Differential Effects of Early Life Stress and Schizophrenia on the Development of Impulse Control Disorder

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2024

PREFACE

The experimental work described in this thesis was conducted at the University of KwaZulu-Natal-Westville (Durban, South Africa), under the supervision of Dr. Thabisile Mpofana.

This work has not been submitted in any form for any degree to any tertiary institution, where use has been made of the work of others, it is duly acknowledged in the text.

20th February 2024

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Fredrick Otieno Oginga

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Date

As the candidate's supervisor, I agree to the submission of this thesis

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……

Dr. T. Mpofana
PhD, MPH
(Supervisor)

Date

DECLARATION

I **Fredrick Otieno Oginga (221017678)** declare that:

i. The research reported in this thesis, except where otherwise indicated, is my original work.

ii. This thesis has not been submitted for any degree or examination at any other university.

iii. This thesis does not contain other persons' data, pictures, graphs or other information unless specifically acknowledged as being sourced from other persons.

iv. That my contribution to the project was as follows:
Identification of research topics, experimental design, execution, data analysis and interpretation, manuscript and thesis write-up.

v. This dissertation does not contain other persons writing unless specifically acknowledged as being sourced from other researchers. Where other sources have been quoted, their words have been rewritten but the general information attributed by them has been referenced.

vi. This dissertation does not contain text, graphics, or tables copied and pasted from the internet unless specifically acknowledged and the source being detailed in the dissertation and the reference sections.

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20th February 2024

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Date

Fredrick Otieno Oginga

DEDICATION

In Appreciation of My Dear Father Isaac Oginga Manaseh

This thesis is dedicated to my Dear father, ***Isaac Oginga Manaseh and Family****, whose unwavering support, encouragement, and belief in my abilities have been the pillars of my academic success. Your guidance and love have shaped my educational journey, and I am profoundly grateful for the opportunities you've provided and the values you've instilled in me. Your presence in my life is a constant source of strength and inspiration, and I am fortunate to have you as my father. Thank you for everything, Dad. This dedication is a testament to our enduring bond and your invaluable influence on my path to achievement.*

PUBLICATIONS AND PRESENTATIONS

List of published peer reviewed articles

Fredrick Otieno Oginga and Thabisile Mpofana. The Impact of Early Life Stress and Schizophrenia on Motor and Cognitive Functioning: An Experimental Study *Frontiers in Integrative Neuroscience Journal Learning and Memory Special issue 2023 Front. Integr. Neurosci. Volume 17 - 2023 | doi: 10.3389/fnint.2023.1251387 This article is part of the Research Topic Learning and Memory 2023 - Volume II Impact Factor-3.213*

List of submitted peer-reviewed articles under review

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Fredrick Otieno Oginga and Thabisile Mpofana. Understanding the Role of Early Life Stress and Schizophrenia on Anxiety and Depressive like Outcomes: An experimental study. *SAGE- Neuroscience Insights.*

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List of conference presentation

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LIST OF ABBREVIATIONS

ACE: Adverse Childhood Experiences

BDNF: Brain-Derived Neurotrophic Factor

CRH: Corticotropin-Releasing Hormone

CNS: Central Nervous System

DL: Developmental Impact

EPM: Elevated Plus Maze

ELS: Early Life Stress

GS: Gestational Stress

GRADE: Grading and Recommendations Assessment, Development and Evaluation

GABA: Gamma-Aminobutyric Acid

HPS: Hippocampal-Prefrontal System

HPA: Hypothalamic-Pituitary-Adrenal Axis

KSZ: Ketamine Induced Parental Schizophrenia Like Symptoms

MDD: Major Depressive Disorder or Depression

MeSH: Medical Subjects Headings

MDD: Major Depressive Disorder

MS: Maternal Separation

NPD: Neuro-Psycho-biological Dysregulation

OFT: Open Field Test

PRISMA-P: Preferred Reporting Items for Systematic Review and Meta-Analysis Protocols

PSZ: Parental Schizophrenia

PFC: Prefrontal Cortex

PTSD: Post-Traumatic Stress Disorder

RCT: Randomized Controlled Trial

SPT: Sucrose Preference Test

SZ: Schizophrenia

ABSTRACT

Background: Early life stress (ELS) and parental psychopathology, such as schizophrenia, can profoundly affect neurobiological and behavioral outcomes in later life. While previous studies in human have explored the individual effects of ELS and parental schizophrenia (PSZ), this study investigates their interactive effects, recognizing that real-life situations often involve multiple simultaneous stressors.

Objectives: This study aimed to comprehensively examine the impact of ELS and schizophrenia like symptoms on locomotion, anxiety and depressive like behavior, spatial memory, social interaction, cognitive flexibility and neuro-inflammation in Sprague-Dawley rats**.**

Methods: Male and female Sprague-Dawley pups were randomly assigned to eight groups: control, ELS, Ketamine to induce schizophrenia like symptoms (KSZ), and ELS + KSZ. ELS was induced through prenatal stress and maternal separation (MS), while schizophrenia-like behaviour was induced by ketamine administration (KSZ). Ketamine was administered intraperitoneal to the dams, while subcutaneous to the pups as per previously published studies. Behavioral assay, including open field, Morris water maze, social interaction behaviour, and sucrose preference test, was conducted. The researchers assessed neuro-inflammation through quantification of glial fibrillary acidic protein astrocytes density and specific inflammatory biomarkers. **Results:** ELS and KSZ on dams exhibited enduring effects on particularly psychomotor retardation ($p < 0.05$). Anxiety and depressive like behavior was elevated in the ELS ($p = 0.023$) and KSZ on dams ($p = 0.017$) groups compared to controls. The combined ELS and KSZ groups showed the highest anxiety and depressive like outcomes ($p = 0.006$). Additionally, spatial memory and cognitive impairment in pups were observed due to the combined impact of ELS and KSZ, which was associated with a decrease in astrocyte density and dysregulation of neuro-inflammatory markers ($p < 0.05$).

Conclusion: This study highlights the interactive effects of ELS and parental psychopathology (KSZ) on behavior, neurodevelopment, and neuro-inflammation in rats. Both ELS and KSZ in parents were linked to anhedonia, subsequently anxiety-like behavior, and ultimately psychomotor, spatial memory, and cognitive decline in rats. Positive parenting was associated with astrocyte regeneration ($p < 0.05$) and cognitive improvement. Understanding these complex interactions provides insights into the challenges associated with these stressors and offers potential therapeutic avenues.

Keywords: Anhedonia; Anxiety like behaviours; Cognitive Psychopathology; Early life Stress; Parental Mental illness; Psychopathology; Ketamine induced Schizophrenia like symptoms

THESIS OUTLINE

This Doctoral dissertation adopts an article-based format, comprising a series of manuscripts presented as thesis chapters:

Chapter One: Introduction

This chapter outlines the background information; it consists of a comprehensive literature review encompassing the foundational concepts of this study. It outlines the study's aims and objectives, presents hypotheses, and underscores the potential research benefits. Manuscript submitted to Brain and Behaviour Wiley's Journal.

Chapter Two: Experimental Study 1

Chapter Two represents the initial segment of the PhD experimental research. It focuses on assessing the Impact of Early Life Stress and Schizophrenia on Motor and Cognitive Functioning: An Experimental Study. This section constitutes the first part of the PhD experimental work, which has been published in the Frontiers in Integrative Neuroscience Journal. DOI; https://doi.org/10.3389/fnint.2023.1251387

Chapter Three: Experimental Study 2

This chapter corresponds to the subsequent segment of experimental research. It centers on the investigation of Understanding the Role of Early Life Stress and Schizophrenia on Anxiety and Depressive like outcomes: An Experimental Study. This manuscript has been submitted to the SAGE-Neuroscience Insights Journal for publication. Pre-Print DOI; https://dx.doi.org/10.2139/ssrn.4603943

Chapter Four: Experimental Study 3

Chapter Four delves into the final study/manuscript, titled "Beyond Psychopathology: Examining the Complex Interactions of Early Life Stress, Schizophrenia, and Neuro-Inflammation on Cognitive and Motor Functioning." This manuscript has been submitted Neuroscience Science Direct

Chapter Five: Conclusion and Recommendations

This chapter serves as a comprehensive synthesis of all the manuscripts presented in this thesis. It offers a culmination of research findings, discusses limitations, and provides valuable recommendations for future studies.

CHAPTER ONE:

INTRODUCTION AND LITERATURE REVIEW

1.1 Introduction to Impulse Control Disorders (ICDs)

A healthy population is an end in itself (Dean, 2013; The King's Fund, 2019); along with being one of the most basic requirements for quality of life, it is the foundation for a country's economic growth and development. One in every four people on the planet will experience mental or neurological disorders at some point (World Health Organisation, 2017). Mental illness refers to conditions that affect cognition, emotion, and behaviour (e.g., schizophrenia, depression, autism) (Manderscheid et al., 2010). Some of the most common mental disorders include schizophrenia (SZ), Attention-Deficit/ Hyperactivity Disorder (ADHD), bipolar disorder (BD), and disruptive, impulse-control, and conduct disorders (DICD) (American Psychiatric Association, 2022). Among these common mental disorders, the prevalence estimates of disruptive impulse-control and conduct disorders in children and adolescent are rife globally and have shown a steady increase over recent years (American Psychiatric Association, 2013; Fariba & Gokarakonda, 2022; Theron et al., 2022). Initially classified as impulse control disorders, the following are common examples of DICD: opposition defiant Disorder (ODD), conduct disorder (CD), pyromania, intermittent explosive disorder (IED), pathological gambling (PG), and kleptomania (Sebastian et al., 2014; Woods et al., 2016).

These conditions impair an individual's daily functioning and have far-reaching consequences on relationships, financial stability, and overall quality of life (Defar et al., 2023). It is estimated that about 400 million people live with ICD globally. That is slightly more than one in twenty people globally (Parra-Díaz et al., 2021). Almost half of this burden lies in low- and middle-income countries because of extreme scarcity of health resources and investment (Blair & Raver, 2016; Mabrouk et al., 2022). Childhood and adolescence are critical stages of life that is characterized with synaptic growth and brain (Konrad et al., 2013; Whittle et al., 2013). Early life stress or parental psychopathologies like schizophrenia have been thought to be the major contributors to developmental problems in children such as DICD (Scott et al., 2016; Watkeys et al., 2022). Hence, understanding the neurobiological mechanisms of this link is crucial because it may offer targets for new therapeutic approaches, given the prevalence of both childhood abuse and adult mental illness. In light of this, there is a pressing need for dependable and robust animal models that researchers can use to uncover key brain processes that may be underscored by ethical limits on conducting these human investigations (Bremner, 2016; Van Der Horst & Van Der Veer, 2008). The silver lining is that rodent models of ELS have gained traction lately (Murthy & Gould, 2018). Maternal separation (MS) has been one

of the most commonly used manipulations to create a rodent model of ELS. Research has shown that MS resulted in increasing anxiety and depressive-like behaviors in rats later in life, implying that it has translational validity (Bölükbas et al., 2020; Janus, 1987; Lehmann & Feldon, 2000). Furthermore, humans and rats are biologically similar; they share more than 98% DNA similarity (Verbitsky et al., 2020). These challenges beckon for comprehensive research endeavors that explore the multifaceted factors of ELS and PSZ contributing to ICD and shed light on potential avenues for intervention and treatment.

1.2 Impact of Early Life Stress on Motor, Memory and Cognitive Functioning

Early life, characterized by the formative years of infancy and childhood, is a critical period in an individual's developmental journey (National-Academy-of-Sciences, 2015). During this time, the human and rodent brains undergo remarkable growth and refinement, laying the foundation for cognitive and behavioral patterns that persist throughout life (McEwen, 2003; Semple et al., 2013; Tierney & Nelson, 2009). However, this period is not immune to adversity, and exposure to various stressors can significantly influence the trajectory of development. MS, limited nesting time, and Gestational Stress have been accepted paradigms in mimicking Adverse Childhood Experiences (ACE) in humans (Biggio et al., 2014; Walker et al., 2017). ACEs involve negative/traumatic experiences experienced as a child (before 18) and encompass a spectrum of adverse experiences, including but not limited to prenatal stressors, maternal separation, neglect, abuse, and trauma (Lange et al., 2019). These ACEs are increasingly being linked to adverse perinatal outcomes; this is similar to results reported in rodent studies on the development of anxiety and depression in pups due to MS. Therefore, understanding the impact of these stressors on cognitive and behavioral development is a paramount concern within the field of mental health research.

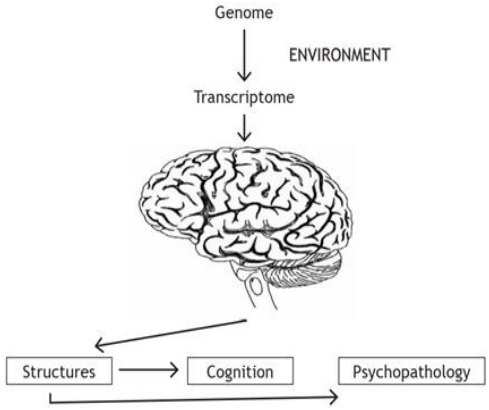

Figure 1-0-1:The environment interacts with the genome to regulate gene transcription. When these processes occur during development, the structure and function of the brain are changed, predisposing individuals to psychopathology (Brietzke et al., 2012).

One of the primary domains affected by ELS, as reported in rodents and human studies, is the cognitive domain. Cognitive functions encompass a wide array of mental processes, including attention, memory, reasoning, problem-solving, and decision-making (Brunson et al., 2005; Cowell et al., 2015). In rodents, cognitive functions can be evaluated through behavioral assays such as the MWM, EPM, social interaction, and SPT. These assays provide valuable insights into aspects of cognition such as spatial memory, learning and memory, executive functioning, and cognitive flexibility. Early stressors can disrupt the delicate balance of cognitive development, leading to cognitive impairments that may persist into adulthood. For example, research has shown that exposure to stress during critical periods of brain development can lead to alterations in brain structure and function, particularly in regions responsible for memory and executive functions, such as the limbic system, hippocampus, and prefrontal cortex (Malave et al., 2022; Tsuda & Ogawa, 2012). Such alterations may manifest as deficits in attention and concentration, difficulties in learning and academic achievement, and impaired executive control, which includes the ability to regulate impulses and make sound decisions.

Beyond cognitive effects, ELS also profoundly impacts behavioral development. A complex interplay of genetic, environmental, and experiential factors shapes behavioral patterns and tendencies. Early stressors can disrupt this interplay, leading to maladaptive behavioral responses. Human subjects and rodent models exposed to early life stress may exhibit heightened emotional reactivity, increased vulnerability to anxiety and mood disorders, and difficulties in emotion regulation (Smith & Pollak, 2020). For instance, a study by Hisey and colleagues exposed male mice to chronic social defeat stress during their juvenile period revealed a socially avoidant phenotype using open-field tests (Hisey et al., 2023). Human studies have also reported that children experiencing ICDs due to ELS often exhibit lower

levels of emotional (internalizing) difficulties and externalizing problems (Marceau et al., 2015; Schreiber et al., 2011). Both rodent and human studies have consistently indicated that lower levels of externalizing and internalizing behaviors are correlated with reduced cognitive and motor functions. Externalizing behaviors often involves actions that are disruptive, aggressive, or rule-breaking for examples aggression, defiance, impulsivity, and conduct problems. While internalizing behaviours also called inward behaviours are often associated with emotional or psychological distress for instance; anxiety, depression, withdrawal, and somatic complaints. It's the cognitive and psychomotor domains affected in both (Liu et al., 2011; Whittle et al., 2020).

In rodents, these functions can be effectively measured through various tests, including the OFT, MWM, and social interaction assessments. Kokubo and collegues study (2018) showed that mice subjected to early-life stressors, such as maternal deprivation (MD) during lactation, exhibited motor coordination dysfunction (Kokubo et al., 2018). In a similar vein, pre-clinical models of early stress (ES) also demonstrate subsequent impairments in cognition, especially in tasks reliant on hippocampal function, such as the MWM and object location tasks (Bonapersona et al., 2021; Othman et al., 2022). These disruptions in memory, motor, and cognitive functioning could contribute to the impulsive behaviors characteristic of ICDs as reported in humans. Hence, understanding the complex interplay between stress exposure, neurobiological changes, and subsequent behavioral outcomes is vital for informing targeted interventions and therapeutic approaches. To achieve this in the present study, we modelled ELS in SD rats because they are scientifically acceptable, are highly tractable and can be easily manipulated pharmacologically, enabling precise control over experimental variables. Moreover, their social structure and cognitive abilities facilitate the investigation of behavioral and social aspects of schizophrenia. These models provide a valuable means to dissect the complex interplay of biological, environmental, and behavioral factors that contribute to developing developmental disorders later in life, ultimately advancing our understanding of these disorders and informing potential therapeutic interventions for humans.

1.3 Parental Schizophrenia Impact on Memory and Cognitive Decline

Schizophrenia is a severe neuropsychiatric disorder that affects about 1% of the global population and has been implicated as one of the key causes of parental psychopathology. Schizophrenia manifests through a spectrum of symptoms categorized into positive, negative, and cognitive domains. Positive symptoms involve hallucinations, such as auditory perceptions not grounded in reality, and delusions, where strongly held false beliefs resist reason. Negative

symptoms encompass affective flattening, leading to reduced emotional expression, anhedonia, indicating a diminished capacity for pleasure, and volition, resulting in decreased motivation for purposeful activities. Cognitive symptoms include impaired memory formation and retrieval, challenges in executive functioning involving decision-making and planning, and difficulties sustaining attention on specific tasks (Popovic et al., 2019).

Schizophrenia is complex and multifactorial; its etiology involves a strong genetic predisposition and environmental influences (Rantala et al., 2022). Schizophrenia is known to impact cognitive and emotional functioning and has been associated with an increased risk of development of mental illness in the offspring (Hussain, 2020). Even though limited research shows the relationship between parental psychopathology and ICD, previous evidence demonstrates that parental mental illness negatively impacts children's cognitive and motor functions (Rusengamihigo et al., 2021). A study by Nieto-Rucian and furness (Nieto-Rucian & Furness, 2019) revealed a strong association between PSZ and psychiatric disorders in offspring. Genetic predisposition, epigenetic modifications, altered neurodevelopment, and environmental stressors have been proposed as contributors to this elevated risk (Alves et al., 2022). Common genetic risk factors are most likely responsible for part of the association between parental mental disorders at any time point (including the perinatal period) and child vulnerability. However, there is evidence that the environment plays an important role in developing mental disorders (Hsu et al., 2014). In addition, epigenetic changes (alteration of gene expression without alteration of genetic sequence, e.g., by methylation) are one mechanism proposed to explain the long-term effects of early life experiences, including the perinatal environment, on biological and behavioral phenotypes. Most of the research comes from animal studies; however, preliminary studies in humans show that prenatal stress and anxiety increase methylation (i.e., silencing) of glucocorticoid receptors (involved in stress responses) in children (Braithwaite et al., 2015; Palma-Gudiel et al., 2015; Szyf, 2013). An additional neurobiological concept in the perinatal period is that of epigenetics, or genetic modifications induced by environmental factors that impact the expression of genes. The predisposition to both health and illness is significantly influenced by the genetic makeup inherited from a child's parents. According to Bale, genetic susceptibility to both mental and physical illnesses encompasses three key components: (1) genetic predisposition, (2) prenatal stress, and (3) exposure to stress after birth (Bale, 2014).

1.4 Maternal Separation, Gestational Stress, and Parental Schizophrenia (PSZ) Impact on Motor and Cognitive Functioning

Considering the common occurrence of childhood maltreatment and adult mental illness, exploring the neurobiological links between them is crucial, as such understanding could potentially unveil opportunities for innovative therapeutic approaches. One of the frequently employed techniques to create a rodent model for ELS involves maternal separation. Research has demonstrated that when MS is applied to rats for an adequate duration, typically lasting 3 hours per day throughout the first two postnatal weeks, it leads to heightened anxiety- and depressive-like behaviors in adulthood. Therefore, this suggests that MS possesses translational validity for studying these conditions (Daniels et al., 2004; Mpofana et al., 2016). Nevertheless, it is important to note that other investigations involving both rats and mice have revealed significant variability in behavioral outcomes stemming from maternal separation (Lehmann & Feldon, 2000). Several of these reports have indicated the absence of any discernible behavioral effects (Lehmann et al., 1999). In response to concerns regarding the unspecified nature of maternal separation as a manipulation, researchers have devised an alternative approach known as the limited bedding/nesting model (Brunson et al., 2005; Cui et al., 2006; Ivy et al., 2008; Rice et al., 2008). In its most extreme form, this model involves housing mother rodents in cages with wire mesh flooring devoid of bedding material and nesting resources. Variations of this model include simply restricting the availability of nesting material (Walker et al., 2017). Consequently, this leads to heightened maternal anxiety and disrupted caregiving behavior, sometimes bordering on abusive actions towards the pups (Rice et al., 2008).

