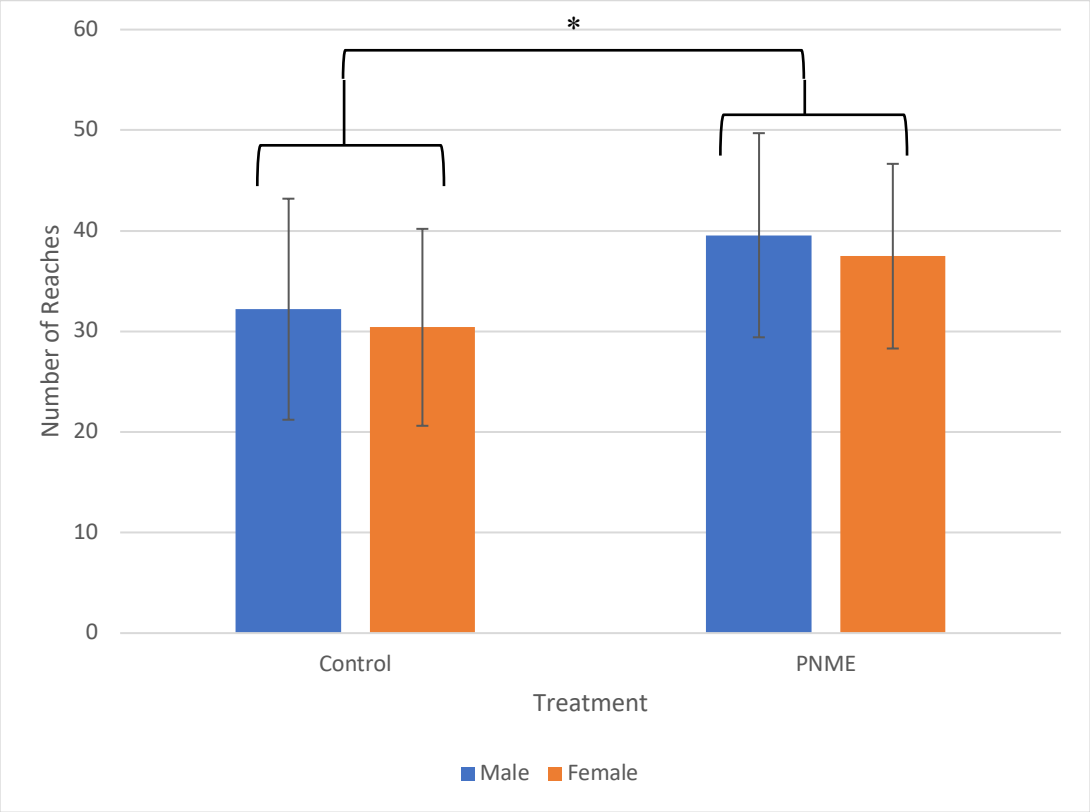
Note. Total number of reaches by treatment group showed a significant difference as compared to controls, $F(1,67) = 4.092$, p = 0.047. Error bars represent the standard error of the mean. Analysis ran via two-way ANOVA. ***$p<.001$; *$p<.05$.

Effects of Prenatal Enrichment on Successful Reaches in WTR

There was a significant effect of treatment observed as compared to controls, $F(1,67) = 8.622$, $p = 0.005$ (Fig. 5.3), suggesting that there was a significant effect of maternal prenatal enrichment on successful number of reaches in WTR; successful reaches were higher in the prenatal enriched animals. There was no effect of sex observed, $F(1,67) = 0.624$, $p = 0.433$. There was no Treatment by Sex interaction observed, $F(1,67) = 0.003$, $p = 0.954$.

Figure 5.3

Successful Reaches in WTR of Animals Exposed to Prenatal Enrichment as Compared to Controls



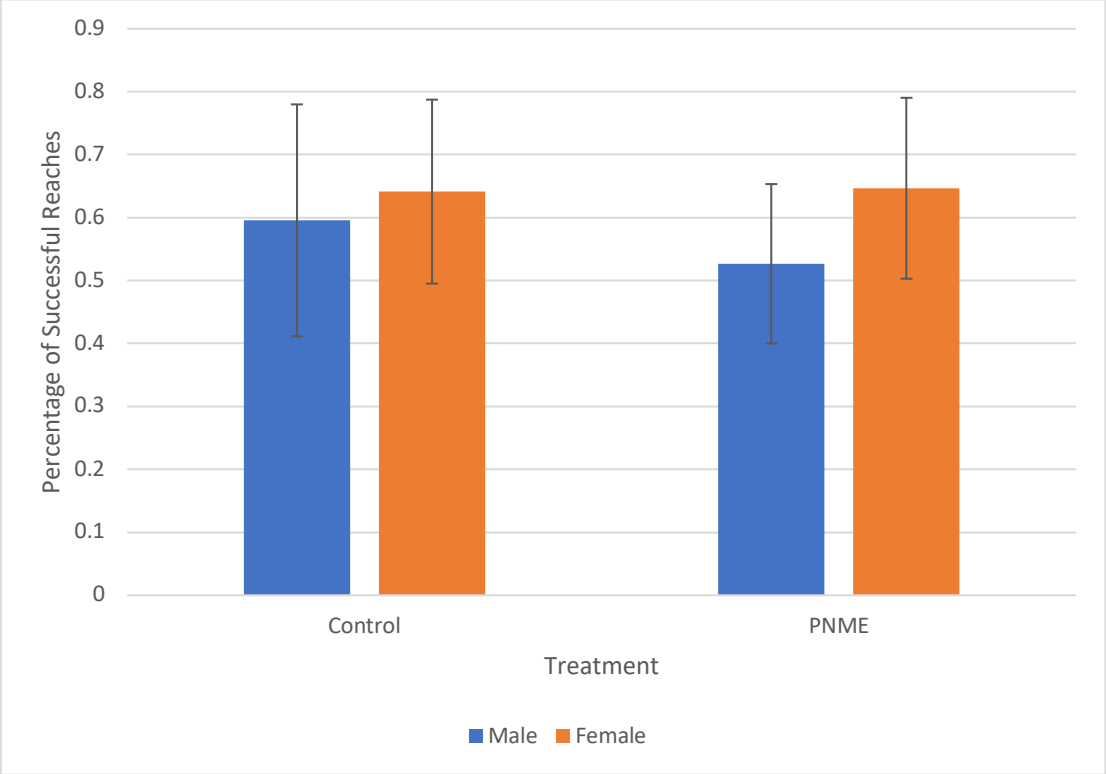
Note. Successful number of reaches by treatment group showed a significant difference as compared to controls, $F(1,67) = 8.622$, p = 0.005. Error bars represent the standard error of the mean. Analysis ran via two-way ANOVA. ***$p<.001$; *$p<.05$.

Effects of Prenatal Enrichment on Percentage of Successful Reaches in WTR

There was no significant effect of treatment observed as compared to controls, $F(1,67) = 0.019$, $p = 0.892$ (Fig. 5.4), suggesting that there was no significant effect of maternal prenatal enrichment on percentage of successful reaches. There was no effect of sex observed, $F(1,67) = 2.310$, $p = 0.134$. There was no Treatment by Sex interaction observed, $F(1,67) = 0.079$, $p = 0.780$.

Figure 5.4

Percentage of Successful Reaches of Animals Exposed to Prenatal Enrichment as Compared to Controls



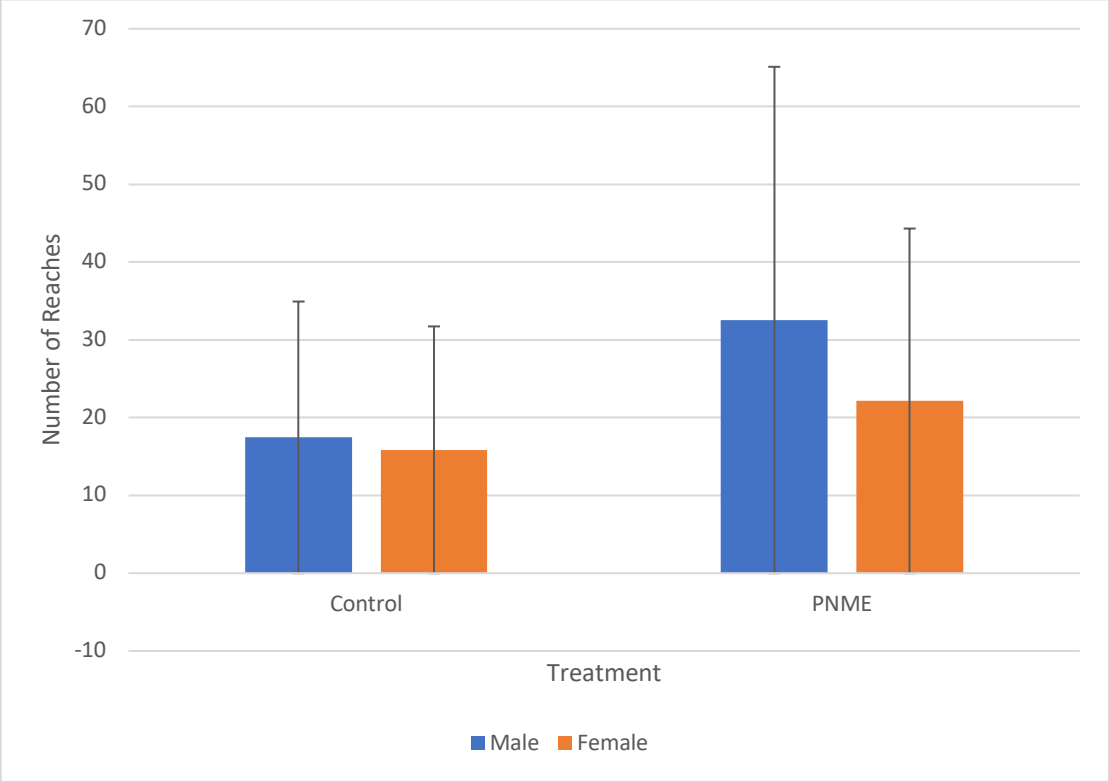
Note. Percentage of successful reaches of treatment group showed no significant differences as compared to controls, $F(1,67) = 0.019$, $p = 0.892$. Error bars represent the standard error of the mean. Analysis ran via two-way ANOVA.