Similar to earlier studies employing the MS, some investigations using this limited bedding/nesting manipulation have reported increased anxiety- and depressive-like behavior in adulthood (Orso et al., 2020), supporting its translational relevance in understanding these conditions. Furthermore, numerous studies have endeavored to replicate human prenatal stress using gestational stress (GS) models involving techniques such as restrain stress and forced swimming during specific days after the dams become pregnant (Darnaudéry et al., 2004). These investigations have consistently revealed that such stressors are associated with diminished learning and spatial memory retention in the offspring (Reincke & Hanganu-Opatz, 2017).

While there have been numerous studies exploring the impact of NMDA antagonists such as phencyclidine (PCP), dizocilipine (MK-801), and ketamine in inducing schizophrenia-like symptoms in rodent models (Becker et al., 2003; Hunt et al., 2015; Lisek et al., 2017), there is

a notable absence of research specifically modeling the effects of schizophrenia like symptoms from dams (parents) to pups (offspring) in rodents or even the impact of normal dams on inducing schizophrenia like symptoms in pups. In modelling schizophrenia like symptoms in rats, ketamine is capable of inducing positive, negative, and cognitive symptoms in the rodents, through neurotransmitter disturbances, and other characteristic features of the illness which are classical to human schizophrenia (Frohlich & Van Horn, 2014). In fact, a plethora of preclinical studies in rodents have indicated that ketamine injections, whether acute or sub-chronic, result in memory impairments in rodents (Białoń et al., 2020; Cao et al., 2021; Kozela et al., 2020). Similar results have been reported in human studies (Ranganathan et al., 2017). Ketamine has been observed to hinder spontaneous alterations related to spatial working memory and learning (Ben-Azu et al., 2018; Oshodi et al., 2021). Furthermore, ketamine can induce anxiety-like behavior (Azimi Sanavi et al., 2022; Wąsik et al., 2019), modify social behavior (Pitsikas et al., 2022; Xu et al., 2019; Zoupa et al., 2019), trigger hyper locomotion (Gruca et al., 2011)(Riehl et al., 2011), increase in anxiety behavior and impair sensorimotor gating (Damazio Pacheco et al., 2019)(Fujikawa et al., 2021) in rats . Ketamine therefore, demonstrates construct validity, face validity, and predictive validity in its application in inducing schizophrenia like symptoms in rats as shown in figure 2 below. Thus, this model mimics all behavioral disturbances observed in patients with schizophrenia.

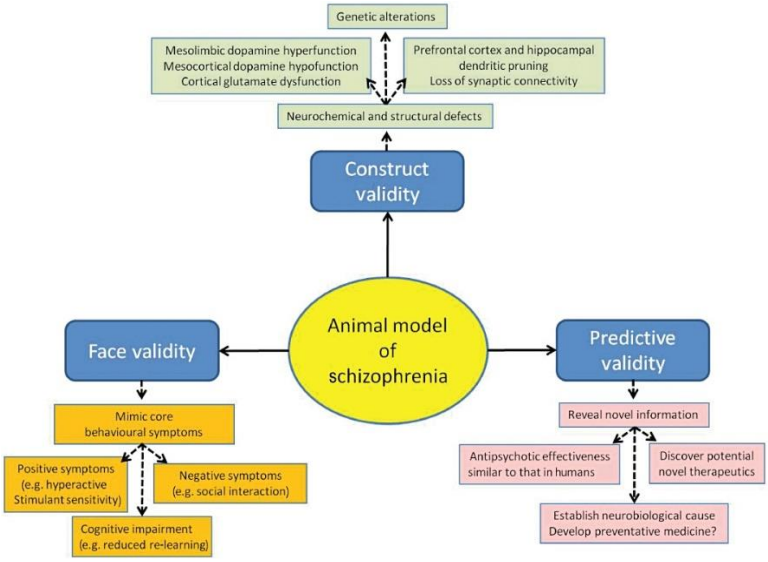

Figure 1-0-2: Illustration depicting the anticipated behavioral, neurochemical, and structural alterations believed to correspond with the three primary symptom domains of schizophrenia within an animal model mimicking the disorder (Jones et al., 2011).

Therefore, this current research is novel in its approach, as it aims to create a novel rodent model to study the consequences of parental schizophrenia, shedding light on this aspect of

human disease. However, there are limitations to using rat models for parental psychopathology. Rats may not fully capture the complexity of human behaviors and psychiatric conditions. The translation of findings from rats to humans requires caution, as species differences exist.

1.6 Immune System and Inflammation on Psychopathology

The immune system plays a pivotal role in the body's defense mechanisms and contributes significantly to maintaining homeostasis. There is a burgeoning interest in understanding the etiological and prognostic implications of the immune system in mental disorders. Previous research indicates the presence of immune response dysregulation and a pro-inflammatory state in individuals with mental disorders (Gibney & Drexhage, 2013). Additionally, neuropsychiatric symptoms are elevated in patients with autoimmune diseases or those undergoing immune-related treatments (Najjar et al., 2013; Shin & Kim, 2023). The intricate relationship between the immune system and psychopathology, specifically in the context of ELS, is a multifaceted area of investigation. ELS can induce profound changes in the immune system, triggering a cascade of inflammatory responses (Soria et al., 2018). This phenomenon is of particular interest due to its potential implications for psychopathology. ELS may lead to immune dysregulation, characterized by alterations in immune cell populations and cytokine production. Two critical ratios, the Neutrophil-Lymphocyte Ratio (NLR) and the Monocyte-Lymphocyte Ratio (MLR), serve as indicators of systemic inflammation and can be affected by ELS. The NLR and MLR are hematological indices that provide insights into the balance between pro-inflammatory and anti-inflammatory responses in the body (Brinn & Stone, 2020). An elevated NLR suggests an increased neutrophilic response, often associated with inflammation, while an elevated MLR indicates heightened monocyte activity (Bhikram & Sandor, 2022). Both ratios have been implicated in various psychiatric conditions and may serve as potential markers of psychopathology risk (Fahiem & Mekkawy, 2022; Gennaro Mazza et al., 2018). Molecular genetic advances have revealed links between immune genes, including those involved in regulating the inflammatory response, and the risk of mental illnesses like schizophrenia and bipolar disorders (Peterson, 2020). The pathogenesis of ICD remains poorly understood. The discovery of biomarkers that can be used as predictors or diagnostics of treatment response in patients presenting with ICD will be a critical step in developing a personalized treatment, which will aid in developing the new treatment. A recent study hypothesized an association between inflammation and its contribution to the etiopathogenesis of ADHD by measuring cytokines and found cytokine gene polymorphisms in ADHD patients. Interleukin-1 (IL-1) and interleukin-6 (IL-6) are pro-inflammatory

cytokines produced by immune cells, including monocytes and neutrophils (Anand et al., 2017; Dursun et al., 2021). ELS can stimulate the production of these cytokines, contributing to a state of chronic inflammation.

Elevated levels of IL-1 and IL-6 have been linked to mood disorders, cognitive impairments, and the pathogenesis of various psychiatric conditions (DiSabato et al., 2016). Furthermore, dysregulation of tissue remodelling enzymes such as Matrix Metalloproteinase-9 (MMP-9) can contribute to excessive synaptic pruning and neuronal damage (Gross et al., 2022; Yabluchanskiy et al., 2013). Astrocytes are actively involved in regulating this neuro-inflammatory response. Astrocytes are critical for maintaining synaptic plasticity, which underlies learning and memory processes. Neuroinflammation, driven by alterations in the immune system and inflammatory markers, can disrupt the finely tuned balance between synaptic strengthening and weakening, potentially leading to cognitive deficits, as shown in Figure 2 (Kwon & Koh, 2020; Liberman et al., 2019). These disruptions can manifest as impairments in cognitive function and memory formation. The exact mechanisms underlying these effects are an active area of research, as understanding these processes is essential for developing targeted interventions to mitigate cognitive and memory deficits associated with neuro-inflammation.

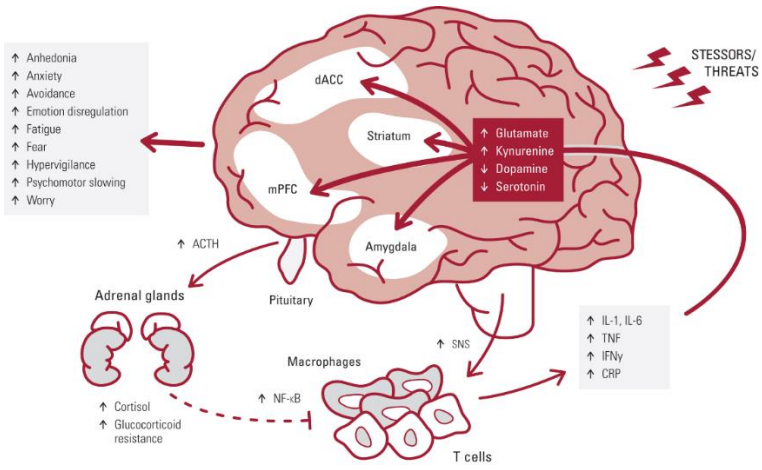

Figure 1-0-3: Impact of Stress on Release of Inflammatory Biomarkers and Activation of the Stress Response System Affecting the Hippocampus (Ravi et al., 2021).

This schematic diagram illustrates the cascade of events triggered by stress exposure. Stress leads to the release of inflammatory biomarkers, such as interleukins and MMP-9, which initiate immune responses in the body. Concurrently, activation of the stress response system, including the Hypothalamic-Pituitary-Adrenal (HPA) axis, occurs. These physiological responses have significant effects on the hippocampus, a brain region crucial for memory and cognitive functions.

1.8 Summary and Research Gap

Early life stress and parental psychopathology are considered important risk factors for children's functioning and have been consistently linked with externalizing problems, internalizing problems, and social skills in children and adolescents (Breaux et al., 2014; Dougherty et al., 2013). ICD is the most common mental health problem among school-going children and adolescents (Gorzny, 2016; Grant et al., 2010; Schulte-Körne, 2016). Due to the high poverty index in Africa, prenatal and postnatal stress levels pose an immense potential risk to children's mental health. Furthermore, the current body of research provides valuable insights into the impacts of ELS and parental schizophrenia on cognitive and behavioral outcomes. Nevertheless, there remains a significant research gap concerning the interactive effects of these factors. Given the multifaceted and complex nature of the impact of ELS and parental schizophrenia, it is crucial to elucidate how they may act synergistically or additively in the developing of ICD-like conditions. This study aims to address this research gap by employing a rat model, allowing for controlled investigations into the interactive effects of ELS and parental schizophrenia on motor, memory, and learning aspects, ultimately shedding light on the effects of inflammatory markers on psychopathology and cognition.

1.9 Research Objectives and Questions

Study aims: To assess the distinct effects of Early Life Stress and Schizophrenia on motor, memory, and cognitive functioning in rat models.

Specific Objectives

1. To examine the influence of prenatal stress on attention, motor skills, and cognition in a rat model.
2. To investigate the impact of maternal separation on both motor abilities and cognitive functions in rats.
3. To evaluate the impact of parental psychopathology (KSZ) on motor and cognition in a rat model.
4. To evaluate the effects of early life stress and Parental Psychopathology on inflammatory biomarkers in blood, specifically examining interleukin 1 and 6, Matrix Metalloproteinase-9, and Brain-derived Neurotrophic Factor (BDNF), and to assess their potential as predictors for the onset of psychopathology.
5. To evaluate the influence of early life stress and Parental Psychopathology (KSZ) on Prefrontal Cortex (PFC) and Hippocampal Astrocyte Density.

2.0 Significance and Relevance

Children and adolescents acquire cognitive and social-emotional skills that shape their future early in life, a period in which robust neuroplasticity exists in the brains This helps them in assuming adulthood and dealing with day-to-day stress in society.

The quality of the environment where children and adolescents grow up shapes their well-being and development. Early negative experiences in homes, schools, or digital spaces, such as exposure to violence, the mental illness of a parent or other caregiver, bullying, and poverty, increase the risk of mental illness. This study seeks to

1. Enhance the understanding of early prenatal stress in children's development of mental illness.
2. Interpolate the effects of parental psychopathology on the development of disruptive, impulse control, and conduct disorder.
3. Comprehend the maternal effects of schizophrenia on the children's learning and memory.
4. Enhance mental health discussion in Africa, as most countries do not have mental health policies.
5. Investigating the intricate interplay between early stress and schizophrenia in the development of impulse control disorders is of paramount importance. This research delves into a field with limited knowledge, potentially advanci understanding of the etiology and mechanisms underlying these disorders. By employing a rat model, this study bridges the gap between animal and human research, offering insights that can be translated into clinical settings.

2.1 Rationale for Animal Model (Rat Model)

Sprague-Dawley rats offer an invaluable model for studying human health, with over 98 percent of their DNA closely mirroring that of humans (Pan et al., 2018). These biological similarities extend to the susceptibility to a range of health issues, including stress, cancer, diabetes, and heart disease. This unique parallel allows researchers to delve into the impact of stress on various biomarkers using Sprague-Dawley rats, shedding light on potential correlations and mechanisms relevant to human health. Rats exhibit behavior resembling human motor skills and cognitive processes, making them ideal for controlled experiments. Additionally, rat models offer insights into the neurobiological mechanisms underlying these impacts, allowing for the examining genetic and environmental factors. The controlled and reproducible experimental environment provided by rat models enhances research reliability while addressing ethical concerns related to human studies. Ultimately, findings from rodent

studies hold translational potential, offering opportunities to understanding neurobiological mechanism and developing therapeutic interventions for psychiatric disorders in humans.

2.2 Brief overview of methodology and study design

Established laboratory methods and protocols were strictly adhered to accomplish the study objectives, as illustrated in chapters 2, 3, and 4 of this dissertation. All animal experimentation was carried out with the approval of the UKZN Animal Research Ethics Committee (AREC/00003119/2021) following the guidelines outlined by the National Institute of Health for the care and use of laboratory animals in South Africa.

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CHAPTER TWO

Chapter two is a research article titled The Impact of Early Life Stress and Schizophrenia on Motor and Cognitive Functioning: An Experimental Study. This article is published on Frontiers in Integrative Neuroscience Journal Learning and Memory Special issue 2023. *Integr. Neurosci. Volume 17 - 2023 | doi: 10.3389/fnint.2023.1251387*

Abstract

The Impact of Early Life Stress and Schizophrenia Like Behaviours on Motor and Cognitive Functioning: An Experimental Study

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Background: Early life stress (ELS) and parental psychopathology, such as schizophrenia (SZ), have been associated with altered neurobiological and behavioral outcomes later in life. Previous studies have investigated the effects of ELS and parental SZ on various aspects of behavior, however by combining these stressors aimed to explore their interactive effects, as individuals in real-life situations may experience multiple stressors simultaneously.

Objective: The aim of this study was to investigate the impact of ELS and ketamine induced schizophrenia like symptoms (KSZ) on locomotor activity, anxiety-like behavior, exploratory tendencies and spatial memory in Sprague Dawley (SD) rats.

Methods: Male and female SD pups were randomly assigned to eight groups: control, ELS, schizophrenia, and ELS + schizophrenia. ELS was induced by prenatal stress (maternal stress) and maternal separation (MS) during the first two weeks of life, while SZ like symptoms was induced by subcutaneous administration of ketamine. Behavioral tests included an open field test (OFT) for motor abilities and Morris water maze (MWM) for cognitive abilities. ANOVA and post hoc Tukey tests were utilized to analyze the data.

Results: This study results show that ELS and parental psychopathology had enduring effects on SZ symptoms, particularly psychomotor retardation ($p < 0.05$). The OFT, revealed increased anxiety-like behavior in the ELS group ($p = 0.023$) and the parental psychopathology group ($p=0.017$) compared to controls. The combined ELS and parental psychopathology group exhibited the highest anxiety-like behavior ($p=0.006$). The MWM analysis indicated impaired spatial memory in the ELS group ($p=0.012$) and the combined ELS and parental psychopathology group ($p=0.003$) compared to controls. Significantly, the exposure to ELS

resulted in a decrease in the population of glial fibrillary acidic protein-positive (GFAP+) astrocytes. However, this effect was reversed by positive parental mental health.

Conclusion: These study findings highlight the interactive effects of ELS and parental psychopathology on anxiety-like behavior and spatial memory in rats. ELS was linked to increased anxiety-like behavior, while SZ was associated with anhedonia-like behavior. Positive parenting augment neuroplasticity, synaptic function, and overall cognitive capacities.

Keywords: Anxiety like behaviours; Cognitive; Early life stress; Motor; Parental mental illness; Psychopathology; Schizophrenia-like symptoms; Spatial memory task, Learning; Spatial navigation

1. Introduction

Stress is the body's response to various internal or external stimuli, often stressors that disrupt the organism's physiological equilibrium or homeostasis (Chrousos, 1992; McEwen. et al., 2020). When faced with a stressor, the body activates its stress response system, which triggers a cascade of physiological and hormonal changes (Chrousos, 2009). This response is primarily regulated by the Hypothalamic-Pituitary-Adrenal (HPA) axis and the sympathetic-adrenal-medullary (SAM) system (Kuhlman et al., 2018; Stults-Kolehmainen et al., 2014).

ELS encompasses detrimental occurrences or difficult situations that occur during the initial stages of development, commonly in childhood or infancy, that have the potential to disrupt the physical, emotional, or social well-being of the child for example maltreatment, neglect, separation, parental loss, extreme poverty, starvation, domestic/community/school violence (Saleptsi et al., 2004). Importantly, the impact of ELS extends beyond the immediate context. Recent studies have revealed that intricate cognitive and emotional functions linked to brain regions with extended postnatal development are particularly at risk due to ELS. Lastly, enduring deficits, particularly in emotional realms, persist long after ELS and could heighten the chances of future psychopathology. Considering this, the utilization of MS, GS, and chronic unpredictable stress (CUS) as models for early-life stress in rodent research has gained considerable traction. These models unveil that progeny subjected to varying stressors within this paradigm tend to exhibit submissive and passive stress responses during their adult phases (Abramova et al., 2021; Cabrera et al., 1999; Gardner et al., 2005; C. A. Wilson et al., 2013). Early life stress has consistent effects on neural development. It disrupts the functioning of crucial systems like the HPA, autonomic nervous system (ANS), and immune system (Alkon et al., 2014; Danese & Lewis, 2017; Koss & Gunnar, 2018), which are essential for shaping

psychological and behavioral responses to environmental challenges (McEwen, 2019). These effects are rooted in changes within neural circuits associated with stress response, including the prefrontal cortex, hippocampus, amygdala, and striatal circuits (Fareri & Tottenham, 2016; Mcewen & Mcewen, 2017). The hippocampus is integral to the formation, consolidation, and retrieval of memories. While the prefrontal cortex is involved in higher-order cognitive functions, including attention, executive function, decision-making, and working memory (Preston & Eichenbaum, 2013; Weilbächer & Gluth, 2017). In schizophrenia, structural abnormalities, such as reduced hippocampal volume, have been consistently observed. These structural changes can impair various aspects of memory, including episodic memory (memory for specific events or experiences) and spatial memory (Ledoux et al., 2014).

SZ is a prominent neuropsychiatric disorder, marked by cognitive, emotional, and perceptual disruptions that profoundly affect individuals' thoughts, behaviors, and overall functioning. Its intricate origins involve genetic, neurobiological, environmental, and psychosocial factors (Faden, 2019; Pan, 2022). Numerous candidate genes related to neurotransmitter regulation, synaptic plasticity, and neurodevelopment contribute to its complexity. Notably, disturbances in dopaminergic signaling, particularly within the mesolimbic pathway, correlate with positive symptoms like hallucinations and delusions. The interplay of these factors underscores the multifaceted nature of the disorder (Konopaske & Coyle, 2022; Plomin & Daniels, 1987; Zamanpoor, 2020). Conversely, parental psychopathology such as SZ refers to cases where children are born to parents that experience this mental disorder. In these situations, the offspring may have an increased risk of inheriting a genetic predisposition for SZ. Additionally, environmental factors and the quality of parental care may also play a role in influencing the child's development and susceptibility to mental disorder later in life (Bakoula et al., 2009; Breaux et al., 2014; Doyle, 2016). Research on ELS in rodents has provided valuable insights into the underlying mechanisms of stress-related disorders in humans (Alfonso & Schulze, 2021; Phillips & Roth, 2019; Roth, 2012).

ELS represents a critical phase in an individual's developmental journey, with the potential to significantly impact motor and cognitive functioning. This developmental window is characterized by heightened neuroplasticity and vulnerability, rendering it a pivotal period for shaping cognitive trajectories (Pechtel & Pizzagalli, 2011). Astrocytes, integral components within the tripartite synapse structure, assume pivotal roles in the regulation of synaptic transmission dynamics. Empirical evidence suggests astrocyte involvement in psychiatric disorders (Bender et al., 2016; Cotter et al., 2001), exemplified by altered morphology in mood disorders and their role in anxiety-like behavior through amygdala activation. The

hippocampus, central to anxiety, features granule and CA1 pyramidal cells linked to anxiolytic effects (Jimenez et al., 2018). While neural circuits implicate hippocampus in anxiety behaviors, the contribution of hippocampal astrocytes remains unexplored.

The objective of this study was to utilize a rat model to investigate the impact of early life stress and parental psychopathology on motor and cognitive functioning. The rationale for combining early life stress and parental psychopathology in this rat model study is to simulate the multifaceted nature of human experiences and examine potential interactive effects between these stressors on memory, motor and learning. This investigation aimed to evaluate the impact of ELS and KSZ on motor, spatial memory, cognitive function, and hippocampal astrocyte density and processes intersections, utilizing behavioral assays such as the OFT and MWM.

2. Materials and Methods

I. Experimental Animals and Treatment

Sprague Dawley (SD) strain are widely recognized for their susceptibility to a range of health ailments, such as stress, cancer, diabetes, and cardiovascular disorders, which closely mirror human conditions (Brower et al., 2015). In alignment with this motivation, a total of twenty-four SD rats, comprising of sixteen nulliparous females in the fertile phase and eight stud male, were procured from the Biomedical Resource Unit (BRU) breeding centre at the University of KwaZulu-Nata (UKZN). The animals were bred and raised within the controlled environment of the Animal House, located at the UKZN's BRU. For this study, a pure breed of the SD strain was used to ensure consistency and uniformity in the experimental cohort. All animals were subjected to standard laboratory conditions, including being housed in pairs within standard cages (29 cm by 22 cm by 14 cm). The temperature was maintained at a constant level, and a 12-hour dark photoperiod was implemented. The rats were provided with unrestricted access to both food and water ad libitum. The present study adhered to ethical guidelines and obtained prior approval before commencing the research. The study protocol was assigned the reference number AREC/00003119/2021.

II. Animal Housing and Surgery

The study involved mating a total of sixteen nulliparous females in the fertile phase and eight proven stud male rats to obtain 64 pups for the experiments. After the animals were collected from the BRU, the females were paired in cages for one week to acclimatize and minimize stress, and to synchronize their oestrous cycles. The stud males were individually housed for one week prior to the mating to build up sperm count and maximize their fertility. Vaginal

smears were taken to assess their oestrous cycle, and when they were at proestrus, two nulliparous females were transferred into a stud male cage. On the subsequent morning, the researchers examined the presence or absence of an ejaculatory plug within the vaginal cavity, as outlined in the study conducted by Paccola and colleagues in 2013 (Paccola et al., 2013). Upon the discovery of a plug, the female was promptly confined within a separate cage, and subsequently labeled with a designated breeding date. The initiation of the plug formation was designated as the starting point of gestation, denoted as day one. After mating, males were removed from the cages and euthanized to avoid stalk contamination.

III. Groupings and Stressors

The study consisted of eight distinct groups, each comprising of eight pups. On the day of delivery 4 pups were randomly selected from each dam and labeled accordingly for proper follow up, this was to increase the translatability and generalizability to human disease. However, female rats often experience hormonal fluctuations during their estrous cycle, than can introduce variability in behavioral and also neurochemical responses compared to male rats (Simpson & Kelly, 2012). The distribution of these groups and the corresponding number of pups are presented in Table 1. Dams and any additional were pups euthanized post weaning.

a) Gestational Stress (Maternal restrainer stress)

To mimic human prenatal stress pregnant dams were subjected to daily GS induction from days 15 to 18. The experimental procedure consisted of subjecting the pregnant dams to a stress protocol, which entailed confining them within transparent cylindrical restrainers (8-10 cm in diameter and 20-24 cm in length) for 45 minutes (Murmu et al., 2006). This confinement took place in a well-illuminated environment maintained at a temperature range of 21-22°C. The dimensions of the restrainers were modified in accordance with the body mass of the laboratory rats throughout the gestational phase of the experimental investigation, which was anticipated to span a duration of approximately 21 to 23 days.

Table 1:Experimental Design and Behavioral Test in ELS. The tables outline different groups and the specific stressors the animals were exposed to.

GROUPS	Number of pups (n)	Treatment Conditions	STRESSORS
Control	8	No stressor	No stressor
Prenatal maternal stress + ketamine injected pups (Group 1)	8	Early Life Stress	Restrainer maternal stress and ketamine injection on the pups PND-7,9,11 & 15.
Prenatal maternal stress (Group 2)	8	Early Life Stress	Restrainer maternal stress
Maternally separated pups (Group 4)	8	Early Life Stress	Maternal separation for 3 hours from PND-1 to PND- 14
Maternal separated + ketamine injected pups (Group 5)	8	Early Life Stress	Maternal separation for 3 hours from PND-1 to PND- 14 & ketamine injection on the pups PND-7,9,11 & 15.
ketamine injected parent (Group 6)	8	Parental Psychopathology	ketamine was be injected to the parents on PND-2,4 to 6
ketamine parent + ketamine Pups (Group 7)	8	Early Life Stress + Parental Psychopathology	ketamine was be injected to the parents on PND-2,4 to 6 & ketamine pups.
ketamine (Positive control) (Group 8)	8	Early Life Stress	ketamine injection on the pups PND-7,9,11 & 15.

b) Maternal and Childs Psychopathology

This study examined the association between parental psychopathology and the likelihood of passing down mental health issues. Offspring susceptibility to disorders arises from a mix of genetic, environmental, and psychosocial factors (McKinney & Stearns, 2021; Sorcher et al., 2022). Moreover, even though the impact of parental mental illness (ELS) on child's development is often the primary end point for most research (Al-Amin et al., 2016; Kamis, 2021), little attention is paid on impact of positive parenting on childhood psychiatric disorders. The researchers therefore modelled this phenomena using group 8 (positive control) as per table 1. Schizophrenia like symptoms was induced in dams from post-natal day 1 to 7. The dams and their offspring were grouped per table 1 conditions. We utilized ketamine, an N-

methyl-D-aspartate (NMDA) receptor antagonist, which mimics cognitive, positive, and negative symptoms of SZ (Kocsis et al., 2013; Neill et al., 2010). Dams received (30 mg/kg, ip) daily basis for five consecutive days while the control group received saline (0.5 ml/kg, i.p) (Chan et al., 2008), and pups were injected with ketamine (16 mg/kg, subcutaneously) three times a week (on Mondays, Wednesdays, and Fridays). The animals were weighed on a daily basis and the does were calculated per body weight for both the dams and pups. This treatment protocol was initiated on postnatal day 1 and continued until postnatal day 14. The selection of dosage, route of administration, and injection schedule were determined through a comprehensive analysis of prior scientific investigations that have reported the induction of psychotic-like alterations subsequent to a 5-day regimen of ketamine treatment (Koh et al., 2016; Lisek et al., 2017; Schobel et al., 2013). On postnatal day 21 the pups were weaned while the maternal subjects were subjected to euthanasia. The animals were left undisturbed until postnatal day 60, at which point they were subjected to behavioral testing.

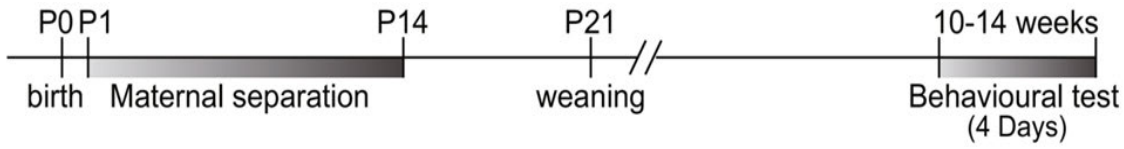

Figure 2-0-1: Timeline of Maternal Separation, Weaning, and Behavioral Test Schedule in ELS Study

c) Maternal Separation (MS)

MS is research paradigm that is frequently employed in animal studies to examine the impact of ELS and the lack of MC on the development of offspring. According to a recent study conducted by Mejja and colleagues in 2021 (Mejía-Chávez et al., 2021), it was observed that MS has the potential to disrupt the attachment bond between the mother and offspring. To study MS effects, we separated dams from their offspring for 3 hours daily (9 a.m. to 12 p.m.) from postnatal day 1 to 14. The dams were relocated from their original cages. The pups were subsequently removed from the experimental rooms and relocated to a distinct room (to establish a barrier to communication between the offspring and their respective mothers).

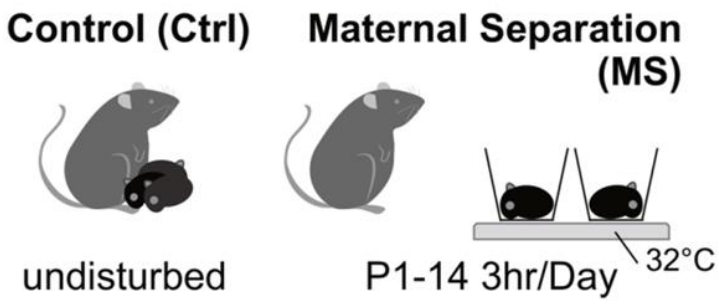

Figure 2-0-2: Illustration of the Maternal Separation Paradigm in Early Life Stress Study.

Following a period of 3 hours (9 a.m. to 12 p.m.), the pups were reintroduced to the designated animal room, and the dams returned to their respective home cages (Kestering-Ferreira et al., 2021; Vetulani, 2013). Figure 2 presents a visual representation showcasing the MS paradigm utilized in the investigation of ELS (figure 1).

IV. Behavioural Test

a) Open Field Test

The OFT is a valuable and widely used behavioral assay, that is fundamental for assessing various aspects of rodent behavior, offering insights into multiple psychological and physiological dimensions (Kraeuter et al., 2019b; Seibenhener & Wooten, 2015). In this test, the animal's spontaneous locomotor activity and exploratory behavior are measured in a novel open arena. The experiment used a 100 cm x 100 cm rectangular enclosure with 40 cm high plywood walls. This box had a clean, opaque plastic floor, divided into 25 even squares by a white grid. A 60 W bulb, strategically located 100 cm above the enclosure's center, served as the main light source. Before introducing each animal, the apparatus was cleaned using a 10% cider vinegar solution. The rat was strategically positioned within one of the corners of the designated apparatus. Subsequently, their behavioral patterns and activities were observed and recorded for 5 minutes. The following parameters were measured; i.e. the total distance traveled (cm), the number of entries to the center of the field, serving as an indicator of the rat’s exploratory behavior and willingness to venture into unfamiliar territory. Furthermore, we measured the time spent in the center of the field (seconds), allowing for anxiety evaluation. The experimental methodology described herein has been previously employed in various scientific investigations (Gould et al., 2009; Võikar & Stanford, 2023).

b) Morris water maze (MWM)

Spatial learning and memory cognitive abilities of the rats were evaluated at postnatal day 60 through the utilization of the MWM paradigm (Barnhart et al., 2015; Nunez, 2008; Vorhees & Williams, 2006). The MWM entailed subjecting the laboratory rats to a pool filled with water that had a milky appearance. The primary objective was for the rats to navigate and locate a submerged escape platform. In this study the researchers utilized a circular pool with a diameter of 2 meters, ’and a depth of 0.5 meters. The water temperature within the pool was maintained at a constant value of 26°C throughout the experiment. The experiment had a training phase and a test phase, during the training phase, the platform was deliberately elevated by a marginal distance of 0.03 meters above the water surface. This was done to effectively direct the rats towards the platform, serving as the designated exit point.

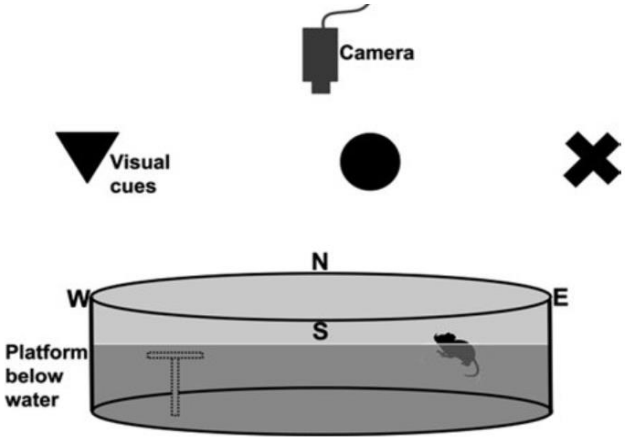

Figure 2-0-3: Illustrates the setup of the Morris Water Maze, which consisted of a circular pool placed in a room with external cues for learning. An overhead camera recorded the rats' swimming paths. To accommodate animals with reduced visual acuity, high-contrast, large shapes served as cues against the background. The transparent platform, depicted as a gray outline, remained invisible to the rats. Starting from north (N), south (S), east (E), and west (W) points, the rats used cues to navigate and find the hidden platform. (Barnhart et al., 2015; Sherrard, 2011).

Following the completion of the training phase, the experimental platform was positioned approximately one inch below the surface of the opaque water. The objective of this experiment was to assess the rats' ability to retain and recall the spatial information associated with the platform's location. Each individual rat was subjected to three consecutive trials; the initial starting positions were randomly assigned as either north, south, east, or west. The rats were placed in water and allowed to = swimming for up to 60 seconds. If the rat failed to locate the platform within 60 seconds, appropriate intervention was promptly administered, ensuring the rat's safety and the time taken was recorded as one minute. After the 3 trials the rat was dried

utilizing a towel (Nunez, 2008; Salman et al., 2019). These parameters measured included the latency to reach the platform, the time spent in the target quadrant, and the swim speed.

V. Sacrifice and Neurochemical Analysis

a) Animal Sacrifice and Tissue Collection

The animals included in this study were subjected to humane care in accordance with the guidelines established by the Institutional Animal Use and Care Committee of the School of Laboratory Medicine and Medical Science at UKZN. The study protocol was assigned the reference number AREC/00003119/2021. Prior to sacrifice, animals were anesthetized using a combination of ketamine and xylazine, inducing a state of deep unconsciousness to minimize any potential distress or pain. Once the desired level of anesthesia was confirmed, transcardial perfusion was performed on 3 animals per group while decapitation procedure was performed in the remaining 5. Sacrifice was done by trained personnel experienced in the procedures, maintaining a focus on precision and compassion. Detailed records of each sacrificing event, including animal identification, date, time, and personnel involved, were documented for regulatory compliance and transparency. The date of sharpening of the guillotine was not more 3 than weeks from the Decapitation date.

b) Transcardial Perfusion Fixation

Twenty-four hours following the conclusion of the behavioral assessment, a cohort of SD rats was injected with ketamine (80 mg/kg, i.p) and xylazine (10 mg/kg, i.p) using a 27-gauge needle and a 1 cc syringe.

Once the anesthesia took effect, the animal was positioned supine, and a thoracotomy was performed to expose the heart. A cannula is then inserted into the left ventricle of the heart to allow for perfusion. The rats were perfused using a solution of phosphate-buffered saline (PBS) and 4% paraformaldehyde (PFA). To optimize perfusion, a minor laceration was created at the caudal aspect of the left ventricle following the exposure of the heart. A perfusion needle (15 gauge) with either a blunt or olive tip, specifically designed for this purpose, was carefully inserted through a surgical incision. The needle was inserted into the ascending aorta. If required, supplementary anesthesia was administered to maintain the rats at an optimal level of surgical anesthesia. The brain was harvested and then separation of the PFC and the Hippocampus, before storage in liquid nitrogen.

c) Decapitation, Post fixation, Tissue Collection and Storage

At postnatal day 60, the SD rats were anesthetized using combination of ketamine and xylazine in accordance with their body weight. The head of each rat was separated from the body using a sharp guillotine. Blood samples were collected from the jugular vein and stored in vacutainers for further analysis. To expose the skull, a midline incision was made along the skin from the neck to the nose, and the neck muscles were trimmed to reveal the base of the skull. An identical incision was made on the opposite side, extending to the posterior edge of the skull surface.

Subsequently, the brain was carefully removed from the skull The brain was then placed in a vial containing fixative solution, ensuring that the volume of the fixative was at least 10 times greater than the brain's size. The vial was intermittently swirled while kept in the fixative solution for 24 hours at a temperature of 4 °C. The brain was rinsed with phosphate-buffered saline through three media exchanges, with swirling performed after each exchange. The harvested hippocampal astrocytes were stored in phosphate-buffered saline with sodium azide and kept at a temperature of 4 °C for further use. (van Rijn et al., 2011).

d) Immunohistochemistry and Immunofluorescence Analysis

i. Tissue preparation

Following euthanasia, the rats were subjected to transcardial perfusion using a solution of 0.1 M phosphate buffer (PB) with a volume of 50 ml. This was subsequently followed by perfusion with a fixative solution composed of 4% paraformaldehyde in 0.1 M PBS (pH 7.4), with a total volume of 100 ml. The fixative solution was maintained at a low temperature, specifically ice-cold, to ensure optimal preservation of the samples. The cerebral organs were meticulously removed from the cranium, followed by their exposure to a subsequent fixation procedure within the identical solution used for initial fixation, spanning a time period of 3 hours.

To acquire coronal sections of the brain that encompassed the hippocampus, we employed a vibratome (model VT1200, manufactured by Leica). The following sections were preserved in a 0.1 M PB solution supplemented with 0.1% sodium azide for the purpose of immunofluorescence labeling. The sections had a thickness of 60 micrometers. Alternatively, some sections were stored in a 0.1 M PB solution without any additives for the purpose of intracellular fills, with a section thickness of 100 micrometers.

ii. Cresyl Violet Staining and Volume Estimation

Cresyl violet was used as a staining technique to effectively visualize and demarcate the boundaries of the hippocampus within the examined sections. An anatomical microscope with

a magnification of 1.25× was used to determine the number of grid points within the hippocampus, as depicted in Figure 2-4.

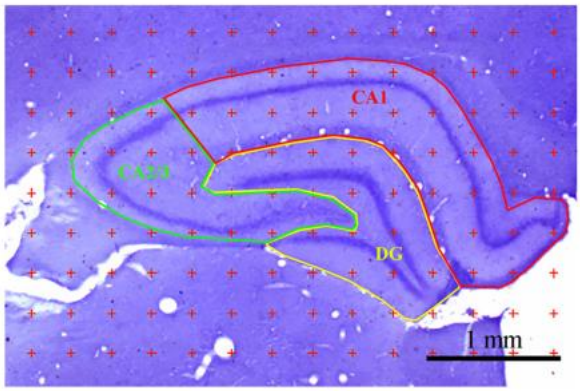

Figure 2-0-4: Illustration depicting the stereological method employed for hippocampal volume estimation. Grid points were counted across the entire hippocampus, Scale bar = 1 mm.