Effects of Prenatal Enrichment on Right Forelimb Reaches in WTR

There was no significant effect of treatment observed as compared to controls, $F(1,67) = 3.649$, $p = 0.061$ (Fig. 5.5), suggesting that there was no significant effect of maternal prenatal enrichment on number of right forelimb reaches, though this difference was trending toward significance ($p < 0.1$). There was no effect of sex observed, $F(1,67) = 1.149$, $p = 0.288$. There was no Treatment by Sex interaction observed, $F(1,67) = 0.618$, $p = 0.435$.

Figure 5.5

Number of Right Forelimb Reaches of Animals Exposed to Prenatal Enrichment as Compared to Controls



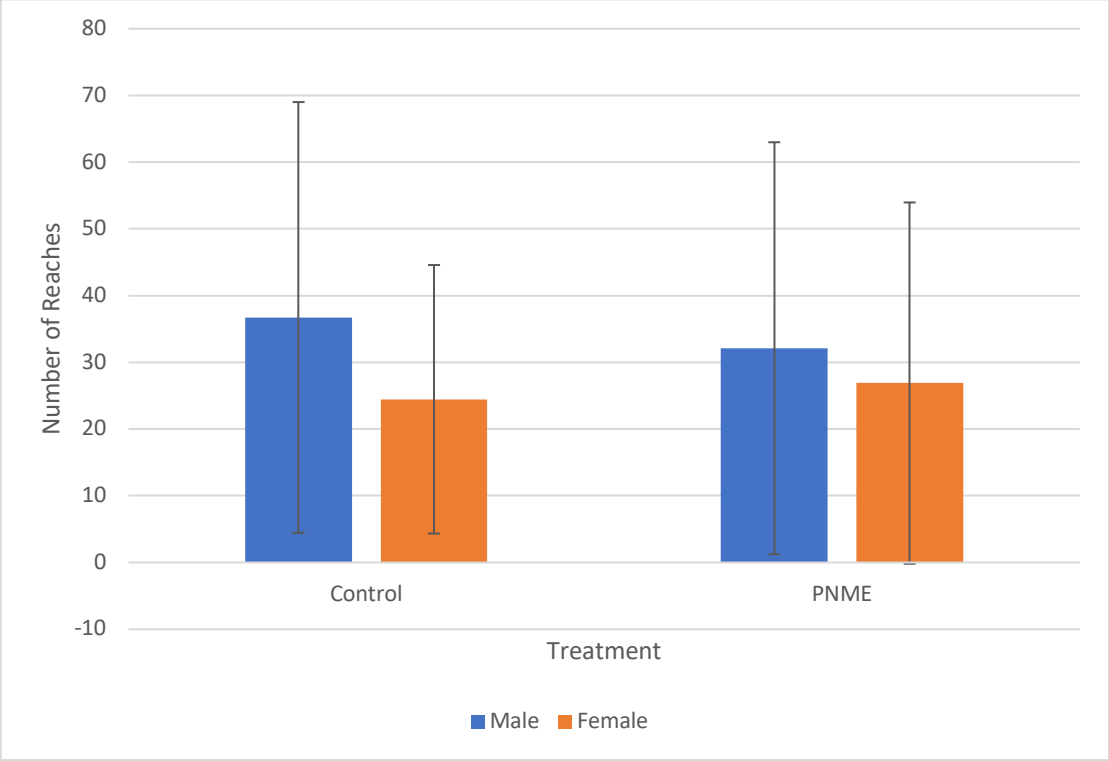
Note. Number of right forelimb reaches of treatment group showed no significant differences as compared to controls, $F(1,67) = 3.649$, $p = 0.061$. Error bars represent the standard error of the mean. Analysis ran via two-way ANOVA.

Effects of Prenatal Enrichment on Left Forelimb Reaches in WTR

There was no significant effect of treatment observed as compared to controls, $F(1,67) = 0.025$, $p = 0.874$ (Fig. 5.6), suggesting that there was no significant effect of maternal prenatal enrichment on number of left forelimb reaches. There was no effect of sex observed, $F(1,67) = 1.605$, $p = 0.210$. There was no Treatment by Sex interaction observed, $F(1,67) = 0.261$, $p = 0.611$.

Figure 5.6

Number of Left Forelimb Reaches of Animals Exposed to Prenatal Enrichment as Compared to Controls



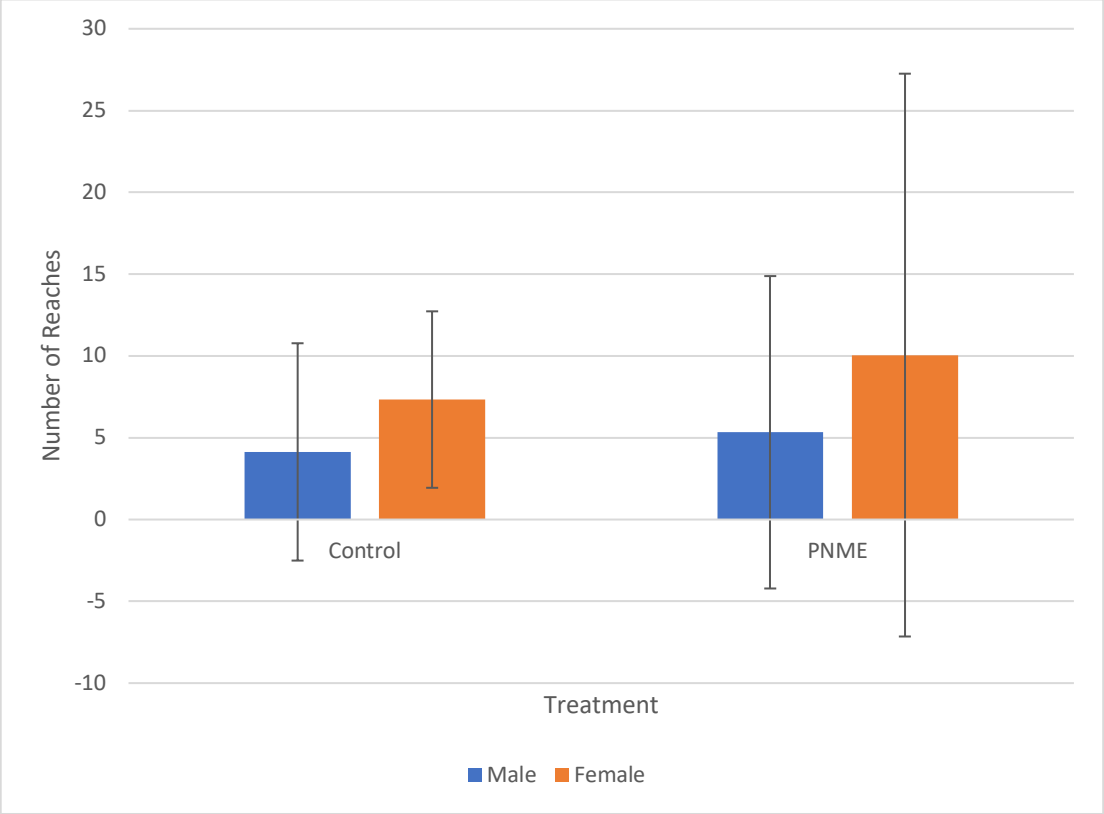
Note. Number of left forelimb reaches of treatment group showed no significant differences as compared to controls, $F(1,67) = 0.025$, $p = 0.874$. Error bars represent the standard error of the mean. Analysis ran via two-way ANOVA.

Effects of Prenatal Enrichment on Both Forelimb Reaches in WTR

There was no significant effect of treatment observed as compared to controls, $F(1,67) = 0.507$, $p = 0.479$ (Fig. 5.7), suggesting that there was no significant effect of maternal prenatal enrichment on number of both (simultaneous right and left) forelimb reaches. There was no effect of sex observed, $F(1,67) = 2.070$, $p = 0.155$. There was no treatment by sex interaction observed, $F(1,67) = 0.076$, $p = 0.783$.

Figure 5.7

Number of Both Forelimb Reaches of Animals Exposed to Prenatal Enrichment as Compared to Controls



Note. Number of both forelimb reaches of treatment group showed no significant differences as compared to controls, $F(1,67) = 0.507$, $p = 0.479$. Error bars represent the standard error of the mean. Analysis ran via two-way ANOVA.

Discussion

The present chapter examines the effects of maternal prenatal environmental enrichment on offspring performance in the WTR test, a sensitive measure of skilled forelimb use and motor coordination. Results revealed a significant increase in both the total number of reaches and successful retrievals in offspring from enriched dams compared to controls. However, there were no significant differences between groups in the percentage of successful reaches or in the lateralization of forelimb use, including right, left, or bilateral reaches, suggesting that while enrichment may enhance overall motivation or motor engagement, it may not directly influence reaching precision or limb preference.

These findings contribute to a sparse but growing body of literature examining the impact of maternal enrichment on fine motor behaviour. While WTR has not been widely applied in maternal enrichment studies, related research offers insight into how early environmental conditions might shape sensorimotor outcomes. One study that notably does exist in this domain, Ulupinar et al. (2015) observed sex-dependent effects of postnatal EE on reaching ability in prenatally stressed animals: female offspring exposed to enriched rearing conditions demonstrated enhanced reaching performance, while general motor learning outcomes were negatively affected in some enriched groups. Tasks used in Ulupinar et al.'s (2015) study include rotarod, string suspension, and skilled reaching. Increased reaching activity observed here aligns with Ulupinar et al.'s findings, suggesting that prenatal enrichment alone may be sufficient to enhance some aspects of skilled motor output, at least at the level of attempted and successful reach frequency.

Interestingly, despite increases in total and successful reaches, the percentage of successful attempts did not differ significantly between groups. This suggests that while enriched

offspring were more behaviourally active or motivated during the task, their reaching accuracy or efficiency was not necessarily improved. This observation may align with evidence from Schuch et al. (2016), who found that animals required task-specific training in addition to environmental enrichment to see significant improvements in reaching accuracy and error reduction. Their enriched rehabilitation group, which combined daily reach training with EE, outperformed both non-rehabilitated and non-enriched animals in fine motor tasks. Our findings support the idea that general maternal enrichment, while sufficient to increase motor engagement, may not provide the targeted sensorimotor feedback necessary to improve reaching precision.