The estimation of the total volume of the hippocampus was conducted using the Cavalieri's principle, as described by (Gundersen et al., 1988; Von Bartheld, 2002). In this principle, the volume (V) of either the hippocampus or its sub regions was determined. The thickness (t) of the tissue block used for analysis was set at 0.6 mm. Each grid point was associated with an area (a(p)) of 0.09 mm2. The total number of grid points (ΣP) present in the hippocampus per rat was calculated.

iii. Immuno-Histochemical Labeling

The tissue slices were subjected to a sequential washing protocol, which comprised three consecutive 10-minute washes in a 0.1 M PB solution supplemented with 0.5% Triton X-100 (TX). The washing process was followed by a blocking step (3 hours) using PB solution, which consisted of 2% normal goat serum (NGS), 3% bovine serum albumin, and 0.3% Triton X-100 (TX). Tissue was then incubated for 48 hours at 4°C. During this incubation, a mouse monoclonal antibody (MAB360, Millipore) specifically designed to target GFAP was utilized. The antibody was diluted to a ratio of 1:10,000 in a working buffer solution composed of PB containing 2% NGS (normal goat serum) and 0.3% TX (Triton X). Following the incubation period, the sections were subjected to a series of three 10-minute washes in the working buffer, then transferred to a working buffer maintained at a temperature of 25°C. The working buffer contained a 1:500 dilution of goat anti-mouse IgG conjugated to Alexa Fluor 633 (Inqaba Biotechnical SA). After a 3-hour incubation period, the sections were subjected to a subsequent 15-minute incubation step at 25°C in a working buffer solution that was prepared by diluting

4',6-diamidino-2-phenylindole (Sigma) at a ratio of 1:2000. The sections were washed and then carefully mounted in Prolong Gold antifade reagent.

Astrocytes and principal neurons were identified using a confocal microscope, which relies on the distinct characteristics of their soma in terms of size and shape. Immunofluorescence labeling was used to detect the presence of the astroglial marker S100b. To achieve this, free-floating sections were subjected to an overnight incubation with a rabbit anti-S100b antibody (Switzerland), at a dilution of 1:400. Tissue was then incubated for 1 hour with 20 mg/ml carbocyanine (Cy) 2-conjugated donkey anti-rabbit IgG (Dianova). To inhibit the unoccupied binding sites of Cy2-anti-rabbit IgG, the sections were incubated for 3-hour with 50% rabbit antiserum. The sections were subsequently subjected to an overnight incubation with 10 mg/ml rabbit anti-GFAP antibodies. The sections were subjected to a 1-hour incubation period with Cy3-anti-digoxin (20 mg/ml; Dianova) to detect the presence of GFAP immune-reactivity.

After stereological analysis, further quantification of GFAP+ astrocytes were performed using ImageJ software. High-resolution confocal images of tissue sections were captured, and regions of interest containing GFAP-labeled cells were selected for analysis. Using ImageJ, individual astrocytes were identified and counted based on their specific fluorescence signal intensity and morphology. The number of GFAP+ cells within each region of interest was quantified using automated cell counting algorithms available in the software, ensuring accurate and reproducible measurements (Olude et al., 2015).

iv. Astrocytes Density and Processes Counting

High-quality digital images of the stained tissue sections are acquired using a microscope equipped with a camera. The acquired images are imported into ImageJ, an open-source image analysis software. Using ImageJ, astrocytes stained positive for the GFAP marker are identified and counted manually or using automated image analysis algorithms.High-resolution images capturing astrocyte processes within the hippocampus were processed to enhance contrast and highlight the intricate nature of these structures. The thresholding technique was applied to distinguish astrocyte processes from the background, and subsequent skeletonization reduced them to a one-pixel width for improved visibility. The "Analyze Skeleton" tool in ImageJ was employed to conduct an intersection analysis, providing quantitative data on the number of intersections within the astrocyte processes. Parameters such as "Prune Cycle" were adjusted for optimal identification. Manual validation of the results was performed to ensure accuracy, and the outcomes were documented, including the average number of intersections per astrocyte or per specified area. This rigorous approach aimed to provide a quantitative

assessment of astrocyte process intersections, contributing to a comprehensive understanding of their distribution and morphology within the studied hippocampal region.

VI. Data Analysis and Statistical Measures

All statistical analysis was performed in Paleontological statistics software Package (PAST-4) and the significance level was set at $p < 0.05$. Results were expressed as mean ± SEM. To address the issue of multiple comparisons and to control the familywise error rate, the adjusted significance level was set at $p < 0.0018$. Comparisons within the groups on behavioural test and astrocytes were done by one-way ANOVA. Power analysis was conducted to test for the effect size in results. Furthermore, after the ANOVA result if the results indicated a significant overall effect we performed a Post hoc Tukey's test to further analyze and compare the means of different groups. Image-J was used to quantify hippocampal astrocytes density (HAD) and the processes intersections. The data on the effects on cognition, memory, and motor were recorded and plotted in bar graphs, box plots and radar plots.

3. Results

I. ELS and KSZ Increased Motor on the SD using Open Field Test

a) Results on the Distance Covered

The OFT is an important tool for assessing anxiety-like behavior and locomotor activity in rodents. The analysis of variance conducted (Control, group1, group 2, group 4, group 5, group 6, group 7 & group 8) revealed a significant difference on distance covered ($p < 0.001$), indicating a decrease in motor performance in the stressed groups [control vs group 1 (prenatal stress and KSZ pups) ,$p < 0.001$; control vs group 4 (maternal separated pups-MSP) , $p < 0.001$; control vs group 5 (MSP & schizophrenic parents), $p < 0.001$; control vs group 6 (KSZ parents only), $p < 0.001$, control vs group 7 (KSZ parents and pups),$p < 0.001$; and control vs group 8 (normal parents but SZ pups), $p < .001$]. There was also a significant difference between group 5 and group 6 ($p=0.168$) as well as between group 6 and group 7 ($p=0.119$).

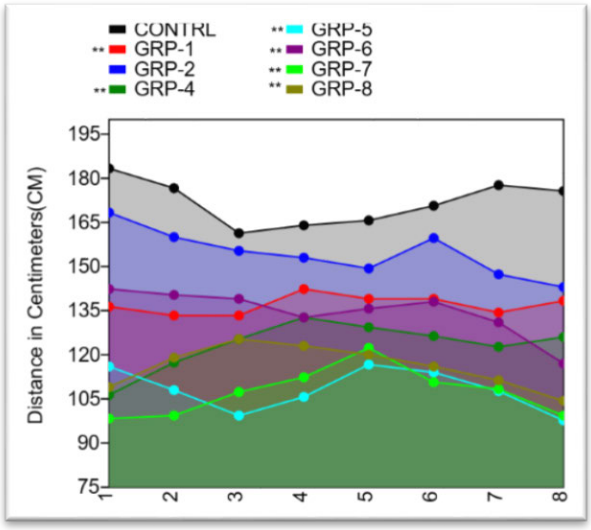

Figure 2-0-5: Distance covered by Sprague Dawley rats in an open field test. The line graph shows the mean distance covered by rats in each of the eight treatment groups plotted against the standard deviation (SD). The distance covered by rats ranged from a minimum of 88 to a maximum of 200 cm. The mean distance covered ranged from 107.25 to 171.875 cm, with a coefficient of variation ranging from 7.04% to 14.00%. Statistical significance was determined using one-way ANOVA, with Bonferroni correction. (*: P value<0.05; **: P value <0.01)

b) Results on Number of Entries into the Center and Peripheral Zones

In order to conduct a comprehensive examination of the effects of ELS on anxiety-like behavior and general locomotor activity, we conducted additional measurements by recording the frequency of entries into both the central and peripheral zones (PZ) of the experimental field. The statistical analysis revealed a significant difference between groups [Control (non-stressed) group vs Groups 1, 4, 5, and 6 ($p < 0.001$), Group 2 and Group 4 ($p > 0.05$), Group 2 vs Group 5 ($p=0.056$). In contrast, the observed data did not reveal any statistically significant distinction between the groups subjected to stress and the group diagnosed with KSZ (Group 8) ($p > 0.05$).

On the time spent in the center zone the analysis revealed significant difference between the control group vs the rest of the groups 1, 4, 5, 6, 7 and 8 ($p < .001$) with the exception of control vs group 1($p < 0.05$). Interestingly, there was a significant difference between Group 7 and group 8, ($p < 0.001$), Group 6 vs Group 7 ($p < .001$), as well as Group 5 vs Group 6 and Group 8 ($p < 0.001$).

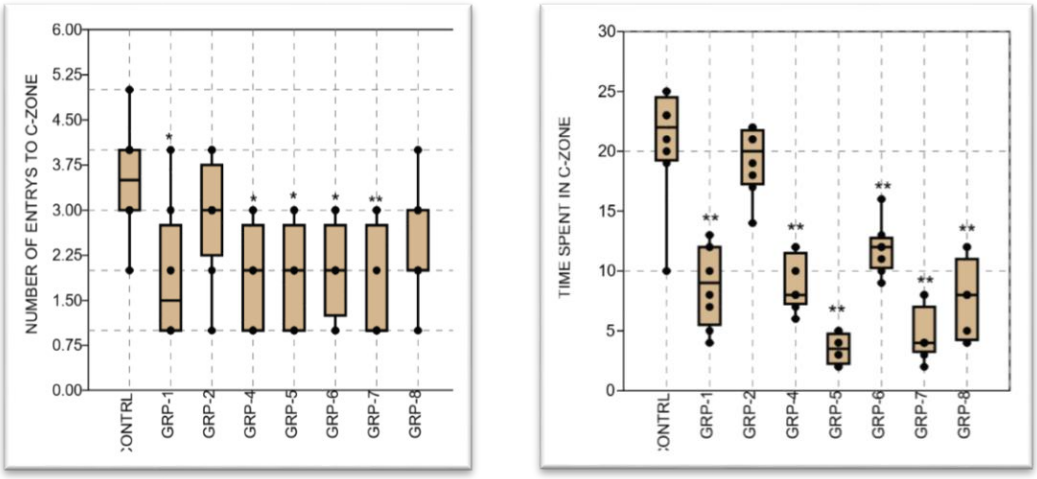

Figure 2-0-6: (A) The graph represents the mean number of entries to the centre of the OFT by eight groups of animals: a control group and groups 1-8, respectively. The control group had a mean value of 3.5 entries, while groups 1-8 had mean values ranging from 1.875 to 2.875 entries. The graph (B) figure displays the mean time spent at the centre, along with the standard error of the mean (SEM) The control group spent the most time at the centre with a mean of 20.75 minutes, while the experimental groups had lower mean times ranging from 8.875 to 4.5 minutes. Statistical significance was determined using one-way ANOVA, with Bonferroni correction. (*: P <0.05; **: P <0.01)

Furthermore, the ANOVA on the number of entries into the PZ revealed significant difference in the means of the groups ($p < 0.001$). There were significant differences in the number of entries to the PZ between the control (non-stressed) and the rest of the groups i.e. Control Vs groups 1 ($p < .001$); group 2 ($p=0.018$); group 4; group 5; group 6; group 7; group 8 ($p < .001$). Group 2 was significantly different from group 6 ($p=0.017$) and group 7 ($p=0.033$).

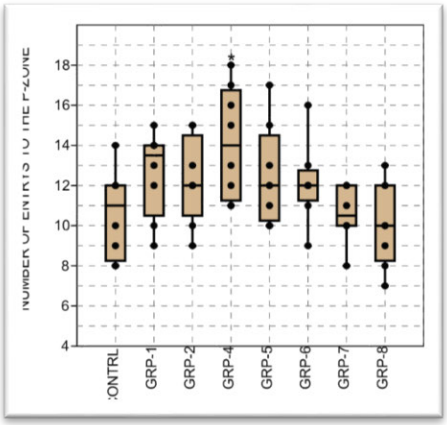

Figure 2-0-7: The figure represents the results of the OFT assessing the number of entries to the peripheral zone in different groups. The data shows a significant difference in the sample medians ($H = 14.51$, $p = 0.035$). Statistical significance was determined using one-way ANOVA, with Bonferroni correction. (*: P <0.05; **: P <0.01)

II. Early Life Stress impairs Learning, Spatial Memory and Cognition (MWM)

The present study aimed at investigating the impact of ELS and SZ on spatial learning and memory in the MWM paradigm. During the acquisition phase, a series of repeated measures were conducted to gather data and analyze the effects of the time taken to find the platform. The ANOVA conducted demonstrated a statistically significant effect of trial ($F(5, 60) = 8.41$, $p < 0.001$), suggesting that there was a noticeable improvement in performance across the different trials. The rats exhibited a notable reduction in the time taken to locate the concealed platform and traversed a shorter distance during repeated trials. These findings provide evidence for the acquisition of learning and spatial memory, as depicted in Figure 9. In the subsequent probe trial, a one-sample t-test was conducted to examine the animals' time spent in the target quadrant. The results indicated a statistically significant difference, as the animals spent a significantly higher proportion of time in the target quadrant compared to chance levels ($t(15) = 4.62$, $p < 0.001$ $\omega^2=0.235$). Furthermore, the observed animals demonstrated a notably higher frequency of traversals over the designated platform area ($t(15) = 3.24$, $p < 0.01$), suggesting a spatial inclination towards the quadrant containing the target.

In order to evaluate the extent of memory retention, a memory recall trial was executed to compare the performance across eight distinct groups. These study findings indicate that there were no statistically significant variations in the means observed between the different groups on day 1 ($p=0.9683$). Significant variations in the means were observed among the groups on day 2 ($F=15.65$, $df=99.21$, $p < 0.001$ $\omega^2=0.635$). There were significant differences in the duration required to locate the platform on day 2 and day 3 among the various experimental groups [control group vs Group 1 ($p < 0.001$), control vs Group 2 ($p=0.036$), control vs Group 4 ($p<.001$), control vs Group 5 ($p=0.197$), control vs Group 6 ($p < 0.001$), and control vs Group 7 ($p < 0.001$). However, there was no difference between Group 8 the control group ($p > 0.05$).

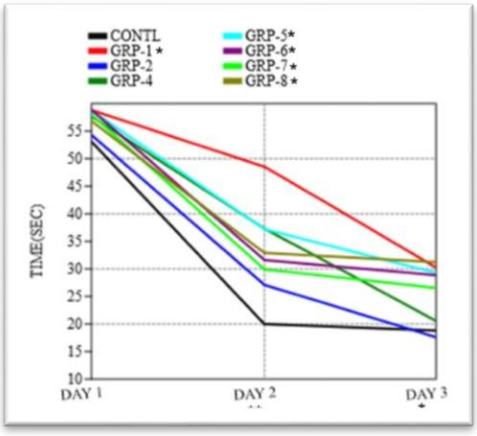

Figure 2-0-8: Time taken to find the platform in the Morris Water Maze test. The graph shows the mean time taken (in seconds) for rats to find the platform in the water maze. The ANOVA showed significant difference between the groups ($F(7,16) = 0.2404$, $p = 0.9683$). Statistical significance was determined using one-way ANOVA, with Bonferroni correction. (*: $P < 0.05$; **: $P < 0.01$)

In the course of the probe trial, there were no statistically significant disparities observed in the duration of time spent in the target quadrant across the various groups ($F(2,24) = 2.332$, $p > 0.05$; Fig. 9). Furthermore, there were no statistically significant variations in swimming speed observed among the eight experimental groups ($F(2,24) = 3.077$, $p > 0.05$) during the initial day of the training period. The analysis of the data indicated that there was no statistically significant variation in the average distances traveled by the different groups on the first day ($F(7,16) = 0.1249$, $p = 0.995$ $\omega^2 = 0.1348$). In the course of the experiment, it was observed that there existed a substantial disparity in the distance to the platform between the control group and various experimental groups on both days two and day three [group 1 ($p < 0.001$), group 2 ($p < 0.001$), group 4 ($p < 0.001$), group 5, group 6, group 7, and group 8 ($p < .001$)]. These findings indicate a significant divergence in the distance to the platform measurements between the control group and the aforementioned experimental groups. The results of the experiment indicate that there was no statistically significant difference in the time taken to find the platform among group 4, group 5, and group 6 ($p > 0.05$). However, group 8 exhibited a significant difference in the time taken to find the platform compared to Group 7 ($p = 0.007$).

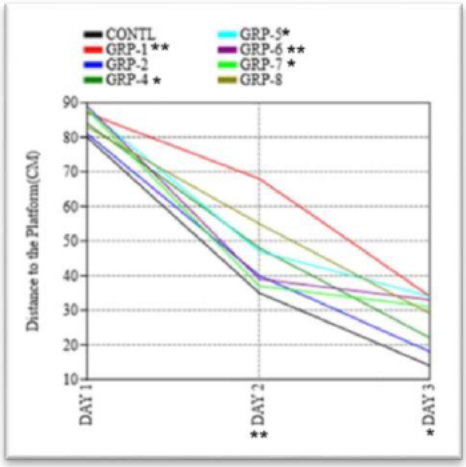

Figure 2-0-9: The figure shows the results of the Morris Water Maze experiment on the distance traveled by rats to find the hidden platform. Statistical significance was determined using one-way ANOVA, with Bonferroni correction. (*: P value<0.05; **: P value <0.01)

III. Early Life Stress Induced Hippocampal Astrocytes Volume Loss

We used the sampling scheme in Table 3 to quantify astrocytes density in the hippocampus. The table includes the number of animals in each group, the sampling method employed (random), the number of sections sampled, the section thickness in micrometers, the number of counting frames sampled, and the number of GFAP+ cells sampled.

Table 2: Sampling Scheme and GFAP+ Cell Analysis in the Hippocampus

Group	Number of Sections Sampled	Section thickness (µm)	Number of counting frames sampled	Total Number of GFAP+ Cells	Sampling Method
Control	8	24.41± 1.55	4	3506 ± 64	Random
Group 1	8	24.41± 1.55	5	2231 ± 44	Random
Group 2	8	24.41± 1.55	4	2920±27	Random
Group 4	8	24.41± 1.55	5	2186±34	Random
Group 5	8	24.41± 1.55	4	1359±35	Random
Group 6	8	24.41± 1.55	5	2126±60	Random
Group 7	4	24.41± 1.55	4	1210±23	Random
Group 8	8	24.41± 1.55	4	1593±55	Random

Section thickness is represented as mean ± standard deviation (SD), whereas the number of counting frames and the numbers of sampled GFAP+ cells are represented as mean with range in parentheses.

The present study aimed to investigate the potential variations in the density of GFAP+ Astrocytes in the hippocampus across different experimental groups. To address the issue of multiple comparisons with 8 groups, we applied the Bonferroni correction method, dividing

the desired significance level (alpha) by the number of comparisons performed. The adjusted alpha level for each individual post hoc test was set at 0.05 / 28 (8 groups choose 2), resulting in an adjusted significance level of approximately $p < 0.0018$. These study findings revealed a statistically significant disparity between the groups ($F(7, 56) = 36.75$, $p < 0.001$ $\omega^2 = 0.7963$), indicating a substantial effect size. This finding suggests that a significant proportion, specifically 79.63%, of the observed variations in HAD can be ascribed to the distinctions among the groups subjected to ELS and those with parental SZ. The post hoc analysis revealed statistically significant differences ($p < 0.05$) among the means of all groups, with the exception of the comparisons between groups 1 and 4 ($p = 1$), groups 1 and 6 ($p = 0.999$), and groups 5 and 7 ($p = 0.002$). There was a significant difference between Group 7 and Group 8 ($p=0.043$).

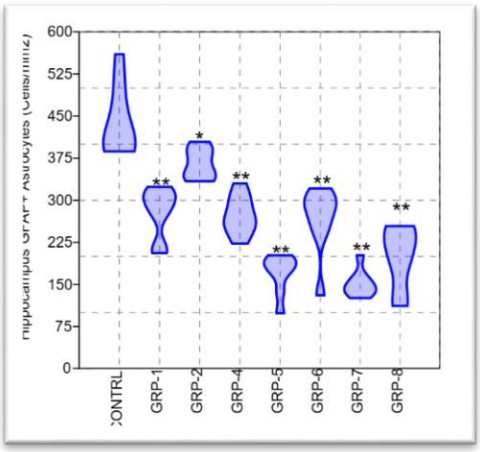

Figure 2-0-10: Violin plot of the astrocyte density in the hippocampus for 8 treatment groups. Statistical analysis revealed significant differences between the groups in both mean and median density ($F(7, 56) = 36.75$, $p < 0.001$; $Hc = 52.03$, $p < 0.001$). Statistical significance was determined using one-way ANOVA, with Bonferroni correction. (*: P value<0.05; **: P value <0.01)

a) Hippocampal Astrocytes Processes Intersections (Lateral and Central)

The analysis conducted on the lateral processes revealed significant findings on the test for equal means. This study results indicate that the control group, exhibited statistically significant differences when compared to the other groups [Group 1 ($p < 0.001$), group 2 ($p=0.031$), group 4 ($p < 0.001$), group 5 ($p < 0.001$), group 6 ($p < 0.001$), group 7 ($p < 0.001$), and group 8 ($p < 0.001$)]. There was no significant difference between group 7 and group 8 ($p=0.004$).