Further, the lack of significant differences in forelimb preference is notable, as reaching lateralization is often thought to reflect cortical asymmetry and motor system maturation. The fact that no group differences emerged in this area may indicate that maternal enrichment alone does not bias the development of lateralized motor circuits.

In sum, these findings provide novel evidence that maternal prenatal enrichment can increase the frequency of skilled reaching behaviour in offspring, which may indicate increased motivation or task engagement in the PE offspring. However, PE did not appear to influence motor precision, limb lateralization, or task efficiency, highlighting the complexity of motor system development and the likely need for task-specific practice to support more refined motor outcomes. Given the limited literature on skilled reaching in the context of maternal enrichment, future work should examine sex differences, training effects, and neural correlates to better understand the mechanisms underlying these behavioural changes.

Conclusion

These results demonstrate a significant effect of maternal prenatal enrichment on number of total and successful reaches in WTR, though no significant effect of percentage of successful reaches or right, left, or both forelimbs reaching preference. These results may suggest that maternal prenatal enrichment does have effect on quantity of reaching in the WTR, but no effect of lateralized movement preference or reaching precision.

Chapter 6

Spatial Memory and Learning in Adulthood: The Morris Water Task and Maternal Prenatal Enrichment

Introduction

The Morris Water Task (MWT) is one of the most widely used behavioural paradigms for assessing spatial learning and memory in rodents. As a hippocampus-dependent task, the MWT provides a robust measure of cognitive abilities by requiring animals to locate a hidden escape platform in a circular pool using spatial cues. Over repeated trials, successful task acquisition is reflected in decreased latency and swim distance, while retention is typically evaluated through a probe trial in which the platform is removed to assess memory for its former location. Because of its sensitivity to neurodevelopmental changes, the MWT is commonly used to investigate the effects of both environmental adversity and enrichment, such as prenatal enrichment, on brain and behaviour.

For example, Sparling et al. (2018) investigated the effects of combined pre- and postnatal enrichment on anxiety-like and cognitive behaviours. In their study, rats raised in enriched conditions showed superior performance in the MWT probe trial, with enriched animals spending more time in the target quadrant, crossing the platform location more frequently, and engaging in fewer thigmotactic (perimeter-circling) behaviours. These findings suggest not only improved spatial memory but also reduced anxiety-like behaviour, both of which contribute to optimal performance in the MWT. Notably, adult female offspring in this study demonstrated particularly strong memory for the platform position and explored the centre of the maze more frequently, highlighting potential sex-specific benefits of enrichment.

Additional support for the cognitive benefits of environmental enrichment comes from Xie et al. (2012), who found that EE could reverse spatial learning deficits in rats prenatally exposed to maternal seizures. Enriched animals in this study demonstrated significantly improved acquisition and probe trial performance, indicating that EE may provide neuroprotective or compensatory effects when prenatal brain development is disrupted. Likewise, Tipyasang et al. (2014) reported that EE attenuated the negative effects of prenatal alcohol exposure, with enriched animals showing faster learning over four days of training and spending more time in the target quadrant during probe trials conducted at multiple time points. These results collectively highlight the potential of enrichment, particularly when applied during sensitive developmental periods, to support long-term cognitive resilience.

Despite this growing body of evidence, relatively few studies have isolated the effects of prenatal maternal enrichment alone, without accompanying postnatal interventions, on spatial learning and memory. Given the demonstrated sensitivity of the developing hippocampus to environmental input, and the consistent improvements observed in spatial navigation following EE in combined paradigms, it is plausible that prenatal enrichment alone could exert measurable cognitive benefits. Moreover, the influence of biological sex remains an important consideration, as several studies suggest differing patterns of response in males and females.

In the present chapter, MWT is used to evaluate whether maternal prenatal enrichment, in the absence of further postnatal stimulation, would influence spatial acquisition and memory retention in offspring. Measures of escape latency, number of platform location crosses, swim velocity, mean distance to platform location, and platform quadrant preference were analysed and interpreted within the context of previous findings.

Specific Hypothesis

Given prior evidence, I hypothesize that animals from enriched dams will demonstrate superior spatial learning and memory relative to controls, with potential sex differences emerging.

Methodology

The MWT is used to assess spatial learning and memory in adult rat offspring. The task is conducted in a quiet behavioural testing room under consistent lighting conditions to minimize external distractions and environmental variability.

Apparatus

The testing apparatus consists of a circular pool measuring about 180 cm in diameter and 60 cm in height, filled to a depth of approximately 40 cm with water maintained at a temperature of 24 ± 1°C. The water was rendered opaque using non-toxic white tempera paint to obscure the location of the submerged platform. A circular escape platform (about 10 cm in diameter) was placed approximately 2 cm below the surface of the water and kept in a fixed location during acquisition trials.

The testing room contains distinct visual cues beyond the maze (e.g., posters, shapes, shelving) that remain constant throughout the experiment to facilitate spatial navigation. A video tracking system (EthoVision XT) is used to record swim paths and behavioural metrics.

Procedure

Habituation and Training

Animals are gently placed into the water at one of four predetermined start positions (northeast, southeast, northwest, or southwest), with start locations varying randomly across

trials. Each animal receives four acquisition trials per day for a total of five consecutive days (approximately P90-94). In each trial, the animal is allowed a maximum of 60 seconds to locate the hidden platform. If the platform is not located within the allotted time, the animal is guided to the platform by the experimenter. Animals are allowed to remain on the platform for 15 seconds before being returned to their holding cage.

Probe Trial

A probe trial is conducted 24 hours after the final training session to assess memory retention (approximately P95). During the probe trial, the platform is removed, and animals are released from start location. Each probe trial lasts 60 seconds.

Measured Variables

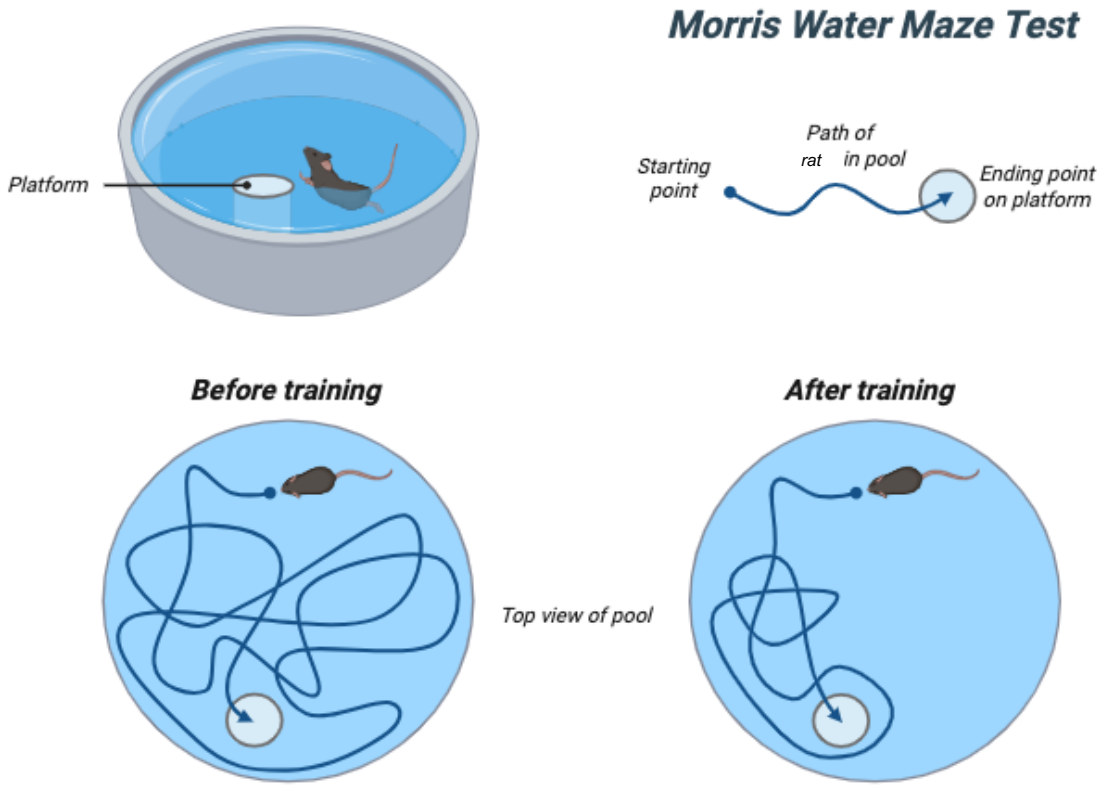
- Escape latency (s) - Time taken to reach platform.
- Mean distance to platform (cm) – Average distance between animal and platform during trial.
- Swim velocity (cm/s) - Average swimming velocity.
- Time in target quadrant (%) - Time spent in the quadrant that previously contained the platform (probe trial).
- Platform crossings (n) - Number of times the animal crossed over the former platform location (probe trial).

Experimental Considerations

All behavioural testing is conducted during the light phase of the light/dark cycle. Experimenters are blinded to group allocation to reduce bias in behavioural scoring and interpretation. Between trials, animals are towel-dried and returned to their holding cages. The water is cleaned and replaced regularly to maintain clarity and hygiene.

Figure 6.1

Morris Water Task



Note. The above image is a depiction of the MWT procedure and apparatus.

Statistical Analysis

Statistical analyses were performed using IBM SPSS Statistics 27 software. Analyses of platform crosses, mean distance to platform, swim velocity, and quadrant preference were conducted using a two-way ANOVA comparing treatment and performance. Analysis of escape latency was conducted using a repeated measures ANOVA comparing testing day, treatment, and performance.