The present study also investigated the central processes intersection and its implications. The obtained results from the ANOVA indicated a statistically significant effect ($F(7,56) = 4.675$, $p < 0.001$, $\omega^2 = 0.3804$). The results of the post hoc test comparisons indicate that there are

significant differences in means between the control group (non-stressed) and groups 1, 2, 4, 5, 6, 7, and 8, $p < 0.001$.

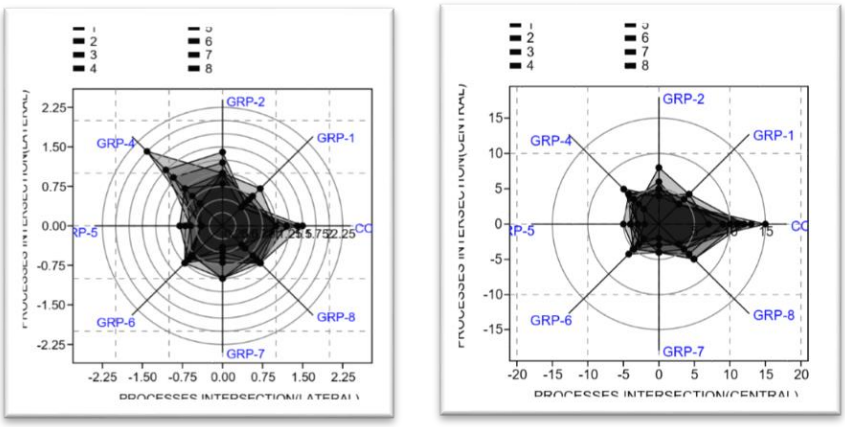

Figure 2-0-11: Analysis of Astrocyte Processes Intersection. (A) Radar Chart, showing the Lateral process. (B) Radar Chart, Showing the Central Process. Two radar chart show the mean length of lateral processes, central processes, and total processes in primary astrocytes. The total length of astrocytic processes was the sum of lateral and central processes and had a mean length of 0.6 μm.

Finally, on the total processes' intersection, we compared means of 8 different groups. Between groups, a significant difference was observed, with $F(7, 56) = 8.714$, $p < 0.001 \omega^2 = 0.458$). A significant difference was observed between most groups, except between groups 1 and groups 5 (p=0.998), 6 (p=1),7 (p=0.625) and 8 (p=1). Finally, we performed a two-sample t-test to compare the means between group 7 and group 8. The results of the t-test revealed a t-value of 1.9042, with a corresponding (p=0.077). Considering we obtained (p=0.077), which is close to the significance threshold, there is marginal evidence to suggest a significant difference between the means of the groups on the total processes' intersections.

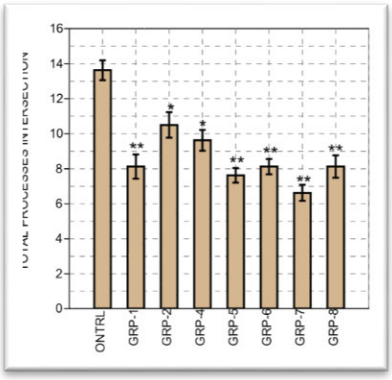

Figure 2-0-12: The bar graph illustrates the total astrocyte intersection processes across eight groups, accompanied by standard errors. Each bar represents the mean value of astrocyte intersection processes, while the error bars indicate the standard errors. The group labels are displayed on the x-axis, and the y-axis represents the total number of astrocyte intersection processes. Statistical significance was determined using one-way ANOVA, with Bonferroni correction. (*: P <0.05; **: P <0.01)

4. Discussion

This study explored the impact of ELS (MS & GS) and KSZ on dams or their interactive nature on locomotor activity, anxiety-like behavior, exploratory tendencies and spatial memory. The study aimed to unravel the complex relationships between these stressors and cognitive outcomes, and also the potential underlying mechanisms. The experimental groups included the control group 1 (non-stressed), group 2 (prenatal stress and KSZ pups), group 3 (prenatal stress), group 4 (MS), group 5 (MS and SZ parents), group 6 (KSZ parents), group 7 (KSZ parents and pups), and group 8 (KSZ pups only). Varied ELS, including MS and GS, exerted distinct effects on SD, resulting in a marked elevation in anxiety-like behavior and a concurrent decrease in locomotor activity. Moreover, spatial memory capabilities exhibited a notable reduction in response to these stressors. In the context of KSZ in dams, heightened anxiety-like behavior and evident psychomotor retardation were observed, along with a concomitant decline in spatial memory function. Interestingly, the influence of standard maternal care emerged to have a reversal effect anxiety-like responses, coupled with an enhancement of motor activity and spatial memory performance, became apparent.

The OFT serves as a valuable tool to assess anxiety-like behavior and locomotor (Seibenhener & Wooten, 2015). In this present study significant differences were observed in the locomotion using OFT indicating reduced motor performance across the groups. These findings suggest that both ELS and parental psychopathology independently contribute to motor deficits in the rat model ($p < 0.05$), with group 2-prenatal stress (GS) exhibiting the least pronounced impact on psychomotor functioning. Also, on the OFT the researchers investigated the differences in

time spent at the center zone among various groups, which is an indicator of the animal's willingness to explore the environment and its anxiety behaviour. A statistically significant difference ($p < 0.05$) was observed between the control group and the remaining groups (ELS and KSZ). Specifically, KSZ pups raised by KSZ dams (group 7) exhibited increased anxiety and reduced exploratory behaviour compared to the KSZ pups that had normal parents (group 8) ($p = 0.046$). This suggests that good parenting (normal parents) may have a reversal effect on anxiety-like behavior and psychomotor retardation in the offspring affected by SZ. The findings imply that the environment and care provided by normal parents may play a crucial role in mitigating anxiety-related responses and improving motor behavior in the context of ELS and parental psychopathology. These findings are similar to previous research in humans that reported that children develop and regulate their emotions when their parents are sensitive and supportive of them. At the same time, it is seen that a greater level of internalizing symptoms like sadness, anxiety, and loneliness, and externalizing symptoms like overreacting, poor impulse control, non-compliance, aggression, and poor relationships with friends are seen in children who do not get proper parenting. It was also noticed that internalizing and externalizing symptoms and problematic peer relationships are associated with harsh, aggressive, or intrusive parenting (Bradley & Corwyn, 2008; Zvara et al., 2018). Furthermore, the researchers observed that groups subjected to differential MS and KSZ (groups 5, 6, and 7) displayed a significantly reduced inclination towards exploratory behavior. The present findings suggest that ELS and parental psychopathology, specifically SZ, exert a lasting influence on the emergence of anxiety and depressive like behaviors later in life.

Previous research has highlighted the impact of ELS such as MS and CUS on motor activity and anxiety-like behavior in rodents (Alves et al., 2022; Rana et al., 2015). For instance study by Rana and colleagues found that rats exposed to MS showed an increase in anxiety- and depressive-like behaviors, leading to decreased exploration in the OFT, which is consistent with this study findings (Rana et al., 2015). In addition, numerous studies have also demonstrated MS and CUS can lead to alterations in the HPA axis and the limbic system, resulting in heightened anxiety-like behaviors and changes in locomotor activity (Orso et al., 2020;Bodegom et al., 2017;Walker et al., 2018). To be specific Van and colleagues reported that ELS induced by prenatal stressors, MS, or the limited nesting model can have significant consequences on the function of the HPA axis in adulthood (Van Bodegom et al., 2017). The effects of ELS on the HPA-axis function are dependent on various factors, including the developmental time window of exposure, the sex of the offspring, and the developmental stage at which effects are assessed. Furthermore, ELS results in HPA-axis hyper-reactivity in

adulthood, similar to what is observed in major depression human studies. This provides empirical evidence on the pathophysiology of anxiety and psychomotor retardation as observed this study. This study results are in line with these studies; hence they reinforce the validity of the rat model for investigating the behavioral consequences of ELS.

As far as our understanding goes, there has not been published studies on modeling the impact of KSZ in rodents. However, over the past few years, a growing body of evidence has lent support to the notion that the malfunctioning of the glutamatergic system constitutes a fundamental pathological alteration observed in SZ. Consequently, the inhibition of the NMDA receptor through the use of non-competitive antagonists like ketamine or PCP triggers the emergence of delusions and hallucinations in individuals who are otherwise in a healthy state, mirroring the symptomatic profile frequently associated with KSZ model in rodents (Kapur & Seeman, 2002; Krystal et al., 1994). Nevertheless, comparisons drawn from unidirectional studies in humans have revealed effects on offspring later in life linked to parental SZ. Children from SZ parents have a higher likelihood of developing specific psychiatric disorders such as anxiety, depression and cognitive impairments during their lifetime (Steenhoff et al., 2021; van der Pol et al., 2016; Weijers et al., 2018). Findings from Vandeeleur and colleagues indicated that offspring of individuals with Bipolar Disorder (BPD) and Major Depressive Disorder (MDD) have elevated rates of mood and anxiety disorders compared to control groups (Vandeleur et al., 2012). This is further supported by Rasic and colleagues who reported that offspring of parents with severe mental illness (SMI) are at increased risk for a range of psychiatric disorders and one third of them may develop a SMI by early adulthood (Rasic et al., 2014).

The MWM test was employed to assess spatial memory and cognitive functioning in the different experimental groups. During the acquisition phase, the researchers observed significant differences in the performance of the experimental groups compared to the control group 1 (non-stressed). The stressed groups showed prolonged latencies and increased path lengths compared to the control group, indicating impaired spatial learning). GS and KSZ pups, exhibited the most substantial impairment in spatial learning ($p < 0.001$) compared to the control group. This finding suggests that the combination of prenatal stress and SZ leads to more severe cognitive deficits in the offspring. Similarly, groups that were subjected to prenatal stress, MS, MS with KSZ parents, and KSZ pups only, respectively, also displayed significant impairments in spatial learning compared to the control group ($p < 0.001$). These results indicate that ELS, irrespective of the presence of parental psychopathology, is sufficient to impair spatial learning.

To assess spatial memory retention, a probe trial was conducted on the third day, during which the platform was removed from the pool. The control group showed a significant preference for the target quadrant, spending more time ($p < 0.001$) and making more crossings ($p < 0.001$) in this area compared to other quadrants. In contrast, all stressed groups displayed reduced preference for the target quadrant, spending significantly less time ($p < 0.001$) and making fewer crossings ($p < 0.001$) in this region. Parental KSZ with KSZ pups (Group 7) exhibited the most severe deficits in spatial memory retention, displaying minimal preference for the target quadrant compared to the other stressed groups. These results indicate that differential ELS and parental psychopathology impair spatial memory retention in the rat model. To assess cognitive flexibility and adaptability, a reversal learning task was conducted. The platform was moved to the opposite quadrant, and the rats were trained to locate the new platform location over three consecutive days. The stressed groups (group 1, group 4, group 5, group 6, and group 8) showed significant deficits in reversal learning compared to the control group ($p < 0.001$). Group 7 again exhibited the most profound cognitive inflexibility, as indicated by significantly longer latencies ($p < 0.001$) and path lengths ($p < 0.001$) compared to the control group. These results suggest that ELS and parental psychopathology not only impair initial spatial learning but also hinder the rats' ability to adapt and relearn a new platform location during reversal learning.

In the realm of rodent behavior and cognitive tasks, mnemonic functions play a crucial role, encompassing both long-term and short-term working memory components. Working memory is believed to depend on sustained neural activity within an active network, while long-term memory and synaptic plasticity are instrumental in shaping the synaptic structure, thereby influencing the spectrum of potential states in memory processing (Sheynikhovich et al., 2023). The deficits observed during the reversal learning task suggest that ELS and parental psychopathology may impair cognitive flexibility and adaptability. The reduced ability to learn a new platform location indicates potential alterations in the rats' executive functions and prefrontal cortex functioning, which are crucial for cognitive flexibility (Piao et al., 2017). Overall, the findings from the MWM provide compelling evidence of the adverse effects of ELS and parental psychopathology or PTSD on spatial learning, memory, and cognitive flexibility in the rat model. These results align with previous research, indicating that ELS can lead to hippocampal dysfunction and alterations in synaptic plasticity, ultimately contributing to cognitive deficits (Floresco et al., 2009; Popescu et al., 2023). As with any scientific study, this research has some limitations that merit consideration. The use of the rat model, was advantageous for controlled experiments, although it does not fully represent the complexity

of human experiences and psychopathology. Additionally, the choice of specific behavioral tests may not fully capture all aspects of cognitive functioning.

The neurochemical analysis aimed to quantify the density of GFAP+ astrocytes in the hippocampus across the different experimental groups. Astrocytes are a crucial glial cell type involved in maintaining neural homeostasis and supporting cognitive functions. The findings from the neurochemical analysis revealed a statistically significant disparity in HAD among the different experimental groups. This substantial effect size can be attributed to the distinctions among the groups subjected to different ELS and those with KSZ parents. These findings indicate that ELS and KSZ independently influence hippocampal astrocyte density. The hippocampus is a brain region crucial for memory formation and spatial learning (Kemp et al., 2022). It is involved in the consolidation and retrieval of spatial memories, processes that were found to be impaired in the behavioral results. Astrocytes play a vital role in synaptic plasticity, neurotransmitter regulation, and maintenance of the brain's microenvironment (Kim et al., 2019; Marathe et al., 2018). The observed reductions in hippocampal astrocyte density in the stressed groups may have implications for hippocampal function. Previous research has shown that chronic stress can lead to astrocyte atrophy and decreased astrocytic processes in the hippocampus, potentially compromising synaptic plasticity and cognitive functioning (Kaplan et al., 2018; B. Li et al., 2022).The hippocampus is also vulnerable to the effects of stress and plays a critical role in emotion regulation and memory processing (Hueston et al., 2017; E. J. Kim et al., 2015). Dysregulation of astrocyte function in the hippocampus may contribute to the cognitive deficits observed in these groups.

In addition to overall astrocyte density, the analysis focused on astrocytic processes, specifically lateral and central processes. Astrocytic processes are essential for synaptic communication and supporting neuronal function (Chiareli et al., 2021). The results showed significant differences in the length of lateral and central processes between the control group and all other experimental groups. The observed alterations in astrocyte density and processes in the hippocampus have potential implications for cognitive function. Astrocytes are known to modulate synaptic transmission, regulate extracellular neurotransmitter levels, and influence synaptic plasticity (Durkee & Araque, 2019; Henneberger & Petzold, 2015). Changes in astrocyte function may affect synaptic communication and compromise the neural circuits responsible for cognitive processes. The hippocampus plays a critical role in spatial learning and memory (Apple et al., 2017; Toda et al., 2019). Reduced astrocyte density and altered astrocytic processes in the hippocampus may contribute to the deficits observed in the MWM (Jahanshahi et al., 2008; Sampedro-Piquero et al., 2014). Impaired astrocyte function could

lead to disruptions in the hippocampal microenvironment and synaptic plasticity, impacting memory formation and spatial learning.

In summary, the research findings indicate a significant association between ELS and KSZ in rats, with notable effects on motor function, memory, learning ability, and the volume of GFAP astrocytes. Henceforth, the findings in this study demonstrate that ELS or KSZ in rats have a negative impact on spatial memory and cognitive abilities. Furthermore, the investigation revealed a significant association between Neuro-Psycho-biological dysregulation and the observed outcomes.

5. Conclusion

In conclusion, this study utilizing a rat model provides significant insights into the impact of early life stress and parental psychopathology on motor and memory cognitive functioning. The results demonstrate that both stressors independently contribute to motor and cognitive deficits in the rat model. The findings highlight the complex and multifaceted nature of the relationships between early life experiences, parental psychopathology, and cognitive development. Importantly, this study introduces a novel perspective by demonstrating that positive parenting interventions can effectively enhance cognitive function and stimulate the hippocampus, contributing to regenerative astrocytes gliosis. Moving forward, further research is warranted to elucidate the underlying molecular mechanisms and to develop effective therapeutic strategies to mitigate the adverse effects of these stressors on cognitive outcomes. Taken together, this novel differential early life manipulation will be able to contribute to a clearer understanding of the negative effects of ELS and parental SZ symptoms on brain health.

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CHAPTER THREE

Chapter three is a research article titled Understanding the Role of Early Life Stress and Schizophrenia on Anxiety and Depressive like Outcomes: An Experimental Study. This manuscript has been submitted to the SAGE-Neuroscience Insights Journal for publication and the pre-print version is published https://dx.doi.org/10.2139/ssrn.4603943. To minimize repetition in the methods section, we make reference to Chapter 2 (Oginga and Mpofana., 2023) where similar procedures have been used.

Abstract

Understanding the Role of Early Life Stress and Schizophrenia on Anxiety and Depressive Like Outcomes: An experimental study

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Background: Adverse experiences due to early life stress (ELS) or parental psychopathology such as schizophrenia (PSZ) have a significant implication on individual susceptibility to psychiatric disorders in the future. However, it is not fully understood how ELS affects social-associated behaviors as well as the developing prefrontal cortex (PFC).

Objective: The aim of this study was to investigate the impact of ELS and ketamine induced schizophrenia like symptoms (KSZ) on anhedonia, social behavior and anxiety-like behavior.

Methods: Male and female Sprague-Dawley rat pups were allocated randomly into eight experimental groups, namely control, gestational stress(GS), GS+KSZ, maternal separation (MS), MS+KSZ pups, KSZ parents, KSZ parents and Pups and KSZ pups only. ELS was induced by subjecting the pups to GS and MS, while schizophrenia like symptoms was induced through subcutaneous administration of ketamine (KSZ). Behavioral assessment included sucrose preference test (SPT) and elevated plus maze (EPM), followed by dopamine testing and analysis of astrocyte density. Statistical analysis involved ANOVA and post hoc Tukey tests, revealing significant group differences and yielding insights into behavioral and neurodevelopmental impacts.

Results: GS, MS, and KSZ (dams) significantly reduced hedonic response and increased anxiety-like responses ($p < 0.05$). Notably, the presence of normal parental mental health demonstrated a reversal of the observed decline in Glial Fibrillary Acidic Protein-positive astrocytes (GFAP+ astrocytes) ($p < 0.05$) and a reduction in anxiety levels, implying its potential protective influence on depressive-like symptoms and PFC astrocyte functionality.

Conclusion: The present study provides empirical evidence supporting the hypothesis that exposure to ELS and KSZ on dams have a significant impact on the on development of anxiety

and depressive like symptoms in Sprague Dawley rats, while positive parenting has a reversal effect.

Keywords: Anhedonia; Anxiety; Astrocytes; Corticosterone; Depressive-like outcomes; Dopamine; Early life stress; Psychopathology; Schizophrenia

1. Introduction

ELS entails a range of detrimental encounters in children such as abuse, neglect, and maltreatment that have profound effects on the brain and mental development, and constitute major risk factors for brain structural changes and adult psychopathology (Agorastos et al., 2019; Pervanidou et al., 2020). It is widely acknowledged that ELS can exert profound and enduring impacts on an individual's emotional and cognitive well-being later in life. For a long time, the precise neural underpinnings of the outcomes stemming from ELS remained inadequately comprehended. However through translational research in rodents (Niwa et al., 2011; Shin et al., 2023), it is postulated that distressing occurrences may induce alterations in the structure of brain regions involved in regulating emotional responses in humans (Krugers & Joël, 2014; Naninck et al., 2015).

In light of this, several studies have shown that rats exposed to MS or GS or chronic unpredictable stress (CUS) exhibit diminished astrocytic volume in the medial prefrontal cortex (Iffland & Neuner, 2020; Poleksic et al., 2021), reduced size of the hippocampus (NCRao et al., 2010; Teicher et al., 2012), heightened activity of the Hypothalamic-Pituitary-Adrenal axis (Finlay et al., 2023; Murphy et al., 2022; Orso et al., 2020), and concomitant impairment of sensory and cognitive functions (Dawes et al., 2015). In a manner akin to the impact of ELS, various parental effects in human such as psychopathology, socioeconomic, and social factors possess the capacity to exert substantial influence on the neurodevelopmental trajectory of children later in life as well (Berg-Nielsen et al., 2002; Harold & Sellers, 2018). In addition, numerous research in humans have established a correlation between parental psychopathology such as SZ and a heightened susceptibility to the development of internalizing and externalizing problems in children (Finsaas & Klein, 2023; McLaughlin et al., 2012; Rusengamihigo et al., 2021) which is seen through disruptive behavior. Schizophrenia stands as a complex neuropsychiatric condition that has a global prevalence of approximately 1% (Charlson et al., 2018; McGrath et al., 2008), moreover its marked by a spectrum of symptoms, encompassing delusions, hallucinations, disorganized speech or conduct, and impaired cognitive function (Fatani et al., 2017; Zhang et al., 2023). Empirical evidence substantiates

the notion that genetic components exert a substantial influence on the development of SZ. Environmental and social factors also potentially contribute to SZ onset, particularly among individuals susceptible to the disorder (Gejman et al., 2010; Shao et al., 2021). Nevertheless, the prevailing body of research predominantly focuses on the isolated examination of these variables and their distinct effects on child psychopathology, behavior, and cognition (Rosello et al., 2021; Slone & Mann, 2016). Building upon this foundation, the current study employs the utilization of GS, MS, and KSZ in rats, exploring their interactive impact on the development of psychiatric illness later in life.

Neurochemical processes intricately govern the complex interplay between emotional and cognitive behaviors within the brain. These processes involve a complex network of neurotransmitters, receptors, and signaling pathways that collectively contribute to the regulation of mood, cognition, and other higher-order functions. Among the various cellular actors involved in these processes, astrocytes have emerged as key players in modulating synaptic activity and maintaining neural homeostasis (Kriegstein & Alvarez-Buylla, 2009; Kucukdereli et al., 2011). Astrocytes assume a pivotal role in normal brain development, hence comprehending the temporal dynamics of their maturation is imperative to understanding how Early Life Adversity (ELA) may influence their lifelong function behaviour (Boghdadi et al., 2020; Yaxing Chen et al., 2020; Eroglu, 2009; Rothhammer & Quintana, 2015). Astrocytic development initiates in the concluding stages of embryonic development and experiences a rapid surge in numbers within the first month after birth. In the hippocampus, the peak generation of astrocytes occurs during the second postnatal week (Catalani et al., 2002; Nixdorf-Bergweiler et al., 1994). In humans astrogenesis is believed to initiate during the final stages of gestation and extends throughout the postnatal period, resulting in a continuous rise in the number of GFAP+ cells across the central nervous system (Menassa & Gomez-Nicola, 2018; Mohn & Koob, 2015; Mottahedin et al., 2017; Patro et al., 2015; Semple et al., 2013).This developmental timeline aligns with early sensitive periods, rendering astrocytes particularly susceptible to the effects of ELS in both humans and rodents. Consequently, it becomes crucial to explore how ELS impacts astrocytes and their potential contribution to ELS-associated brain dysfunction.

In the realm of human health, conditions like depression and anxiety frequently coexist, with impaired social functioning often preceding the onset of these disorders. As a result, the investigation of disrupted social behaviors holds significance in unraveling the intricacies of stress response development. The current investigation endeavors to bridge this existing

knowledge gap by undertaking an empirical inquiry to explore the collective influence of ELS and SZ on the development of anxiety and depressive like symptoms in rats. The rationale for combining ELS and PSZ in this rat model study is to simulate the multifaceted nature of human experiences.

2. Materials and Methods

I. Experimental Animals and Treatment

Twenty-four SD rats, including nulliparous females in the fertile phase and stud males, were sourced from the University of KwaZulu-Natal's Biomedical Resource Unit and mated. They were raised under controlled conditions with unrestricted access to food and water, adhering to ethical guidelines, and the study was granted approval with reference number AREC/00003119/2021.

II. Animal Housing and Surgery

In the study, 16 nulliparous, fertile-phase female rats were mated with 8 stud males to produce 64 pups for experimentation. To synchronize the estrous cycles, the females were acclimatized by being paired in cages for a week, and the males were individually housed to optimize sperm count. The initiation of gestation was marked by the presence of an ejaculatory plug, and males were euthanized after mating to prevent stock contamination. The experimental design and timeline of stressors, including both pre-natal stress and post-natal stress, along with the schedule of behavioral tests, are illustrated in Figure 3.1. This figure provides a visual representation of the chronological order and nature of stressors imposed on the rats, as well as the corresponding behavioral assessments conducted throughout the study. More details regarding the housing of experimental animals have been extensively covered in Chapter Two.

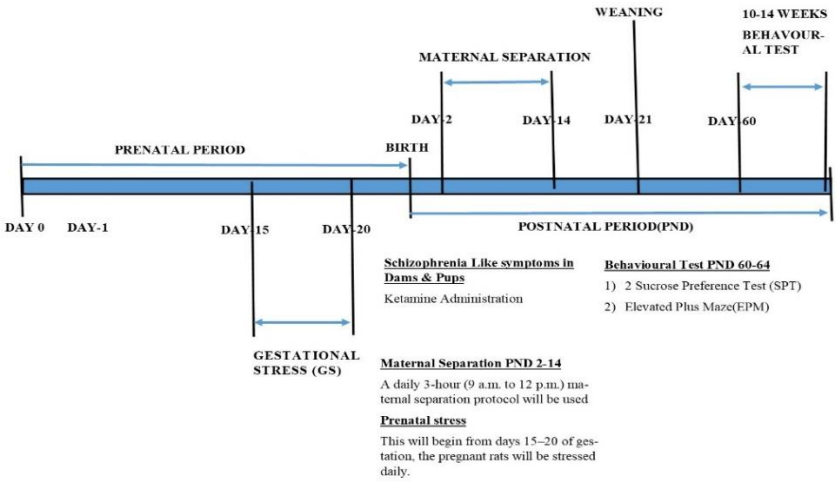

Figure 3-0-1: A schedule of stressors (Pre-natal stress & post-natal stress) and behavioural test.

III. Bedding Change and Urine collection

Regular cleaning and changing of bedding was done weekly from old beddings to new beddings, however on the 3-week interval from week 3 to 15, SD rats were transferred in their respective groups to metabolic cages for urine collection within their experimental rooms during the changing of beddings for a period of 30 minutes. Urine samples were immediately placed in clean, labeled containers for subsequent analysis.

IV. Groupings and Stressors

Rats were randomly assigned to 8 groups: control (non-stressed pups), group 1 (GS + ketamine injected dams), group 2 (GS), group 4 (MS), group 5 (MS + ketamine injected pups, group 6(ketamine injected parent), group 7 (ketamine parent + ketamine Pups) and group 8 [ketamine positive control). The dams and their offspring were grouped per table 1 conditions. We utilized ketamine, an N-methyl-D-aspartate (NMDA) receptor antagonist, which mimics cognitive, positive, and negative symptoms of SZ (Kocsis et al., 2013; Neill et al., 2010). Dams received (30 mg/kg, ip) daily basis for five consecutive days while the control group received saline (0.5 ml/kg, i.p) (Chan et al., 2008), and pups were injected with ketamine (16 mg/kg, subcutaneously) three times a week (on Mondays, Wednesdays, and Fridays). The animals were weighed on a daily basis and the does were calculated per body weight for both the dams and pups. This treatment protocol was initiated on postnatal day 1 and continued until postnatal day 14. The selection of dosage, route of administration, and injection schedule were determined through a comprehensive analysis of prior scientific investigations that have

reported the induction of psychotic-like alterations subsequent to a 5-day regimen of ketamine treatment (Koh et al., 2016; Lisek et al., 2017; Schobel et al., 2013). On postnatal day 21 the pups were weaned while the maternal subjects were subjected to euthanasia. The animals were left undisturbed until postnatal day 60, at which point they were subjected to behavioral testing. A comprehensive discussion of groupings and stressors has been previously presented in an earlier chapter 2 (Oginga and Mpofana., 2023) page 24.

a) Gestational Stress (Maternal restrainer stress)

To simulate human prenatal stress, pregnant dams were subjected to daily restraint stress induction from days 15 to 18. The stress protocol involved confining the pregnant dams in transparent cylindrical restrainers for 45 minutes in a well-illuminated environment with temperature maintenance between 21-22°C, with adjustments in the restrainer dimensions based on the rats' body mass throughout the approximately 21 to 23-day gestational period.

b) Maternal and pups Psychopathology (Parental Schizophrenia)

Dams and pups were injected with ketamine to simulate schizophrenia like symptoms (KSZ). Behavioral testing was conducted on the offspring at postnatal day 60, following a protocol in line with previous studies (Koh et al., 2016; Lisek et al., 2017; Schobel et al., 2013). (Oginga and Mpofana., 2023) page 25.

c) Maternal Separation (MS)

The maternal separation protocol involved daily 3-hour separations from 9 a.m. to 12 p.m. Dams and pups were placed in separate areas to prevent interaction. After the separation, pups were returned to the room, and dams went back to their cage. The light/dark cycle was from 6 a.m. to 6 p.m., with weekly cage cleaning. Weaning occurred at postnatal day 21, and rats were left undisturbed until postnatal day 48 (Begni et al., 2020). Figure 2 visually depicts this separation process.

V. Behavioural Tests

a) Sucrose Preference Test (SPT)

Anhedonia, refers to the inability to derive pleasure from enjoyable activities, is a fundamental symptom of depression observed in humans, rodents can also present with hedonic like response (Markov, 2022; Serchov et al., 2016). Rats were provided with two bottles in their home cages: one containing 5% sucrose solution and the other containing regular water. This setup was initiated on Gestational day 22 for prenatal stress and on Postnatal days 56 to 59 to

test the effects of ELS and KSZ, with a duration of 48 hours (Liu et al., 2018). On the third day, a preference test was conducted by replacing one bottle with plain water while the other bottle still contained the sucrose solution. Prior to the test, the rats' weights were measured, food was provided, and they were left undisturbed in their cages for 24 hours. Afterward, their weights were measured again before the test commenced. Following a 24-hour testing period, the bottles were removed and weighed to determine the sucrose intake. This preference test was conducted over a period of three days (Liu et al., 2018). The preference for sucrose was calculated using the formula: Preference = (sucrose intake/total intake) × 100. This protocol was adapted from (Markov, 2022) and (Eagle et al., 2016).

b) Elevated Plus Maze (EPM)

In the current study, the researchers employed the EPM as a behavioral test to assess anxiety-like behavior in SD. The maze consisted of a plus-shaped platform elevated above the ground with two open arms and two enclosed arms as shown in Figure 3 (Walf & Frye, 2007). Rats naturally exhibit an aversion to open and elevated spaces, making the EPM an effective tool for measuring anxiety-related responses. The rat was placed at the center of the maze facing one of the enclosed arms and allowed to freely explore the maze for 5 minutes. We recorded and analyzed the behavior of the rats during the test.

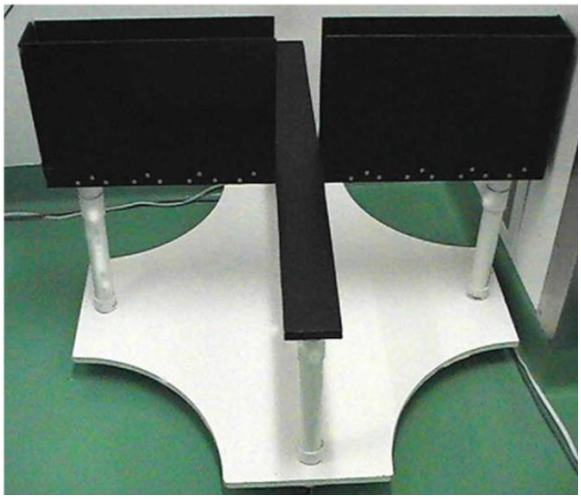

Figure 3-0-2: Picture of the elevated plus maze used for testing rats. The elevated plus maze is placed on the floor on a movable platform with casters (Walf & Frye, 2007).

During the test, we measured parameters such as the time spent in the open arms, number of entries into the open arms, and the latency to enter the open arms as indicators of anxiety-like behavior. Anxiety like behaviour was typically characterized by a reduced exploration of the open arms and an increased preference for the closed arms. Conversely, decreased anxiety was

reflected in the increased level of exploration in the open arms. The EPM has been extensively validated as a reliable and sensitive test for assessing anxiety-like behavior in rodents in previous studies (Walf & Frye, 2007, 2009).

VI. Sacrifice and Neurochemical Analysis

a) Animal Sacrifice and Tissue Collection

Animals were anesthetized with ketamine and xylazine to ensure deep unconsciousness, minimizing distress and pain. Transcardial perfusion or decapitation was then performed by trained personnel. The guillotine blade was sharpened within 3 weeks of use, and animal welfare was prioritized throughout to uphold ethical standards and research integrity.

b) Transcardial Perfusion Fixation

24 hours after behavioral tests were concluded, half of the SD rats were anesthetized with ketamine and xylazine. They were then perfused with PBS and 4% paraformaldehyde (PFA). The perfusion involved making a small incision in the left ventricle, inserting a perfusion needle into the ascending aorta, and removing the sternum and thymus. This procedure aligns with Gage et al. (2012) guidelines, preserving brain tissue for detailed analysis while ensuring blood-free samples for imaging and staining.

c) Decapitation, Post Fixation, Tissue Collection, and Storage

On post-natal day 60, SD rats were anesthetized with combination of ketamine and xylazine and their heads were severed from the body using a guillotine. An incision was made along the neck to expose the skull, and neck muscles were trimmed to reveal the skull base. Blood samples were taken from the jugular vein and stored. The brain was carefully removed, trimmed of any remaining tissue, and placed in fixative solution for 24 hours at 4 °C. Afterward, the brain was washed with phosphate-buffered saline, and the PFC and hippocampus were collected and stored in phosphate-buffered saline or HBHS solution with sodium azide at 4 °C (van Rijn et al., 2011).

d) Corticosterone and Dopamine Levels Quantification

Corticosterone, and dopamine concentrations were measured using ELISA kits. Specifically, we utilized the corticosterone ELISA kit (E-EL-R0269), and dopamine kit (E-EL-R0343) to assess the levels of these hormones in both blood samples and Urine. The ELISA kits provided a quantitative measurement of the concentration of corticosterone and dopamine based on

specific antibody-antigen interactions. These measurements allowed us to examine the levels of these hormones in relation to this experimental conditions.

e) Astrocyte Cell Cultures

i. Tissue preparation:

After the behavioral protocol, animals were anesthetized with ketamine and xylazine overdose and perfused with phosphate buffer and 4% paraformaldehyde fixative (Oginga and Mpofana., 2023). The brain sections, containing the orbi-frontal cortex and dorsal hippocampus, were obtained with a vibratome and stored for analysis.

ii. Cresyl Violet Staining and Volume Estimation:

Cresyl violet stain marked the hippocampus boundaries, and a 1.25× magnification anatomical microscope counted grid points in the hippocampus (Fig. 2-0-4) please see page 31. Cavalieri's principle was employed to estimate the volume of the hippocampus and orbi-frontal cortex, considering tissue block thickness (0.6 mm), area per grid point ($a(p) = 0.09\ mm^2$), and the total grid points (ΣP) in each rat's hippocampus or orbi-frontal cortex (Gundersen et al., 1988; Von Bartheld, 2002).

iii. Immuno-Histochemical Labeling

In this study, the researchers performed immuno-histochemical labeling on tissue slices. The slices were washed with a solution of 0.1 M PB and 0.5% Triton X-100, then blocked them with PB containing 2% normal goat serum, 3% bovine serum albumin, and 0.3% Triton X for 3 hours. Astrocytes were labeled with an anti- GFAP antibody and 4',6-diamidino-2-phenylindole staining. The confocal microscope identified astrocytes and neurons based on size and shape. Astrocyte volume, complexity, and density in the hippocampus were assessed. Regions of interest were defined based on neuron morphology, counted using a stereological system (ZEISS, Germany) at ×2.5 magnification with a 2% area sampling fraction and a 3 μm guard zone. The number of GFAP+ cells was counted over a 15 μm depth (as previously described in chapter 2). Data Analysis and Statistical Measures

Statistical analysis was conducted using PAST-4, with a significance level of $p < 0.05$, but for familywise error control, $p < 0.0018$ was adopted due to multiple comparisons. One-way ANOVA was used for comparisons within groups on behavioral tests and astrocytes, and power analysis assessed effect size. Post hoc Tukey's test was performed if ANOVA indicated a significant effect. Image-J was employed for quantifying hippocampal astrocyte density and process intersections. Results for cognition, memory, and motor effects were presented in bar graphs, box plots, and radar plots. P-values adhered to APA criteria, with three digits after the

comma for values below 0.001 (e.g., $p < 0.001$). A comprehensive procedure of Data analysis has been previously provided in an earlier Chapter 2

3. Results

I. Early Life Stress Amplifies Schizophrenia's Grip on Anhedonia in SD Rats

Anhedonia, a symptom associated with depression, can occur at various stages of the depressive episode (American Psychiatric Association, 2022a). It is characterized by a diminished capacity to experience pleasure or a loss of interest in activities that were previously enjoyable (Treadway & Zald, 2011). To test for anhedonia, we measured the percentage sucrose preference. The analysis of variance between the Control and the treatment groups (group1, group 2, group 4, group 5, group 6, group 7 & group 8) revealed a significant difference on SPT ($p < 0.001$), indicating an increase in anhedonia. Additionally, a student t-test comparison between group 8 (normal dams with KSZ pups) and group 7 (KSZ dams and pups) revealed a significant difference ($p = 0.01$) on SPT.

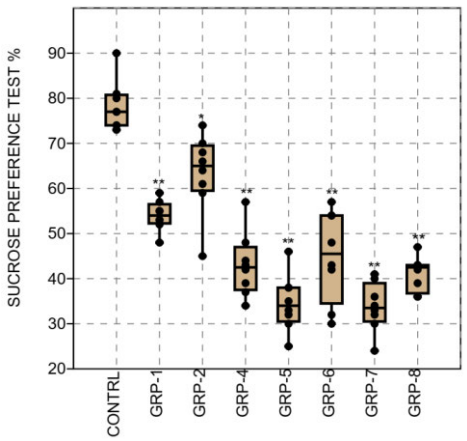

Figure 3-0-3: Percentage Sucrose Preference Test in Different Treatment Groups and Control. The figure presents the results of the percentage sucrose preference test in seven treatment groups compared to the control group. The sucrose preference test was conducted to assess the hedonic like response and reward sensitivity in the different experimental conditions. Statistical significance was determined using one-way ANOVA, with Bonferroni correction. (*: P <0.05; **: P <0.01)

II. Early life stress has a lasting effect of anxiety like response later in life using Elevated plus maze (EPM)

a) Number of entries to the Open Arm and Closed Arm

In the present study the researchers used the EPM to measure anxiety-like behavior in SD rats. EPM takes advantage of the conflict between the rodent's innate fear of open spaces and its motivation to explore novel environments (Kraeuter et al., 2019a; Walf & Frye, 2007). The

statistical analysis revealed no significant difference between the control and the rest of the groups 1, 2, 4, 5, 6, 7 and 8 ($p > 0.05$) on the number of entries to the closed arm. However, group 6 demonstrated a significantly higher number of entries to the closed arm compared to group 4 ($p=0.009$) and group 5 ($p=0.03$).

Interestingly on the number of entries to the open arm, there was a significant difference between the control group vs the other groups i.e. group 1,2,3,4,5,6,7 and8 ($p = 0.429$; $p = 0.339$; $p = 0.015$; $p = 0.02$;1 $p = 0.394$; $p = 0.957$). Moreover, there was also a large effect size.