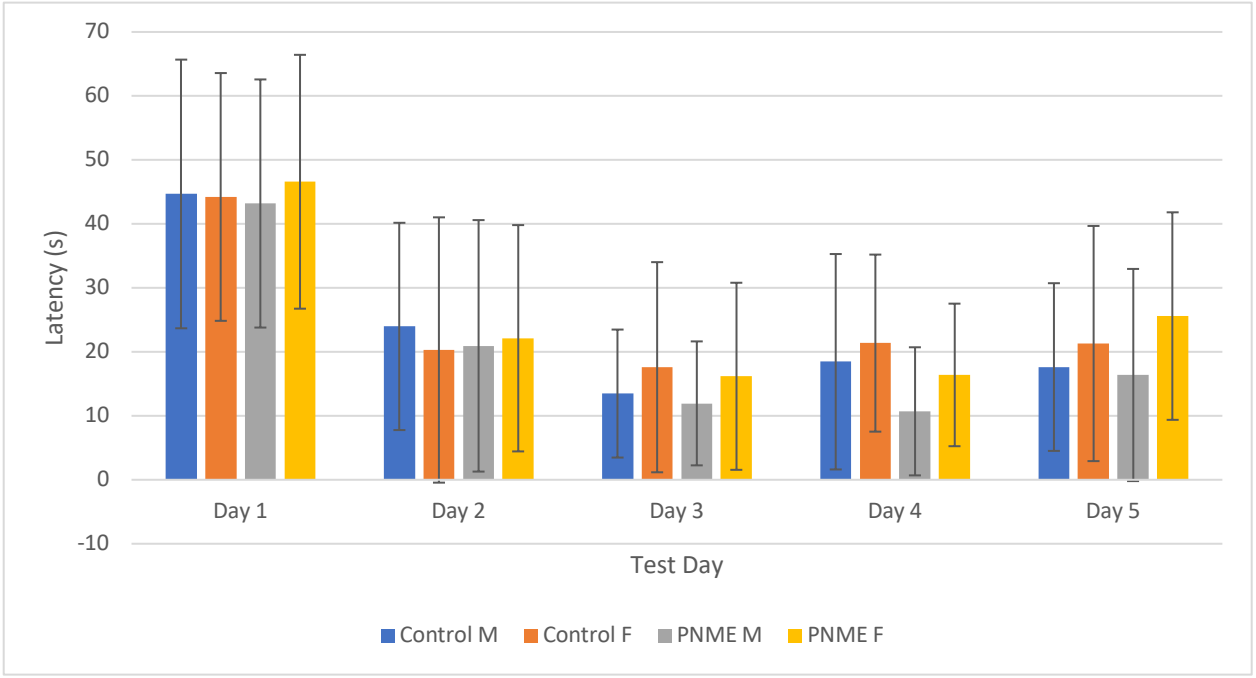
Results

Effects of Prenatal Enrichment on Escape Latency in MWT

There was no significant effect of treatment observed, $F(1,343) = 0.364$, $p = 0.548$ (Fig. 6.2), suggesting that there was no significant effect of maternal prenatal enrichment on escape latency. There was no effect of sex observed, $F(1,343) = 0.1.993$, $p = 0.163$. There was significant effect of test day observed, $F(1,343) = 42.910$, $p = <0.001$, in which escape latency decreased for all animals throughout the duration of the testing days. There was no Treatment by Sex interaction observed, $F(1,343) = 0.641$, $p = 0.426$. There was no Treatment by Test Day interaction observed, $F(1,343) = 0.683$, $p = 0.604$. There was no Test Day by Sex interaction observed, $F(1,343) = 0.650$, $p = 0.627$. There was no Treatment by Test Day by Sex interaction observed, $F(1,343) = 0.083$, $p = 0.988$.

Figure 6.2

Escape Latency of Animals Exposed to Prenatal Enrichment as Compared to Controls



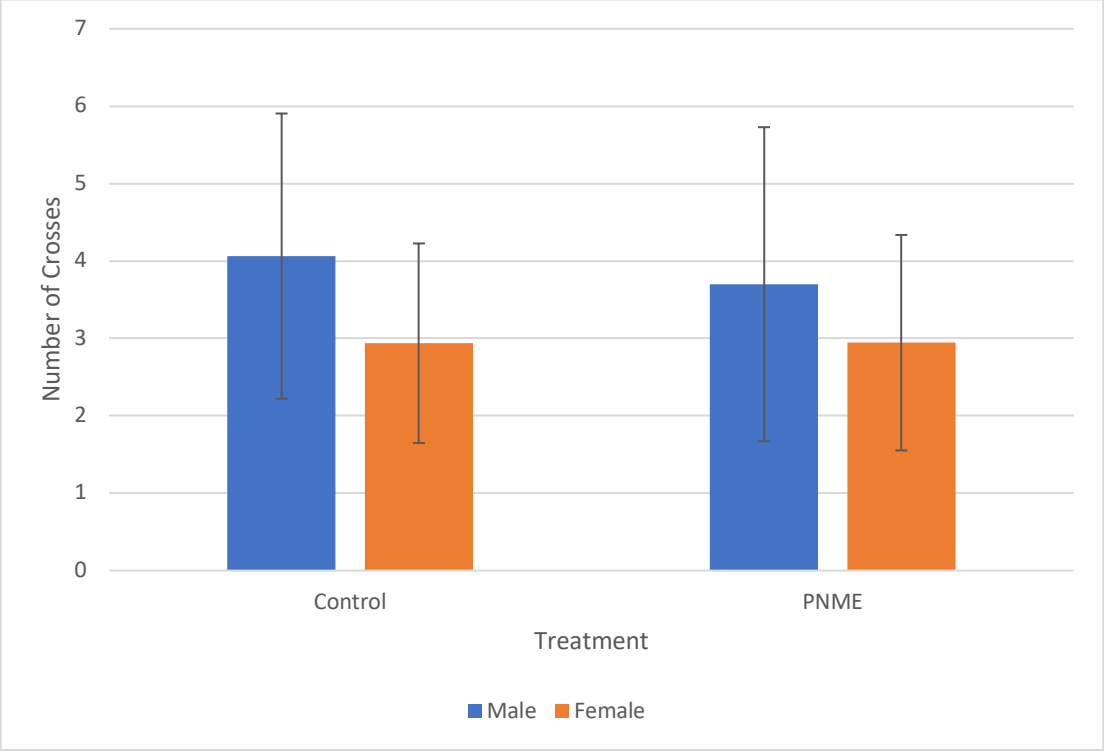
Note. Escape latency of treatment group showed no significant differences as compared to controls, $F(1,343) = 0.364$, $p = 0.548$. Error bars represent the standard error of the mean. Analysis ran via repeated measures ANOVA.

Effects of Prenatal Enrichment on Platform Crosses in MWT

There was no significant effect of treatment observed, $F(1,67) = 0.194$, $p = 0.661$ (Fig. 6.3), suggesting that there was no significant effect of maternal prenatal enrichment on number of platform location crosses. There was an effect of sex observed, $F(1,67) = 5.413$, $p = 0.023$, in which males had more platform crosses than females. There was no Treatment by Sex interaction observed, $F(1,67) = 0.209$, $p = 0.649$.

Figure 6.3

Number of Platform Crosses of Animals Exposed to Prenatal Enrichment as Compared to Controls



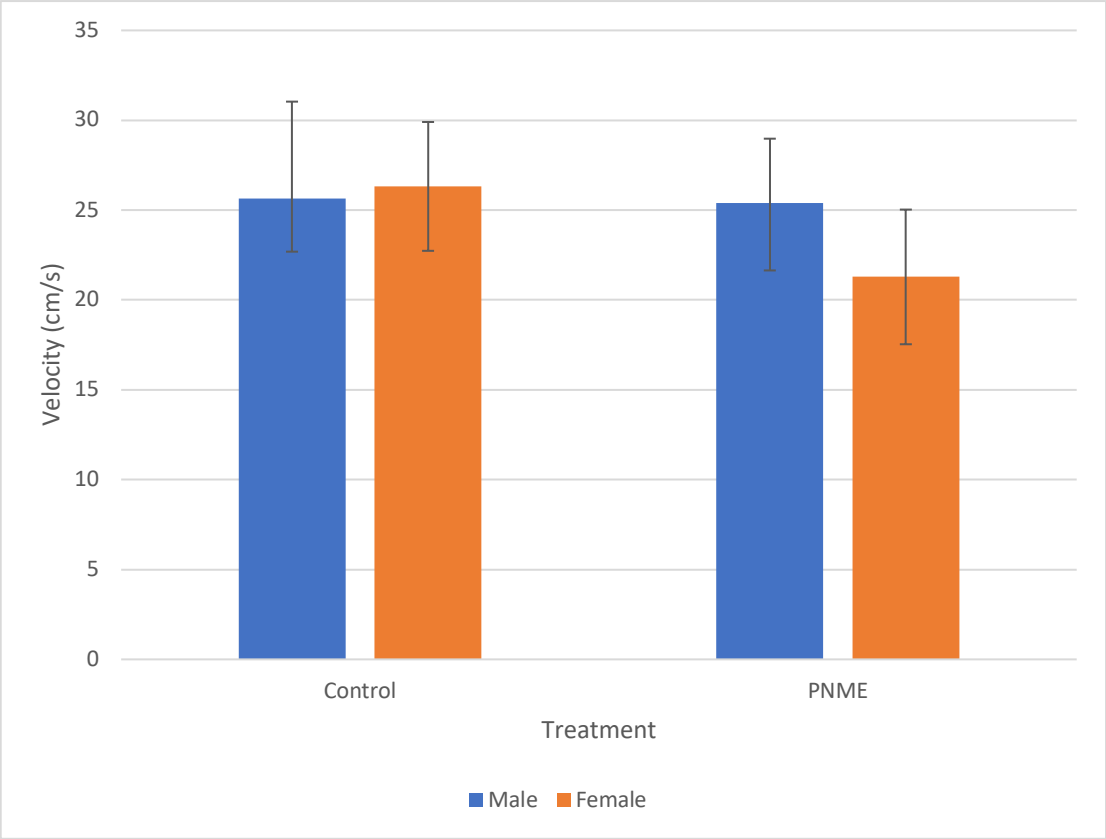
Note. Number of platform crosses of treatment group showed no significant differences as compared to controls, $F(1,67) = 0.194$, $p = 0.661$. Error bars represent the standard error of the mean. Analysis ran via two-way ANOVA.

Effects of Prenatal Enrichment on Swim Velocity in MWT

There was no significant effect of treatment observed, $F(1,67) = 2.169$, $p = 0.156$ (Fig. 6.4), suggesting that there was no significant effect of maternal prenatal enrichment on swim velocity. There was no effect of sex observed, $F(1,67) = 0.915$, $p = 0.350$. There was no Treatment by Sex interaction observed, $F(1,67) = 1.761$, $p = 0.199$.

Figure 6.4

Swim Velocity of Animals Exposed to Prenatal Enrichment as Compared to Controls



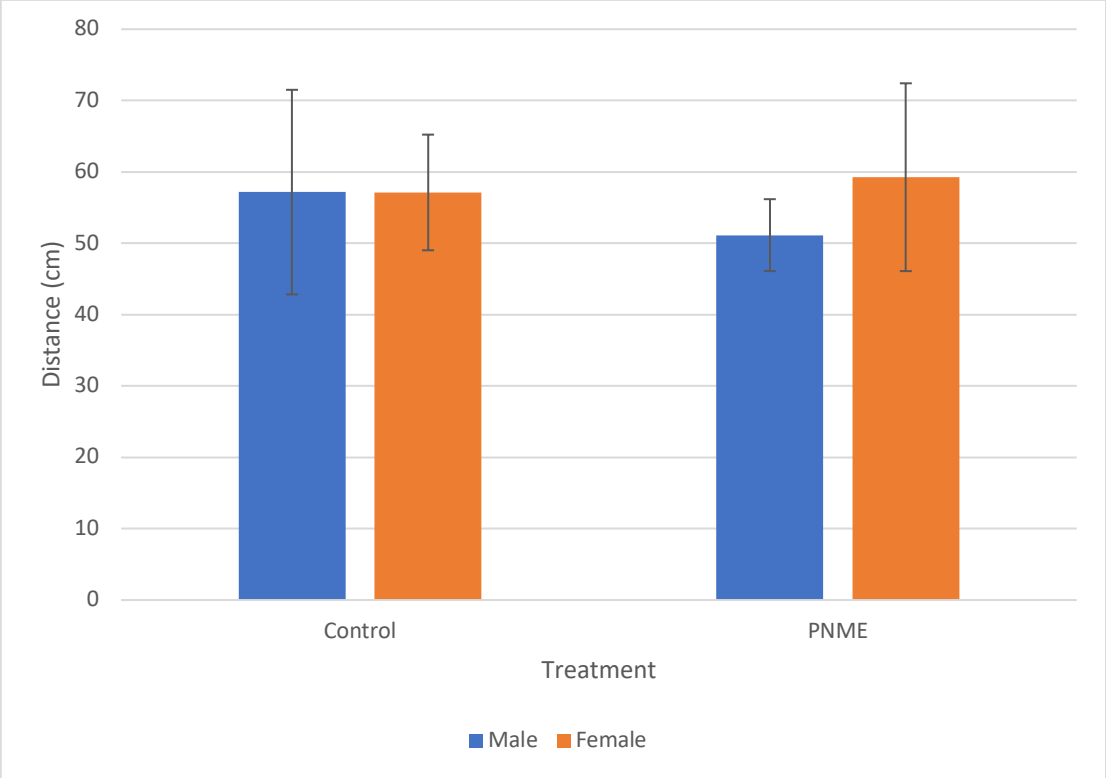
Note. Swim velocity of treatment group showed no significant differences as compared to controls, $F(1,67) = 2.169$, $p = 0.156$. Error bars represent the standard error of the mean. Analysis ran via two-way ANOVA.

Effects of Prenatal Enrichment on Mean Distance to Platform in MWT

There was no significant effect of treatment observed, $F(1,67) = 0.162$, $p = 0.691$ (Fig. 6.5), suggesting that there was no significant effect of maternal prenatal enrichment on mean distance to platform location. There was no effect of sex observed, $F(1,67) = 0.696$, $p = 0.414$. There was no Treatment by Sex interaction observed, $F(1,67) = 0.714$, $p = 0.408$.

Figure 6.5

Mean Distance to Platform Location of Animals Exposed to Prenatal Enrichment as Compared to Controls



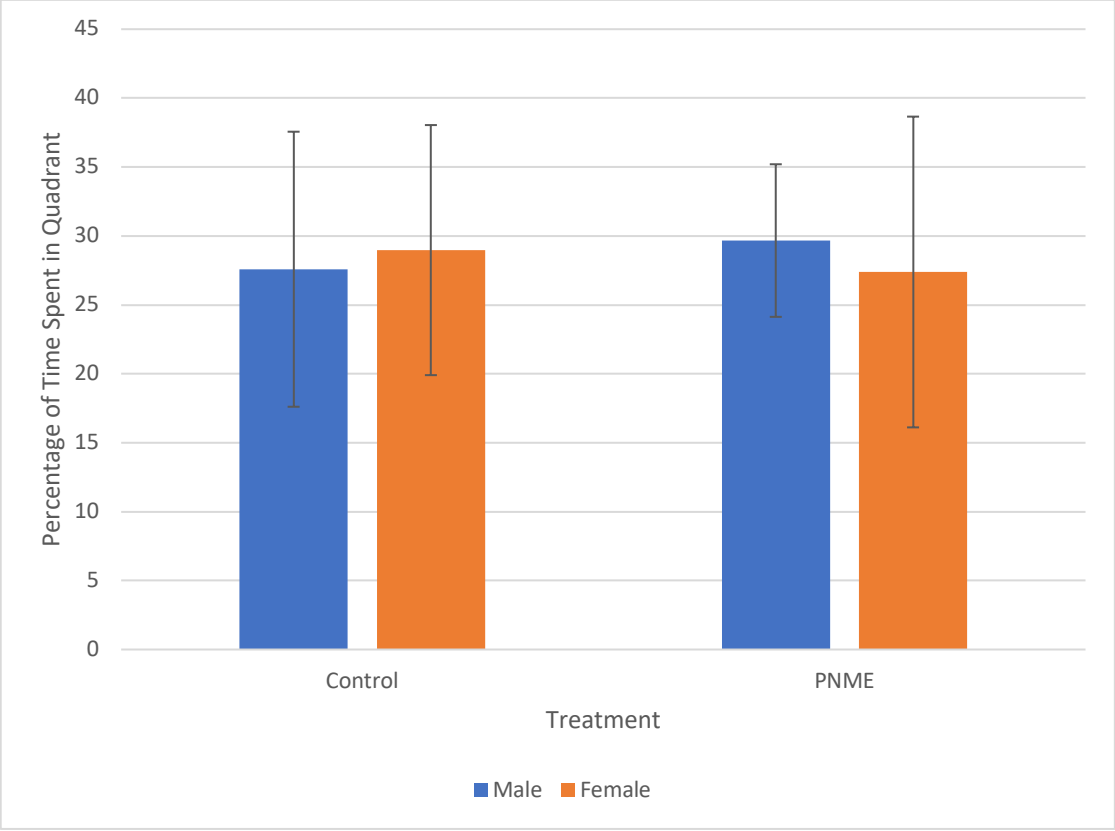
Note. Mean distance to platform location of treatment group showed no significant differences as compared to controls, $F(1,67) = 0.162$, $p = 0.691$. Error bars represent the standard error of the mean. Analysis ran via two-way ANOVA.

Effects of Prenatal Enrichment on Platform Quadrant Preference in MWT

There was no significant effect of treatment observed, $F(1,67) = 0.167$, $p = 0.688$ (Fig. 6.6), suggesting that there was no significant effect of maternal prenatal enrichment on platform quadrant preference. There was no effect of sex observed, $F(1,67) = 0.210$, $p = 0.652$. There was no Treatment by Sex interaction observed, $F(1,67) = 0.641$, $p = 0.433$.

Figure 6.6

Platform Quadrant Preference of Animals Exposed to Prenatal Enrichment as Compared to Controls



Note. Platform quadrant preference of treatment group showed no significant differences as compared to controls, $F(1,67) = 0.162$, $p = 0.691$. Error bars represent the standard error of the mean. Analysis ran via two-way ANOVA.

Discussion

In this chapter, the effects of PE on offspring performance in MWT is evaluated. Specifically, we examined escape latency, swim velocity, distance to platform, platform crossings, and quadrant preference, finding no significant differences between enriched and control groups across any measure.

These findings stand in contrast to prior research employing combined pre- and postnatal enrichment protocols. Sparling et al. (2018) demonstrated that when enrichment spans both gestation and post-weaning, offspring show enhanced spatial memory during the probe trial, evidenced by increased platform crossings, more time in the target quadrant, and reduced thigmotaxis. Importantly, adult female offspring in that study exhibited particularly strong platform memory and centre maze crossings. The absence of similar findings in the present study suggests that prenatal enrichment alone may be insufficient to elicit measurable MWT improvements, particularly if postnatal interventions are omitted.

Research also highlights sex-specific effects in enrichment contexts. Zuena et al. (2016) found that maternal EE influenced behaviour differentially: female offspring showed improved learning, while males exhibited anxiety-like behaviours, with altered motility, including swimming speed, that could affect water maze performance.

Further evidence of EE's spatial learning benefits is demonstrated in Xie et al.'s (2012) study, in which it was observed that EE reversed spatial learning deficits caused by maternal seizures, demonstrating clear improvements in maze performance. Likewise, Tipyasang et al. (2014) reported that EE, following prenatal alcohol exposure, attenuated spatial learning deficits and preserved probe trial preference across multiple post-training time points. These findings suggest that enrichment may be most effective in rescuing impaired or compromised systems,

whereas in otherwise healthy development, its impact may be less pronounced, potentially aligning with our current null result.

Additional support for enrichment's cognitive benefits comes from studies in which maternal care rather than postnatal interventions were assessed. For example, pre-reproductive maternal enrichment has been shown to improve spatial performance in offspring in MWT, associated with elevated BDNF expression in hippocampal regions, suggesting transgenerational effects that augment cognitive development (Cutuli et al., 2015). Again, while promising, these studies involve enrichment before reproduction and/or during parenting, suggesting that timing and context of EE application may critically influence outcomes.

The present findings contribute to the nuanced understanding of maternal enrichment effects. Unlike combined or stress-buffering enrichment paradigms that yield clear MWT improvements, prenatal enrichment alone appears insufficient to alter standard cognitive development trajectories as measured by the MWT, at least under the conditions studied. It remains possible that complementary postnatal interventions, stress exposure, or maternal pre-reproductive enrichment are necessary to elicit performance enhancements in spatial memory tasks.