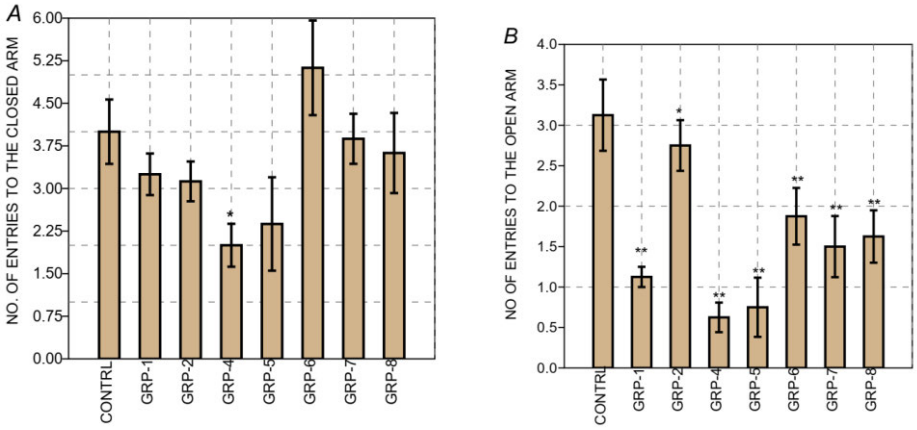

Figure 3-0-4: Number of Entries into the Open and Closed Arms on the Elevated Plus Maze. The results of the Welch F test indicated a significant difference ($p = 0.039$) among the groups, suggesting variations in the measured parameter. Statistical significance was determined using one-way ANOVA, with Bonferroni's correction. (*: $P < 0.05$; **: $P < 0.01$)

b) Time Spent on the Closed Arm and Open Arm

For us to have a comprehensive assessment of anxiety-like behavior that results from the impact of ELS, we further analyzed the time spent on both arms. In this present study we started by analyzing data on the time spent on the closed arm, the groups exposed to ELS and SZ had a significant difference compared to the control ($p=0.05$), group 5 (PSZ and MS) spent the highest time on the closed arm compared to the rest. Additionally, the post hoc analysis revealed that, groups 1,4,5,6,7 and 8 exhibited significant differences compared to the control group.

Finally, on the time spent in the open arm, the post hoc analysis revealed that both the Control ($M = 96.3$, $SD = 4.6$) and Group 2 ($M = 78.8$, $SD = 5.4$) demonstrated significantly higher time spent on the open arm compared to the other Groups ($p < 0.05$), in addition group 8 was slightly significantly different from group 7 ($P=0.04$).

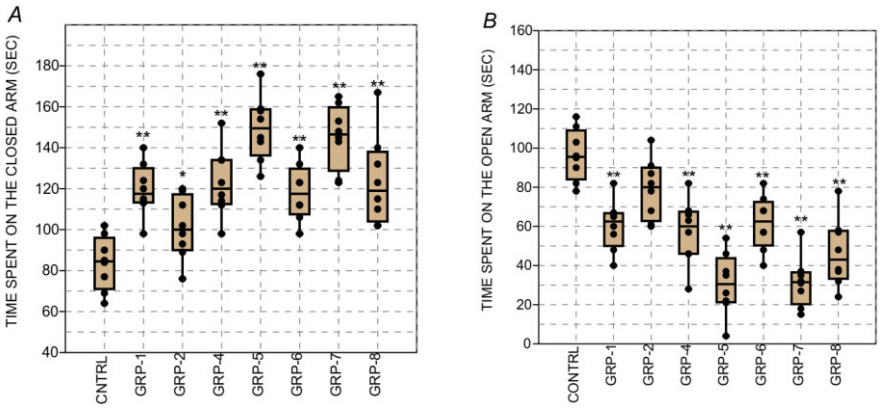

Figure 3-0-5: Box plots illustrating the time spent on the closed arm (Graph A) and time spent on the open arm (Graph B) in the elevated plus maze. Graph A: The boxes indicate the interquartile range (IQR), with the horizontal line inside representing the median. The whiskers extend to the minimum and maximum values, excluding outliers, which are depicted as individual data points beyond the whiskers. Graph B: The box plot in Graph B depicts the distribution of time spent on the open arm across the eight experimental groups. Statistical significance was determined using one-way ANOVA, with Bonferroni's correction. (*: P <0.05; **: P <0.01)

III. Early Life Stress & Parental psychopathology Induced Prefrontal Astrocytes Volume Loss

We examined prefrontal astrocytes density as a potential correlate of the observed behavioral changes. Astrocytes, a type of glial cell in the brain, play a crucial role in the functioning of the prefrontal cortex (PFC) and have been implicated in anxiety and depression (Lu et al., 2016). In addition, these astrocytes have processes and intersections that are essential for maintaining the structural and functional integrity of this brain region. The analysis of variance (ANOVA) revealed significant differences between the control group and each of the experimental groups (MS, GS and KSZ) ($p < 0.001$) moreover with a larger effect size ($\omega^2=0.707$). In addition, a paired student T –test showed that there was also a significant difference between group 7 and group 8 ($p = 0.011$).

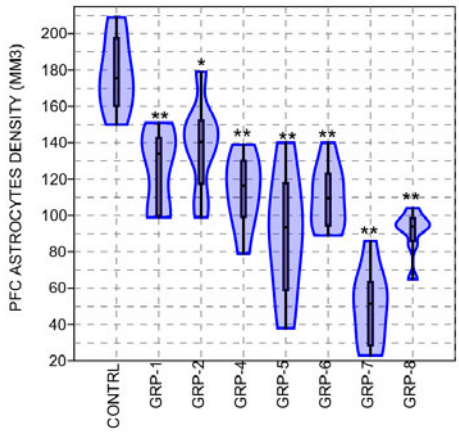

Figure 3-0-6: Violin box plot depicting the distribution of the measured variable among the eight experimental groups. The violin box plot displays the variation in the measured variable across the control group and seven other experimental groups. Each group is represented by a violin-shaped distribution, where the width represents the density of data points at different values. The white dot within each violin represents the median, and the thick horizontal line denotes the interquartile range (IQR). The thin lines extending from the violins indicate the range of the data, excluding outliers. Statistical significance was determined using one-way ANOVA, with Bonferroni correction. (*: $P < 0.05$; **: $P < 0.01$).

IV. Early life stress and PSZ on Increases Corticosterone levels

Quantification of corticosterone levels in early life impact studies using rodent models is essential for understanding the physiological stress response, assessing the effects of early life experiences, studying stress-related disorders, evaluating treatments, and establishing translational relevance (Gong et al., 2015). The Post-hoc comparisons revealed that all groups (1,2,4,5,6,7 and 8)1 significantly differed from the control group ($p < 0.001$). Additionally, Group 1 showed significant differences with group 2 ($p = 0.026$), group 4 ($p < 0.001$), group 5 ($p = 0.998$), group 6 ($p = 0.001$), and group 7 ($p < 0.001$). Group 2 exhibited significant differences with group 4 ($p < 0.001$), group 5 ($p < 0.001$), and group 7 ($p < 0.001$), group 4 showed a significant difference with group 5 ($p = 0.064$), suggesting differences in behavioral outcomes between these two groups. Group 5 demonstrated significant differences with all other groups ($p < 0.001$). Group 6 also displayed significant differences with group 7 ($p = 0.0001$) and group 8 ($p = 0.002$). Finally, group 7 exhibited a significant difference when compared with group 8 ($p < 0.001$).

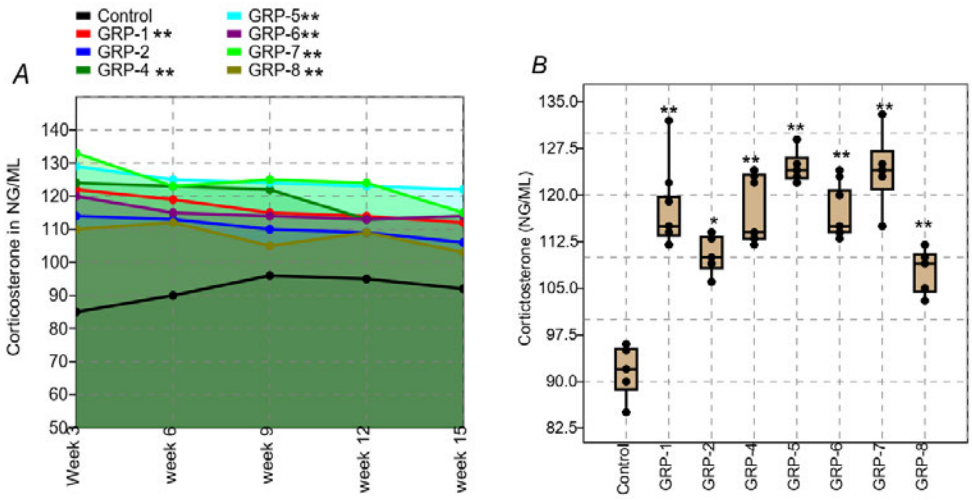

Figure 3-0-7: (A) The line graph depicts the changes in corticosterone levels measured at different time points (weeks 3, 6, 9,12 and 15) in the experimental group. Each data point represents the mean corticosterone level, and the error bars indicate the standard deviation. The corticosterone levels showed fluctuations over time, with an initial increase at week 6, followed by a gradual decrease until week 12. At week 15, the corticosterone levels reached their lowest point. The graph illustrates the dynamic nature of corticosterone levels and provides insights into the temporal patterns of corticosterone regulation. (B) Box plot indicating corticosterone levels Statistical significance was determined using one-way ANOVA, with Bonferroni's correction. (*: $P < 0.05$; **: $P < 0.01$).

V. Early Life Stress effect on Plasma Dopamine level

The specific effects of early life stress on plasma dopamine levels can vary depending on the nature, duration, and severity of the stressor, as well as the developmental stage at which the stress is experienced. The results of the pairwise comparisons yielded statistically significant differences among various experimental groups ($p < 0.05$). Specifically, significant differences were observed between the Control vs group 1 ($p < 0.001$), Control vs group 2($p = 0.057$), Control and group 5($p < 0.001$), Control vs group 7($p < 0.001$), and Control vs group 8($p < 0.001$). Additionally, significant distinctions were evident between the GSSP and GS groups ($p = 0.003$), as well as the group 4 vs group 7($p = 0.007$). Notably, no significant differences were observed between the group 4 vs group 8 ($p = 0.191$).

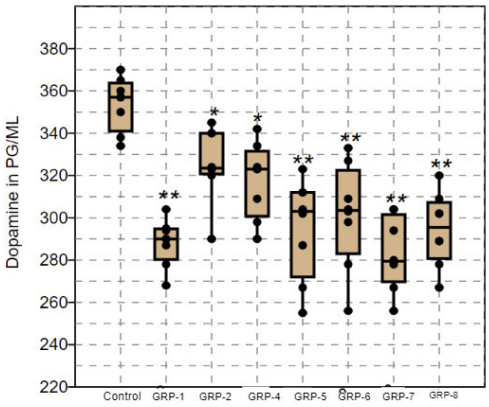

Figure 3-0-8: Plasma Dopamine Concentration Among Eight Experimental Groups Exposed to ELS and Parental Psychopathology. The figure illustrates the plasma dopamine concentration, measured in picograms per milliliter (pg/ml), among eight distinct experimental groups. Statistical significance was determined using analysis of variance (ANOVA) followed by post hoc Tukey tests (*$p < 0.05$, **$p < 0.01$) to assess group differences in plasma dopamine levels. Error bars represent standard error of the mean (SEM) values.

4. Discussion

This study demonstrates that differential ELS and KSZ in dams caused increased anxiety-like behavior in SD rats. These stressors also induced Astrocyte volume reduction in the PFC of SD rats. As for the physiological and neurochemical changes that occur in response to ELS and KSZ in rats, we quantified urine corticosterone from week 3 to 15, on three-week interval and plasma Dopamine. For some time now, MS, GS and social isolation in rats have been employed to mimic early life experiences in humans such as childhood maltreatment or trauma. Several previous studies have reported that MS, GS, and social isolation during early life in rats caused reduced social interaction and anhedonic like behavior (Li et al., 2020; Nishi, 2020). Nevertheless, there remains a dearth of comprehensive exploration into the distinct impacts of MS, GS, and KSZ on social behavior, anhedonia, and anxiety like responses in rat model. Even so to our knowledge no study has postulated the impact of KSZ as reported here on mimic parental psychopathology in humans. Here this study showed that differential stressors such as MS, GS and KSZ, increased anxiety-like (anhedonia) behaviors in ELS SD rats (Figures 4, 5 and 6).

To be specific there was a significant difference in means among the groups, indicating that ELS amplified SZ's grip on anhedonia in SD rats. Moreover, with a large effect size emphasizing the substantial impact of ELS and KSZ on anhedonia. The present study found a significant increase in anhedonia as depicted by the decreased sucrose preference in the rats exposed to stress (MS, GS, ELS and PSZ) compared to the Control ($p = 0.001$). MS and GS have previously underscored the amplifying impact of ELS on schizophrenia-related anhedonia

in later stages of life, as demonstrated in the study by Cui and colleagues (Cui et al., 2020). Furthermore, these methods have been effectively employed in creating depression models, with rodents exhibiting heightened anxiety-like behaviors as a result. (Du Preez et al., 2020). Interestingly in the present study, we also observed a found a significant decrease in anhedonia as depicted by the increased sucrose preference in the pups of Group 8 (consisting of normal parents and SZ pups) compared to the Group 7 (consisting of schizophrenia parents and pups). This finding suggests that normal parenting can potentially reduce the magnitude of depressive like outcomes in later life. These results are particularly noteworthy as they contribute novel data in the context of experimental animal studies, as previous researchers have not modelled PSZ paradigm and therefore not reported such findings. Nevertheless several human studies have reported a positive correlation between positive parenting and children externalizing and internalizing behaviours (Ying Chen et al., 2019) compared to the contrary in PSZ (Hussain, 2020). Moreover, a study by Dong and colleagues examined the impact of positive parenting on children's emotional intelligence and found that higher levels of positive parenting were associated with greater emotional awareness and regulation skills in children (Dong et al., 2020). In addition, Tarver and colleague's autism metaanalysis reported that negative parenting behaviors resulted in a positive correlation with externalizing behaviors, while positive parenting behaviors resulted in a positive correlation with social skills and a weak positive correlation with internalizing behaviors (Tarver et al., 2019).

EPM is the gold standard in measuring anxiety out comes in rodent model, this is because of its good face validity and its test re-test reliability (Figueiredo Cerqueira et al., 2023). As earlier mentioned previous studies have shown that ELS such as MS, GS and social interaction increases anxiety later in life (He et al., 2020). Here this study showed that differential ELS or KSZ have the propensity of increasing anxiety later in life through the number of entries and time spent on both closed and open arm. For instance, on the number of entries into closed arms the results demonstrated a significant effect variation in anxiety levels among the different groups. However, on the number of entries into the open arm, the control group and prenatal stress group exhibited significantly higher time spent on the open arm compared to the other groups with a substantial effect size. Further analysis indicated significant differences in means between the groups, particularly for the time spent on the closed arm.

Several studies have reported similar effects, Bondar et al., 2018 found that brief MS was associated impairment with social behaviour and anxiety in mice. Furthermore, maternal separation had deleterious effects on rodents behavior and demonstrated significant sex-specific effects on social behavior (Kestering-Ferreira et al., 2021).These consistent results

across studies indicate the detrimental impact of MS on anxiety-related behaviors. In addition, a human study by Elysayed and colleagues (Elsayed et al., 2019), investigated the role of familial risk, parental psychopathology, and stress in the first onset of depression during adolescence. The findings indicated that adolescents with a high familial risk for depression were eight times more likely to develop first-onset depression compared to those with low familial risk. Their results showed that maternal behavioral disorders and increased recent life stress directly predicted the initial onset of Major Depressive Disorder (MDD) in high-risk adolescents.

Conjointly, sociality and anxiety are intricately linked and regulated by neural circuits centered around the PFC and Hippocampus (Chavanne & Robinson, 2021; Kenwood et al., 2022). These two areas of the brain have been associated with control of social behaviors and anxiety (Chavanne & Robinson, 2021; Chen et al., 2022). Furthermore, these brain regions are recognized as being negatively affected by trauma, neglect and child maltreatment (Burrus, 2013). In the PFC and Hippocampus of children exposed to ELS and PSZ several prior studies have reported reduced gray matter volume and alterations in neural connectivity (Gorka et al., 2014).

We found a significant decrease in PFC astrocyte density in stressed rats compared to the control, non-stressed rat. This result suggests the possibility that ELS and PSZ may cause inflammation and result in neuronal cell death in the brain. To support this idea, previous studies have reported neuronal cell death due to chronic stress (Lucassen et al., 2014; Miguel-Hidalgo, 2022). Moreover altered astrocyte density in the PFC has been implicated in the pathophysiology of various psychiatric disorders, including SZ (Banasr & Duman, 2008; Di Benedetto et al., 2022; Rahati et al., 2016). In addition, the regenerative astrocytes gliosis potential could explain the difference experienced in reduced anhedonia and anxiety between group 8 (Normal parents and schizophrenia pups) and group 7 (Schizophrenia parents and pups), as supported by several prior studies (Chiareli et al., 2021; Gallo & Deneen, 2014).

In addition, the neuronal underpinnings, we quantified the urine corticosterone levels on a three-week interval from week 3-15 (see figure 10). The results demonstrated a significant overall difference between the means of groups, emphasizing the impact of ELS and PSZ influence on corticosterone levels (see figure 10 and 11) with a high effect size. These findings underscored the substantial impact of ELS and PSZ on the regulation of corticosterone release. All treatment groups showed significant differences when compared to the control group, indicating the disruptive effect of early life stress and parental psychopathology on the stress

response system. Also, we observed a significant difference between Group 7 (SZ parents and pups) and Group 8 (normal parents and schizophrenic pups). This finding suggests that good parenting practices had a reversing effect on the stress response system. Chronic stress can lead to dysregulation of the Hypothalamic-Pituitary-Adrenal (HPA) axis, resulting in increased secretion of corticosterone, a stress hormone. Prolonged activation of the HPA axis can contribute to various physiological and psychological effects associated with chronic stress as seen in figure 11. More stress however can further lead into more corticosterone affecting the PFC astrocytes density in the brain.

Dopamine is often associated with the brain's reward system, contributing to feelings of pleasure and reinforcement. It plays a role in motivation and the anticipation of rewards. Several studies suggested that stress induces alterations in dopamine signaling that may potentially contribute to behavioral changes associated with chronic stress. In the current study, we observed that differential ELS and individuals with KSZ exhibited a significant reduction in plasma dopamine levels compared to the control group and other groups, except for the GS group ($p < 0.05$). However, with not much significance there was a slight difference between group 8 vs group 7. Generally, this signified that the above stressors have a potential role in decrease in anhedonia and increased anxiety as early reported by the SPT and EPM. This study findings are in line with prior studies by (Majcher-Maślanka et al., 2017)(Sinani et al., 2022) that reported that ELS and PSZ causes anxiety through changes in the mesolimbic dopamine reward functions, hype responsiveness of HPA axis stress response, and other stress- and reward-related pathways that affects dopamine levels. In summary depression and anxiety are usually the first key symptoms in development of mental illness. In the context of this study, it's important to acknowledge the limitations associated with using a rat model to explore the relationship between KSZ and later-life anxiety and depression in rodents. While this approach offers valuable insights, several considerations warrant attention. To begin with, the rat model might simplify the complexity of human SZ, potentially limiting its relevance to understanding anxiety and depression. Secondly, while rats do exhibit behaviors associated with anxiety and depression, these might not precisely mirror human emotional experiences.

In conclusion, this study results demonstrate that ELS and KSZ impacts adolescent social behaviors, anxiety-like behaviors, and PFC characteristics in SD rats. This study suggests that ELS causes the alterations in the astrocytes in PFC, leading to social impairments and increased anxiety-like behaviors later in life. Similarly, the interplay of these factors (ELS and PSZ) underscores the multifaceted nature of the disorder experienced in humans. Interestingly, positive parenting has a potential benefit of reducing anxiety and depression later in life.

Additional research employing molecular and cellular techniques, such as gene expression analysis or neurotransmitter receptor profiling on the PFC, hippocampus and basal ganglia, may provide valuable insights into the underlying neurobiological alterations.

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CHAPTER FOUR

Chapter 4 is a research article titled Beyond Psychopathology: Examining the Complex Interactions of Early Life Stress, Schizophrenia, and Neuro-Inflammation on Depressive like Outcomes, this article has been submitted for publication to Elsevier in Behavioral Sciences journal. To minimize repetition in the methods section, we make reference to Chapter 2 (Oginga and Mpofana., 2023) where similar procedures have been used.

Abstract

Beyond Psychopathology: Examining the Complex Interactions of Early Life Stress, Schizophrenia, and Neuro-Inflammation on Depressive like Outcomes

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Background: The brain and the immune system are not fully formed at birth, but rather continue to mature in response to the postnatal environment. The two-way interaction between the brain and the immune system makes it possible for childhood psychosocial stressors [Early Life Stress (ELS) & Ketamine induced like behaviours in rats (KSZ)] to affect immune system development, which in turn can impact brain development and its long-term functioning.