Conclusion

These results show no significant effect of escape latency, number of platform location crosses, swim velocity, mean distance to platform location, or platform quadrant preference. These results suggest that maternal prenatal enrichment has no effect of spatial memory or learning in the MWT.

Chapter 7

Volumetric Measurements of the Adult Offspring Brain Following Maternal Prenatal Enrichment

Introduction

Volumetric analyses of brain regions in the context of maternal experience have predominantly focused on the adverse effects of prenatal stress. This area of research has garnered significant attention due to mounting evidence that stress experienced during pregnancy can lead to measurable changes in neonatal brain development. For instance, a study by Triplett et al. (2022) investigated the impact of prenatal exposure to early life adversity on neonatal brain volume. Their findings revealed a bilateral reduction in hippocampal and amygdala volumes in neonates whose mothers experienced prenatal stress. More specifically, maternal tobacco use during pregnancy was uniquely associated with reduced amygdala volume, whereas social disadvantage, a composite measure including factors such as income, education, and neighborhood environment, was identified as a significant predictor of reduced hippocampal volume. In addition to these region-specific findings, Triplett et al. (2022) also reported broader volumetric changes, demonstrating that social disadvantage during pregnancy was associated with reduced gray matter volume in both cortical and subcortical brain regions. Further structural alterations included reductions in overall white matter volumes and cortical folding, indicating that the adverse effects of prenatal stress are not restricted to isolated regions but rather may reflect widespread developmental disruption across the neonatal brain.

Given the well-established negative consequences of prenatal stress on early brain development, researchers have increasingly begun to question whether the inverse might also stand to be true, namely, whether positive environmental conditions, such as enrichment, might provide protective or even enhancing effects on the developing brain. Supporting this hypothesis, Gonzalez et al. (2019) examined the impact of early life EE following maternal separation, a commonly used model of early life stress. Their results demonstrated significant increases in volume within the mPFC and the ventral hippocampus of enriched animals. Notably, these volume increases were observed both in animals that had experienced early life stress and in those raised under standard (control) conditions, suggesting that enrichment may exert beneficial effects on brain development regardless of early adversity.

Building on this growing interest in the positive effects of enrichment, previous work from the Gibb lab has begun to explore how maternal prenatal enrichment might influence offspring brain development. In a study by Gibb et al. (2014), the effects of prenatal enrichment were explored in the context of recovery from perinatal cortical injury. Although the study did not directly assess volumetric differences, the findings nonetheless indicated promising structural changes. Specifically, prenatally enriched animals displayed increased cortical thickness anteriorly, as well as increased volume in the thalamus, both anteriorly and posteriorly. Additionally, Golgi-Cox staining revealed higher dendritic spine density in the enriched group, suggesting enhanced synaptic connectivity. While volumetric data were not the primary focus of this study, the observed anatomical enhancements point to the potential for prenatal enrichment to positively influence offspring brain structure.

Specific Hypothesis

Based upon the field of available research on neuroanatomical changes in the brain following maternal experience and environmental enrichment, I hypothesize that volume in both Cg3 and PAR1 will be increased in prenatally enriched animals compared to control animals.

Methodology

Volumetric Analysis

Volumetric analysis was conducted in a pilot sample of 15 male subjects (8 control, 7 prenatally enriched). Volumetric analyses of the medial prefrontal cortex (Cg3) and parietal cortex (PAR1) were conducted using Stereo Investigator 10 (MBF Bioscience) in conjunction with a compound light microscope. The procedures were adapted from previously established protocols for measuring cortical thickness and volume in rodent models (Karl et al., 2010; Kolb & Whishaw, 1981; Jenkins et al., 2018). Anatomical identification and delineation were guided by the Rat Brain Atlas (Paxinos & Watson, 1986), with additional reference to stereotaxic coordinates provided by Zilles (1985).

Cg3 Volumetric Protocol

Although area Cg3 is frequently examined in rodent studies, few standardized volumetric protocols exist. To address this, a region-specific tracing and volume estimation protocol was developed by Crump (2023), designed to enhance measurement consistency and anatomical precision.

The anatomical boundaries of Cg3 extend from approximately +4.70 mm to +2.20 mm anterior to bregma. However, only the portion between +4.20 mm and +2.70 mm could be reliably identified across all brains. Within this range, five representative coronal sections were

selected per animal. The total number of sections falling within this region was divided by five to determine equally spaced sampling intervals.

In each selected section, the Cg3 area was manually outlined using clearly identifiable anatomical landmarks, including the corpus callosum and caudate putamen. Tracings were imported into Stereo Investigator, where regional volume was computed using the software's volumetric analysis tools.

PAR1 Volumetric Protocol

Volumetric analysis of the parietal cortex (area PAR1) was conducted using the same general methodology as for Cg3. The region was identified and delineated based on anatomical definitions provided in Paxinos and Watson (1986), with stereotaxic confirmation from Zilles (1985). PAR1 is situated more posteriorly, extending approximately from –2.50 mm to –4.50 mm relative to bregma. For consistency across samples, a reliable range between –2.70 mm and –4.20 mm was selected for analysis.

As with the Cg3 procedure, five coronal sections were chosen from each brain, evenly spaced within the defined range. Manual tracing of PAR1 boundaries was guided by adjacent anatomical structures such as the hippocampal formation depending on the level of the section.

Outlines were analyzed using Stereo Investigator's volumetric tools, allowing for the computation of total volume within the defined range for each brain. This parallel approach ensured methodological consistency between Cg3 and PAR1 measurements, facilitating direct comparison of volumetric data across regions.

Statistical Analysis

Statistical analyses were performed using IBM SPSS Statistics 27 software. Analysis of regional volume was conducted using a two-way ANOVA with hemisphere (right or left) and

treatment (control or prenatally enriched) as independent variables. Statistical analyses of each region were run separately such that comparisons were only drawn between volume and treatment.

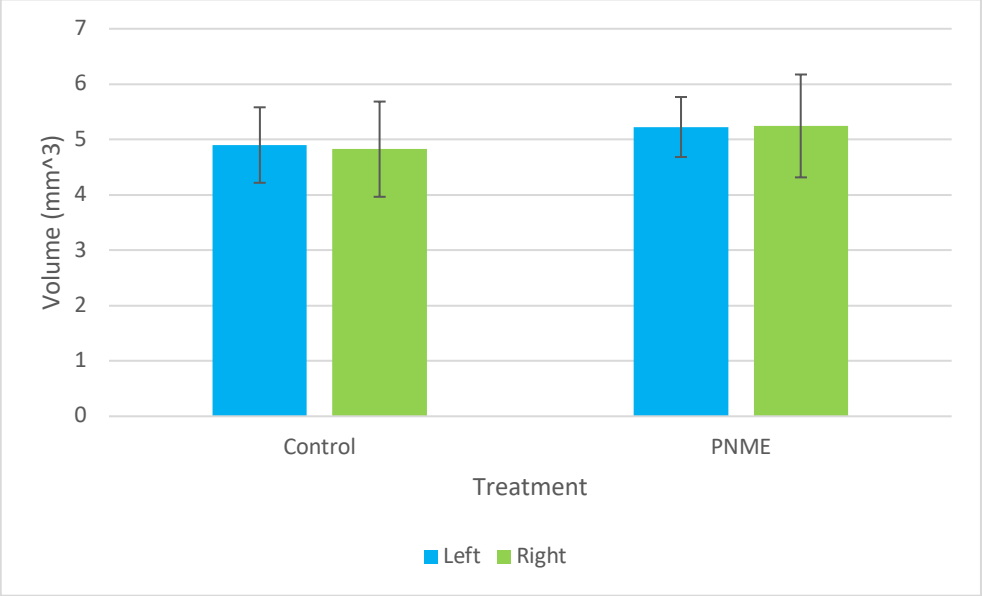
Results

Effects of Prenatal Enrichment on Cg3 Volume

There was no significant effect of treatment observed, $F(1,13) = 1.802$, $p = 0.191$ (Fig. 7.1). There was no effect of hemisphere observed, $F(1,13) = 0.011$, $p = 0.916$. There was no Treatment by Hemisphere interaction observed, $F(1,13) = 0.028$, $p = 0.868$.

Figure 7.1

Changes in Cg3 Volume of Animals Exposed to Prenatal Enrichment as Compared to Controls



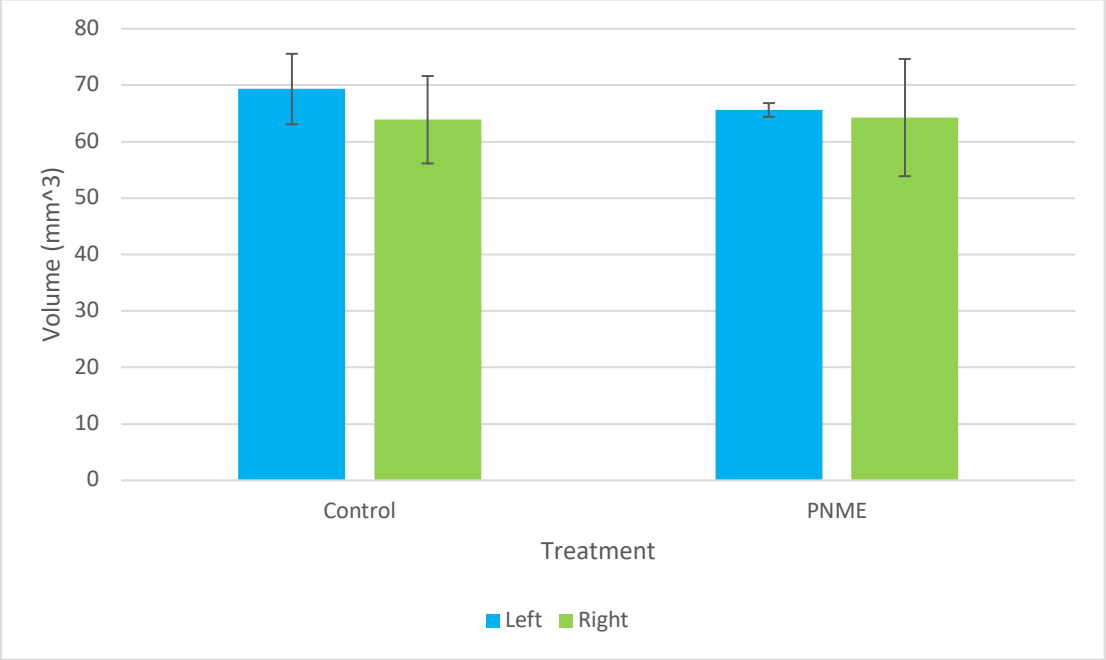
Note. Volume of Cg3 in treatment groups showed no significant differences as compared to controls, $F(1,13) = 0.180$, $p = 0.679$. Error bars represent the standard error of the mean. Analysis ran via two-way ANOVA.

Effects of Prenatal Enrichment on PAR1 Volume

There was no significant effect of treatment observed, $F(1,13) = 0.521$, $p = 0.477$ (Fig. 7.2). There was no effect of hemisphere observed, $F(1,13) = 2.369$, $p = 0.136$. There was no Treatment by Hemisphere interaction observed, $F(1,13) = 1.223$, $p = 0.279$.

Figure 7.2

Changes in PAR1 Volume of Animals Exposed to Prenatal Enrichment as Compared to Controls



Note. Volume of PAR1 in treatment groups showed no significant differences as compared to controls, $F(1,13) = 2.581$, $p = 0.132$. Error bars represent the standard error of the mean. Analysis ran via two-way ANOVA.

Discussion

This analysis aimed to investigate the effect of maternal prenatal enrichment on volume of the Cg3 and PAR1 regions of male Long-Evans rats. No significant changes in volume of either region were observable in the brains of animals prenatally exposed to EE.

Prenatal enrichment yielded no significant changes in volume compared to the control group. Additionally, there was no Hemisphere by Treatment interaction, suggesting that the enrichment experience did not significantly impact right and left hemispheres differently.

However, it should be noted that the sample used for this analysis was a pilot sample of 15 males. Further analysis should be completed to determine if these results are consistent in a larger sample size and in female subjects.

Interestingly, in Maruoka et al.'s (2009) study examining maternal enrichment's effect on prenatal hippocampal proliferation, cell proliferation was found to be influenced by maternal enrichment in female, but not male, offspring. This sex-specific effect suggests that neurodevelopmental responses to maternal enrichment may differ by sex, potentially due to underlying hormonal, genetic, or epigenetic mechanisms that mediate neuroplasticity during the prenatal period. Given this finding, it is possible that the absence of significant volumetric differences in the current study may be attributed to the exclusive sample of male subjects. If female offspring are more responsive to the neurobiological effects of prenatal enrichment, as Maruoka et al.'s findings suggest, the current sample may have underestimated the full impact of enrichment on brain development. Future studies incorporating both male and female subjects will be important for evaluating potential sex-dependent effects and for better understanding the influence of prenatal environmental conditions on structural brain outcomes.

Conclusion

The results of this study demonstrate no significant effect of maternal prenatal enrichment on Cg3 or PAR1 volume. These results may suggest that maternal prenatal enrichment has no long-term effects on volume of the Cg3 or PAR1 regions, however, it is important to note that these results should be replicated in a larger sample with both male and female subjects before coming to that conclusion.

Chapter 8

General Discussion

The previous chapters have outlined the behavioural and neuroanatomical effects of maternal prenatal enrichment in the Long-Evans rat throughout the lifespan. The present chapter will discuss the general trends seen in the findings of this experiment, comment on how these findings fit into the field of maternal experience and offspring brain development and the potential applications of maternal EE as a way to benefit offspring development, reflect on experimental design, outline limitations and potential improvements for future research, and lastly, provide an overview of the potential impacts of this research.

Review of Hypotheses and Predictions

This research was conducted on the premise of neuroplasticity, theorizing that the brain can change in response to the environment, thus driving changes in behaviour as a result. This research aimed to address two main questions:

1. Are there any neuroanatomical effects of maternal prenatal enrichment on the volume of the parietal and frontal lobes of offspring?
2. Are there any behavioural changes in offspring associated with maternal prenatal enrichment?

Based on these questions, I hypothesized:

1. There will be positive long-term effects from maternal prenatal enrichment observable in the offspring brain.

2. There will be positive long-term effects from maternal prenatal enrichment observable in offspring behaviour.

Based on the current literature available in the field, I predicted that any changes found would not be harmful to the animals.

Review of Findings by Behavioural Assessment

The main finding of this research is that maternal prenatal enrichment showed minimal significant effects in observed offspring behaviour.

Open Field Test (OFT)

These results demonstrate no significant effect of maternal prenatal enrichment on total or novel square entries in the OFT (Table 8.1). These results may suggest that maternal prenatal enrichment has no effects on locomotor or exploratory behaviour in the OFT. Additionally, there was no Treatment by Sex interaction, suggesting that maternal prenatal enrichment did not impact males and females differently.

Elevated Plus Maze (EPM)

These results demonstrate no significant effect of maternal prenatal enrichment on duration in open arms, duration in closed arms, duration in outermost ends of open arms, number of entries in open arms, number of entries in closed arms in EPM (Table 8.1). These results may suggest that maternal prenatal enrichment has no effects on anxiety-like behaviour in the EPM. Additionally, there was no Treatment by Sex interaction, suggesting that maternal prenatal enrichment did not impact males and females differently.

Whishaw Tray Reaching (WTR)

These results demonstrate a significant effect of maternal prenatal enrichment on number of total and successful reaches in WTR, though no significant effect of percentage of successful reaches or right, left, or both forelimbs reaching preference. These results may suggest that maternal prenatal enrichment does have effect on quantity of reaching in the WTR, but no effect of lateralized movement preference or reaching precision. Additionally, there was no Treatment by Sex interaction, suggesting that maternal prenatal enrichment did not impact males and females differently.

Morris Water Task (MWT)

These results show no significant effect of escape latency, number of platform location crosses, swim velocity, mean distance to platform location, or platform quadrant preference (Table 8.1). These results may suggest that maternal prenatal enrichment has no effect of spatial memory or learning in the MWT. Additionally, there was no Treatment by Sex interaction, suggesting that maternal prenatal enrichment did not impact males and females differently.

Table 8.1

A Summary of Observed Behavioural Effects

Behavioural Assessment	Treatment	
	Prenatal Maternal Enrichment	Control
Open Field (OFT)		
Total Square Entries	-	-
Novel Square Entries	-	-
Elevated Plus Maze (EPM)		
Duration in Open Arms	-	-

Duration in Closed Arms	-	-
Duration in Outermost Ends	-	-
Open Arm Entries	-	-
Closed Arm Entries	-	-
Whishaw Tray Reaching (WTR)		
Total Reaches	⇑ [1]	⇓ [1]
Successful Reaches	⇑ [2]	⇓ [2]
Percentage of Successful Reaches	-	-
Left Forelimb Reaches	-	-
Right Forelimb Reaches	-	-
Both Forelimb Reaches	-	-
Morris Water Task (MWT)		
Escape Latency	-	-
Platform Crosses	-	-
Swim Velocity		
Mean Distance to Platform Location	-	-
Platform Quadrant Preference	-	-

Note. This table summarizes the behavioural effects observed following maternal prenatal enrichment as described in this thesis. "-" indicates no significant result.
[1]Maternal prenatal enrichment resulted in increased number of total reaches in WTR in the treatment group as compared to controls. [2]Maternal prenatal enrichment resulted in increased number of successful reaches in WTR in the treatment group as compared to controls.

Review of Findings by Anatomical Assessment

The main finding of this research is that maternal prenatal enrichment showed no significant effects in Cg3 or PAR1 volume in the pilot sample of male subjects evaluated (Table 8.2). These results may suggest that maternal prenatal enrichment has no long-term effects on

volume of the Cg3 or PAR1 regions, however, it is important to note that these results should be replicated in a larger sample with both male and female subjects before finalizing that conclusion.

Table 8.2

A Summary of Observed Volumetric Changes

Brain Region	Treatment	
	Control	Prenatal Maternal Enrichment
Cg3 Volume	-	-
PAR1 Volume	-	-

Note. This table summarizes the volumetric changes observed following maternal prenatal enrichment as described in this thesis. "-" indicates no significant result.

General Conclusions

I aimed to investigate the long-term impact of maternal prenatal EE on offspring by assessing behavioural outcomes across several domains, locomotion, anxiety-like behaviour, fine motor control, and spatial learning, as well as by analysing volumetric changes in key cortical brain regions. While the theoretical promise of environmental enrichment has been well-documented across developmental neuroscience, the current findings suggest that prenatal-only EE produces limited observable changes in offspring behaviour or brain volume under these conditions.

Across all behavioural assessments, the effects of maternal prenatal enrichment were modest. No significant effects were observed in the OFT, EPM, or MWT, all of which assess different aspects of motility, exploratory drive, and spatial learning and memory. While previous studies, such as Sparling et al. (2018), reported that pre- and postnatal enrichment can enhance spatial navigation and reduce anxiety-related behaviours (e.g., thigmotaxis) particularly in

females, such findings were not replicated here under a prenatal-only enrichment paradigm. Similarly, Zuena et al. (2016) found maternal EE had sex-specific effects on behaviour, with improved learning outcomes in females and heightened anxiety-like behaviour in males, highlighting the complexity of enrichment responses across sexes. In contrast, the present study found no significant Treatment by Sex interactions, suggesting a more uniform behavioural profile across males and females under prenatal EE alone. It should also be noted that under ethical animal practices, all animals have some form of enrichment in their cages, meaning the control animals and offspring were directly exposed to some environmental enrichment, though not as extensive as the enriched dams.

Interestingly, the only behavioural domain in which maternal prenatal enrichment appeared to exert a measurable effect was skilled reaching. Offspring from enriched dams performed a greater number of total and successful reaches in the WTR task compared to controls. This may indicate heightened motivation or engagement with the task, though measures of lateralization or percentage success did not differ significantly. These findings align partially with Ulupinar et al. (2015), who reported that rearing conditions influenced motor performance in a sex-dependent manner, with improved reaching seen in enriched females. Yet, in the current study, no sex-specific differences emerged, further suggesting that prenatal enrichment may not robustly interact with sex in isolation, or that more refined measures or larger samples may be required to detect such effects.

From a neuroanatomical standpoint, volumetric analysis of the medial prefrontal cortex (Cg3) and parietal cortex (PAR1) revealed no significant differences between enriched and control groups in male offspring. This suggests that prenatal enrichment did not produce lasting macroscopic changes in cortical volume detectable at the resolution used. Although preliminary

in scope, these findings are important, especially when contrasted with prior evidence that links brain volume loss in these regions to impaired cognitive and motor function (Hamezah et al., 2017; Casas et al., 2018). Tajima et al. (1993) also reported reductions in local brain volume associated with behavioural deficits, reinforcing the value of volumetric measurements as proxies for neural health. While no deficits were observed here, the absence of significant volumetric differences may reflect the subtlety of prenatal enrichment effects in the absence of postnatal continuation or stress-related modulation.

These findings are consistent with the broader conclusion from McCreary and Metz (2016), who emphasized that environmental enrichment is most effective when applied as an intervention for stress-related developmental disruptions. They also noted that the timing, type, and individual susceptibility to enrichment play critical roles in determining outcomes. It is plausible that prenatal EE in the absence of adverse prenatal experiences or extended postnatal stimulation may not be sufficient to yield strong or consistent neurobehavioural effects. Furthermore, the absence of postnatal enrichment in the current study may have limited the opportunity for enriched prenatal experiences to translate into measurable long-term advantages.

Additionally, changes in baseline housing requirements mandated by Animal Care Services may have resulted in the control condition functioning as a form of mild enrichment. In contrast to Gibb et al. (2014), whose control animals were housed in smaller, single-level "shoebox" cages with minimal space, control animals in the present study were housed in larger, dual-level cages that provided substantially more room for movement and interaction. These updated housing standards may therefore have reduced the contrast between control and enriched conditions, effectively shifting the control environment toward a moderately enriched baseline

relative to much of the existing environmental enrichment literature. The absence of a truly minimal control housing condition may have further contributed to the null findings observed in this study.

All in all, the results of this thesis underscore the nuanced and context-dependent nature of environmental enrichment effects. While the concept of maternal prenatal enrichment holds promise as a modulator of offspring development, its influence appears constrained under isolated conditions. Future research would benefit from integrating longitudinal designs, larger sex-balanced samples, and combined pre- and postnatal enrichment paradigms. Moreover, the use of stress-sensitive or high-risk models may better reveal the therapeutic potential of enrichment, as has been demonstrated in studies involving prenatal stress or injury (e.g., Xie et al., 2012; McCreary & Metz, 2016).

To conclude, while maternal prenatal enrichment alone does not appear to robustly alter offspring behaviour or brain structure, its limited but positive effect on skilled reaching invites further exploration. As the field moves forward, a more comprehensive and multi-dimensional approach, accounting for the interaction of biological sex, timing of intervention, environmental context, and task-specific demands, will be necessary to fully understand the developmental impact of early enrichment.

Limitations

While this thesis offers important insights into the effects of maternal prenatal EE on offspring behaviour and brain volume, several limitations warrant consideration when interpreting the results and planning future research.

Duration of enrichment in this study was modest compared to the majority of EE research, in which subjects are exposed to the enriched environment for durations of approximately three months. It is possible that the shortened duration of enrichment in this study (21 days) may have limited any elicited effects of EE.

The battery of behavioural tests employed was originally designed to detect deficits, which may have resulted in a ceiling effect in the present study. Given that control animals exhibited baseline, non-impaired performance and the treatment group was enhanced rather than impaired, the tasks may not have been sufficiently challenging. As a result, both groups were able to perform at normal levels, potentially obscuring any measurable benefits in the enhanced group.

Previous studies have reported notable social behaviour alterations following maternal EE. For instance, Zuena et al. (2016) found that male offspring housed in enriched conditions exhibited increased social play and anxiety-like behaviours, while enriched female offspring showed improved learning. Sparling et al. (2018) also reported altered social interaction behaviours, such as increased sniffing and self-grooming, in enriched juvenile rats. In contrast, the present study did not include any social behaviour tests, which limits our understanding of whether prenatal EE may have influenced offspring sociality. Including such measures in future research would provide a more comprehensive behavioural profile.

The sample sizes used in behavioural testing were relatively modest. While adequate for detecting large effects, smaller or more subtle effects, especially potential sex-specific outcomes, may have gone undetected due to limited statistical power. This is particularly important when differences may be nuanced or context-dependent, as suggested by studies reporting sex-specific effects of EE on learning and anxiety-related behaviour (e.g., Zuena et al., 2016; Sparling et al.,

2018). Future studies would benefit from larger, well-powered cohorts to reliably detect such effects.

Our volumetric analyses of Cg3 and PAR1 brain regions were conducted on a pilot sample of only 15 male subjects. This preliminary sample size restricts the generalisability of the structural findings and precludes examination of sex differences in brain volume. Given reported interactions between EE and sex on both behaviour and neuroanatomy (Zuena et al., 2016), future volumetric analyses should include larger and sex-balanced samples to allow robust statistical testing and more representative conclusions.

This study employed a prenatal-only EE paradigm. While this approach isolates the prenatal developmental period, it contrasts with studies combining pre- and postnatal enrichment, which have shown stronger effects, especially in spatial memory tasks such as the Morris Water Task (Sparling et al., 2018). Additionally, EE has been shown to ameliorate deficits induced by prenatal stressors like maternal seizures (Xie et al., 2012) or alcohol exposure (Tipyasang et al., 2014). Without modeling early-life adversity or adding postnatal enrichment, we may have limited the ability to observe more substantial or clinically relevant outcomes.

Volumetric analysis at the level of gross brain regions (Cg3, PAR1) may overlook regional or microstructural changes, such as cell density, dendritic arborisation, spine density, or white matter integrity. Given that early environmental influences can produce subtle synaptic changes (e.g., Gibb et al., 2014), future work could incorporate techniques like diffusion imaging, or immunohistochemistry to capture finer-grained neuroanatomical effects.

Relocating dams from enriched to standard housing prior to parturition occurred outside of their typical light–dark phase housing transition schedule and may have constituted an

additional stressor. This disruption could have influenced maternal physiology or behavior, potentially affecting prenatal conditions and contributing to variability in the observed outcomes.

Future Directions

Building on the findings and limitations of this thesis, several avenues for future research are recommended to deepen our understanding of how maternal prenatal EE influences offspring development, particularly with respect to behaviour and brain morphology.

As rats are nocturnal, housing animals in the enriched environment during the light phase of the 12:12 h light–dark cycle may have reduced engagement with the enrichment. Future studies may benefit from providing enrichment during the dark phase, when animals are naturally more active and therefore more likely to interact with and derive benefit from the enriched environment.

As discussed previously, the control housing employed in the present study differs from that used in much of the existing prenatal enrichment literature. Future research should consider directly comparing traditional "shoebox" style control housing with enriched housing, or alternatively employing a graded housing design that includes shoebox housing, contemporary standard housing, and enriched housing. Such comparisons would allow for examination of dose-dependent or graded effects of environmental enrichment as housing conditions progressively improve.

The battery of behavioural tests used were originally created to show deficits and may have caused a ceiling effect in the subjects, considering the control animals were baseline with no deficits, and the treatment group was enhanced. This may have caused a ceiling effect in any

potential results, where both groups were able to complete the tasks at a normal level, but were not challenged enough to demonstrate any benefits within the enhanced group.

Given the evidence that EE can significantly impact social behaviour, particularly in sex-specific ways (Zuena et al., 2016; Sparling et al., 2018), future studies should incorporate a broader range of behavioural assessments. Social interaction tasks, play behaviour analysis, and social preference paradigms would allow for a more comprehensive evaluation of the behavioural effects of maternal EE.

To enhance statistical power and detect more subtle or sex-specific effects, future experiments should involve larger and more balanced cohorts. A greater sample size would also permit more reliable subgroup analyses by sex, treatment condition, or developmental stage, helping to clarify the complex interactions reported in previous literature (e.g., Zuena et al., 2016).

While this project isolated the prenatal period, combining prenatal and postnatal enrichment, as done in studies like Sparling et al. (2018), may produce more robust behavioural and cognitive outcomes. Furthermore, introducing a prenatal stress model (e.g., maternal restraint stress or exposure to seizures, as used in Xie et al., 2012) could help determine whether EE serves as a protective factor under adverse developmental conditions (McCreary & Metz, 2016).

Future anatomical investigations should go beyond volumetric measurements to include microstructural assessments such as cellular density, dendritic complexity, synaptic density, or myelination. Techniques like immunohistochemistry, or diffusion-weighted MRI could uncover finer-grained structural changes that may not be reflected in gross volume differences. These methods could help explain behavioural findings at the cellular and circuit levels. As an

immediate next step, the brain samples from this study will be used to quantify neuronal density within key cortical regions. This analysis will help clarify whether maternal prenatal EE affects the cytoarchitecture of brain areas involved in motor control, cognition, and emotion, even in the absence of gross volumetric changes. These findings will contribute important insight into how early enrichment influences the neuronal makeup of offspring brains. Additionally, many PE studies report significant differences in HPC neuroanatomy. HPC volume and cellular analysis should be prioritized in future related research.

In summary, while the current research provides an important foundation for understanding the effects of maternal prenatal enrichment, future studies should aim to incorporate more comprehensive behavioural measures, larger and more diverse samples, and advanced neuroanatomical analyses. These next steps will be essential to fully elucidate the developmental impacts of prenatal environmental enrichment and to determine its potential therapeutic relevance for early life adversity.

Conclusion

The research presented in this thesis examines the long-term behavioural and neuroanatomical effects of maternal prenatal environmental enrichment in Long-Evans rat offspring. Although the hypotheses outlined were not consistently supported—given that minimal significant differences were observed across most behavioural measures and no volumetric changes were found in the sampled brain regions—this work contributes to the growing field of developmental enrichment research. By investigating the role of prenatal experience on offspring outcomes, this thesis offers foundational data to guide future studies exploring the timing, mechanisms, and sex-specific effects of early life enrichment.

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