Objective: The present study aimed at exploring the complex multifaceted interactions between early-life stress, schizophrenia (SZ), and neuro-inflammation, and their collective effect on social interaction, aggression and social avoidance.

Methods: Male and female Sprague-Dawley rat pups were randomly assigned to eight groups: control, ELS, schizophrenia, and ELS + schizophrenia. ELS was induced by Gestational Stress (GS)and Maternal Separation (MS) during the first two weeks of life, while KSZ like symptoms was induced by peritoneal and subcutaneous administration of ketamine. To assess depressive like outcomes, social interaction tests were conducted. In addition, Neuro-inflammation was determined through changes in inflammatory bio markers such as Neutrophils Lymphocytes ratio (NLR), expression levels of interleukin-6 (IL-6), matrix metalloproteinase-9 (MMP-9), interferon-gamma (IFN-g) and total astrocytes processes in the hippocampus and Prefrontal cortex(PFC).

Results & conclusion: The study results revealed that both differential ELS and KSZ had a significant impairment in social interaction and social preference on the experimental groups compared to the control group ($p < 0.05$). In addition, there was a decreased GFAP astrocyte processes intersection in the hippocampus and PFC ($p < 0.01$). Furthermore, differential

reductions were observed in the NLR ($p < 0.05$), interleukin-6 (IL-6), matrix metalloproteinase-9 (MMP-9), and interferon-gamma (IFN-g) were altered ($p < 0.05$), indicating an inflammatory response. Gaining insights into the neuro-inflammatory pathways that contribute to depressive like outcomes could offer new therapeutic avenues for addressing the multifaceted challenges associated with this ELS and parental schizophrenia(PSZ).
Keywords: Neuro-inflammation, early-life stress, schizophrenia, Social preference, Social Interaction, astrocytes, Avoidance, cytokines

1. Introduction

The notion that stress could have detrimental effects on the health of children has been present for quite some time. Around fifty years ago, Meyer and Haggerty established a clear dose-response correlation between family-related stress and children outcome (Meyer & Haggerty, 1962). However, even after numerous decades, the understanding on the connections between ELS or parental schizophrenia (PSZ) and immunity remains incomplete. The mechanisms by which ELS or PSZ disrupts the developing immune system, and how these mechanisms might influence brain and behavioral development during childhood, still eludes us. This stands in sharp contrast to the abundance of accumulated research findings in these realms, particularly concerning animals, and to a lesser degree, human studies. Interestingly, several studies have identified both ELS and PSZ as significant risk factors for the development of depression later in life (Graignic-Philippe et al., 2014).

One possible mediator candidate is the immune system. The activation of neuro-inflammatory and immune processes has been proposed as a pivotal factor influencing the progression of diseases within various pathologies that impact the central nervous system (CNS) (Sochocka et al., 2017). A lasting imprint of ELS on inflammation and immune system reactivity is evident. Clinical research has revealed a distinct "immune phenotype" that characterizes the blood of individuals exposed to Childhood Maltreatment (CM), encompassing sustained low-level inflammation, expedited immune aging, and conceivably compromised cellular immunity (Chen et al., 2021; Danese & Lewis, 2017). These alterations in the immune system have been intertwined with the vulnerability of ELS individuals to psychiatric and brain disorders, considering the indispensable role of immune processes and neuro-immune communication in CNS development and homeostasis. Another possible mechanism is that ELS initiates a permanent rewiring of innate immune cells and stress systems, including the sympathetic nervous system and the hyper-activation of the Hypothalamus-Pituitary-Adrenal (HPA) axis, regulators of immune responses (Fagundes & Way, 2014; Veru et al., 2014). Taken together

this rewiring could lead to heightened inflammatory responses upon successive challenges. Such rewiring could potentially trigger amplified or dysfunctional neuro-inflammatory reactions to protein aggregates and tissue damage, potentially sparking the development of CNS pathologies in ELS-affected individuals. Additionally, exposure to ELS or PSZ may act as a triggering factor for brain disorders by altering immune pathways that play a crucial role in the maturation of the brain, particularly affecting astrocytes and microglia. In individuals subjected to maltreatment, irregular neuronal function and synaptic plasticity might prompt premature aging and susceptibility to insults within circuits influenced by neurodegenerative ailments and injuries (Danese & J Lewis, 2017; Müller et al., 2015).

Astrocytes are integral components of the CNS, and also glial cells that play pivotal roles in modulating neuro-inflammatory responses and maintaining CNS homeostasis (Sofroniew, 2020). These star-shaped glial cells possess a remarkable ability to detect shifts in their microenvironment and respond by releasing a diverse array of signaling molecules, including cytokines like interleukins and tumor necrosis factor-alpha, as well as neurotransmitters like glutamate and ATP (Perez-Catalan et al., 2021; Qian et al., 2023). This molecular repertoire empowers astrocytes to significantly influence microglia, the resident immune cells of the brain, thereby intricately shaping the balance between neuroprotection and neurodegeneration (Giovannoni & Quintana, 2020; Linnerbauer et al., 2020). Moreover, astrocytes play a direct role in synaptic function and plasticity. By controlled release of glio-transmitters such as glutamate, they actively engage in synaptic transmission and modulate neural communication. Additionally, they contribute to vital processes like synapse formation, synaptic pruning, and the maintenance of optimal neurotransmitter levels, thereby fundamentally shaping neural circuitry. In the realm of neuro-inflammation, astrocytes manifest dual roles. On one hand, they possess mechanisms to counteract inflammation, releasing anti-inflammatory factors and promoting tissue repair. On the other, they have the potential to escalate inflammatory responses under specific conditions, further underscoring their intricate involvement in CNS health and pathology (Chiareli et al., 2021). Astrocytes are also active participants in neurodevelopmental processes, guiding neuronal migration, supporting synaptogenesis, and regulating neurogenesis. The dysregulation of astrocytic functions due ELS or PSZ periods has been implicated in various neurodevelopmental disorders (Butt et al., 2005; Lapato & Tiwari-Woodruff, 2018; Perez-Catalan et al., 2021).

Multiple animal models, such as MS, GS, and Limited Nesting & bedding time (LBT), have been widely used to replicate ELS and childhood traumatic experiences (Deslauriers et al., 2018; Murthy & Gould, 2018).

These studies underscore the adverse effects of MS or GS on social preference, avoidance, and the architecture of the Hippocampus and PFC, ultimately impacting physical health, yet, the mechanisms by which ELS induces astrocytopathy and related behaviors, including pro-inflammatory mediators like MMP-9, IL-6, and TNF-α, remain not well explored (Brenhouse et al., 2019; S. H. Shin & Kim, 2023). Moreover, the interplay between ELS and PSZ has yet to be modeled in animal research, providing a novel avenue for exploration, of the multifaceted EL experiences in humans.

To address these questions, we evaluated the impact of ELS (MS, GS) and KSZ on development of depressive like outcomes later in life in rats. Depressive like outcomes were monitored through social approaches, social latency and social interaction as well as astrocytes intersection processes on the hippocampus and PFC in rats. Moreover, we evaluated the NLR, platelet-to-lymphocyte ratio (PLR), and lymphocyte-to-monocyte ratio (LMR), as indicators of systemic inflammation.

2. Materials and Methods

I. Experimental Animals and Treatment

Twenty-four SD rats (16nulliparous females in the fertile phase and 8 stud males) were sourced from the University of KwaZulu-Natal's Biomedical Resource Unit and mated. They were raised under controlled conditions with unrestricted access to food and water, adhering to ethical guidelines, and the study was granted approval with reference number AREC/00003119/2021.

II. Animal Housing and Surgical Procedures

In the study, 16 nulliparous, fertile-phase female rats were mated with 8 stud males to produce 64 pups for experimentation. To synchronize the estrous cycles, the females were acclimatized by being paired in cages for a week, and the males were individually housed to optimize sperm count. The initiation of gestation was marked by the presence of an ejaculatory plug, and males were euthanized after mating to prevent stock contamination.

III. Animal Groupings and Stress Factors

Rats were randomly assigned to 8 groups; control (non-stressed pups), group 1 (parental maternal stress + ketamine), group 2 (prenatal maternal stress pups), group 4 (Maternally separated pups), group 5 (MS+ ketamine injected pups), group 6 (ketamine injected parent),

group 7 (ketamine parent + ketamine Pups) and group 8 (Ketamine positive control)). A comprehensive discussion of groupings and stressors has been previously presented in an earlier chapter 2 (Oginga and Mpofana., 2023).

a) Maternal Confinement Stress during Gestation

To simulate prenatal stress in humans, pregnant dams were subjected to daily restraint stress induction from days 15 to 18. The stress protocol involved confining the pregnant dams in transparent cylindrical restrainers for 45 minutes in a well-illuminated environment with temperature maintenance between 21-22°C, with adjustments in the restrainer dimensions based on the rats' body mass throughout the approximately 21 to 23-day gestational period.

b) Dams and Pups Psychopathology

Dams and pups were injected with ketamine to simulate schizophrenia (SZ) symptoms. Behavioral testing was conducted on the offspring at postnatal day 60, following a protocol in line with previous studies (Oginga and Mpofana., 2023; Koh et al., 2016; Lisek et al., 2017; Schobel et al., 2013).

c) Maternal Separation: The Impact of Dams-Pup- Separation

The maternal separation protocol involved daily 3-hour separations from 9 a.m. to 12 p.m. Dams and pups were placed in separate areas to prevent interaction as previously describe by Oginga and Mpofana., 2023 (which forms chapter 2 of this dissertation). After the separation, pups were returned to the room, and dams went back to their cage. The light/dark cycle was from 6 a.m. to 6 p.m., with weekly cage cleaning. Weaning occurred at postnatal day 21, and rats were left undisturbed until postnatal day 48 (Begni et al., 2020). Figure 2 visually depicts this separation process.

IV. Behavioral Assessments

a) Social Interaction Behaviors

Social interactions play a crucial role in the biology of various species, contributing to the structure and stability of social networks and relationships within societies (Wieczorek et al., 2022). The effects of stress on social interaction was assessed using Crawley's sociability and preference for social novelty test. On postnatal day 60 (PND 60), the rat cages were transferred to the behavioral room 30 minutes prior to the commencement of the experiment. The experimental setup consisted of three chambers created using Plexiglas walls: one in the middle, and one on each side. The subject rat was placed in the center of the middle chamber

for a 5-minute adaptation period. Following adaptation, the walls separating the chambers were removed, allowing the subject rat to freely explore all three chambers. During the experiment, the following parameters were monitored and recorded: the duration and number of direct (active) contacts between the subject rat and the containment cup housing either a stranger rat or remaining empty, recorded separately for each chamber. Active contact was counted when the subject rat made direct contact with the containment cup or stretched its body within 3-5 cm of the cup. Other behaviors of the subject rat in each compartment, such as walking, self-grooming, freezing (no body movements for more than 5 seconds), as well as any unusual behaviors like hyperactivity or increased aggression, were also recorded in terms of duration and frequency. Additionally, the duration and number of entries into each compartment were documented. In the experimental design, rodents underwent a series of behavioral tests to evaluate social behaviors. A schematic representation of these tests, including assessments for social preference, social avoidance, and social fear, is presented in Figure 5-5

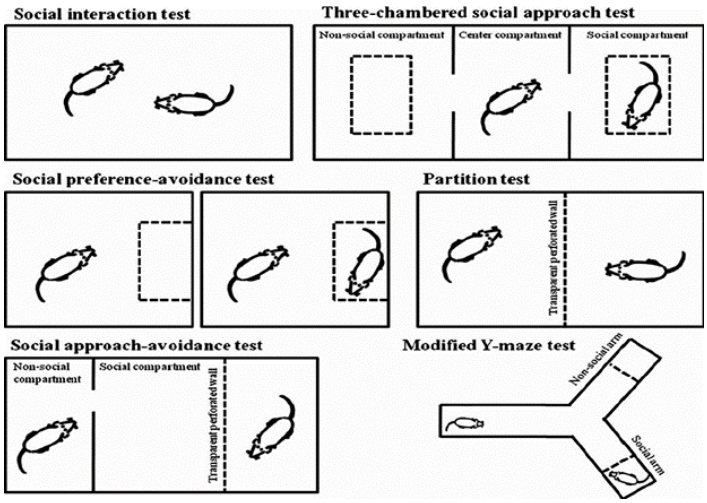

Figure 4-0-1: Schematic representation of the behavioral tests used to assess social preference, social avoidance and social fear in rats

After each trial, the chambers were cleaned using 70% ethanol (between rats) and Ortho-phthalaldehyde1:5:1 (between cages) to prevent olfactory cues and ensure proper disinfection, respectively. A rat was considered to be in a chamber when its head and all four paws had entered. Each session of the test lasted for 10 minutes (Kaidanovich-Beilin et al., 2010).

V. Sacrifice and Neurochemical Analysis

a) Animal Sacrifice and Tissue Collection

Animals were anesthetized with ketamine and xylazine cocktail (KX rat cocktail 0.1 mL/100g rat wt. IP. Contains: 91 mg/kg Ketamine. 9.1 mg/kg Xylazine) to ensure deep unconsciousness,

minimizing distress and pain. Transcardial perfusion or decapitation was then performed by trained personnel as previously detailed (Oginga and Mpofana., 2023). The guillotine blade was sharpened within 3 weeks of use, and animal welfare was prioritized throughout to uphold ethical standards and research integrity.

b) Transcardial Perfusion Fixation

After 24 hours of behavioral tests, half of the SD rats were anesthetized with a mixture of ketamine and xylazine. They were then perfused with PBS and 4% paraformaldehyde (PFA). The perfusion involved making a small incision in the left ventricle, inserting a perfusion needle into the ascending aorta, and removing the sternum and thymus. This procedure aligns with Gage et al. (2012) guidelines, preserving brain tissue for detailed analysis while ensuring blood-free samples for imaging and staining.

c) Decapitation, Post fixation, Tissue Collection and Storage

On post-natal day 60, SD rats were anesthetized with combination of ketamine and xylazine, and their heads were severed from the body using a guillotine. An incision was made along the neck to expose the skull, and neck muscles were trimmed to reveal the skull base. Blood samples were taken from the jugular vein and stored. The brain was carefully removed, trimmed of any remaining tissue, and placed in fixative solution for 24 hours at 4 °C. Afterward, the brain was washed with phosphate-buffered saline, and the PFC and hippocampus were collected and stored in phosphate-buffered saline or HBHS solution with sodium azide at 4 °C (van Rijn et al., 2011).

d) Complete Blood Cell count

The trunk blood was harvested, NLR and MLR was measured. NLR: NLR is often used as an indicator of systemic inflammation, psychiatric diagnosis and stress (Brinn & Stone, 2020; Bulut et al., 2021). We calculated the NLR by dividing the absolute neutrophil count by the absolute lymphocyte count. MLR, like NLR, is also a marker of inflammation and immune system activity. It is calculated by dividing the absolute monocyte count by the absolute lymphocyte count.

e) ELISA Kits

ELISA kits were employed to quantify the expression levels of IL-1b, IL-6, MMP-9, and Brain-Derived Neurotrophic Factor (BDNF) in rat samples.

VI. Astrocyte Cell Cultures

1. Tissue preparation:

After the behavioral protocol, animals were anesthetized with ketamine and xylazine overdose and perfused with phosphate buffer and 4% paraformaldehyde fixative. The brain sections, containing the orbi-frontal cortex and dorsal hippocampus, were obtained with a vibratome and stored for analysis.

2. Cresyl Violet Staining and Volume Estimation:

Cresyl violet stain marked the hippocampus boundaries, and a 1.25× magnification anatomical microscope counted grid points in the hippocampus (Fig. 2-0-4) please see page 31. Cavalieri's principle was employed to estimate the volume of the hippocampus and orbi-frontal cortex, considering tissue block thickness (0.6 mm), area per grid point ($a(p) = 0.09\ mm^2$), and the total grid points (ΣP) in each rat's hippocampus or orbi-frontal cortex (Gundersen et al., 1988; Von Bartheld, 2002).

3. Immuno-Histochemical Labeling

In this study, we performed immuno-histochemical labeling on tissue slices. We washed the slices with a solution of 0.1 M PB and 0.5% Triton X-100, then blocked them with PB containing 2% normal goat serum, 3% bovine serum albumin, and 0.3% Triton X for 3 hours. Astrocytes were labeled with an anti-glial fibrillary acidic protein (GFAP) antibody and 4',6-diamidino-2-phenylindole staining. Astrocyte volume, complexity, and density in the hippocampus were assessed and analyzed using Image J. Regions of interest were defined based on neuron morphology, counted using a stereological system (ZEISS, Germany) at ×2.5 magnification with a 2% area sampling fraction and a 3 μm guard zone. The number of GFAP+ cells was counted over a 15 μm depth. A comprehensive procedure of Immuno-Histochemical Labeling has been previously provided in an earlier Chapter 2.

VII. Data Analysis and Statistical Measures

Statistical analysis was conducted using PAST-4, with a significance level of $p < 0.05$, but for familywise error control, $p < 0.002$figure was adopted due to multiple comparisons. One-way ANOVA was used for comparisons within groups on behavioral tests and astrocytes, and power analysis assessed effect size. Post hoc Tukey's test was performed if ANOVA indicated a significant effect. Image-J was employed for quantifying hippocampal astrocyte density and process intersections. Results for avoidance, social interaction and social preference were presented in bar graphs, box plots, and while the astrocytes processes were presented in radar

plots. P-values adhered to APA criteria, with three digits after the comma for values below 0.001 (e.g., $p < 0.001$).

3. Results

I. Social Interaction Behaviour

In this study examining social approaches among various groups (Control, group1 (prenatal maternal stress + ketamine injected pups), group 2 (GS), group 4 (MS), group 5 (maternal separated + ketamine injected pups), group 6 (KSZ in dams), group 7 (ketamine parent + ketamine Pups), and group 8 (KSZ) pups, the ANOVA revealed that both the stressed and schizophrenia groups exhibited significantly different social approach patterns when compared to the control group (non-stressed) ($p < 0.001$, **Figure 4A)** with a substantial effect size ($\omega^2=0.653$). On the social approach latency post hoc analysis between the control group Vs the treatment (group 1,2,4,5,6,7 and 8) yielded significant findings ($p <0.001$ **Figure 4B**). Notably, control differed significantly from group 4 ($p < 0.001$), Group 5 ($p = 0.001$), Group 6 ($p < 0.001$), and Group 7 ($p < 0.001$). Group 1 and Group 2 also exhibited significant differences compared to Group 4 ($p = 0.017$ and $p < 0.001$, respectively), while Group 5 differed significantly from Group 6 ($p = 0.002$). However, no significant differences were observed between group 5 and Group 8 ($p = 0.404$), as well as group 6 and group 7 ($p = 0.004$).

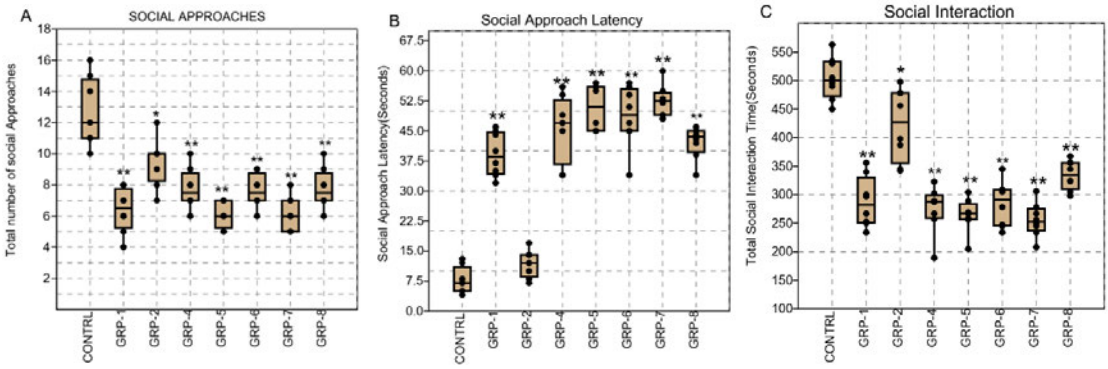

Figure 4-0-2: Boxplots and Jitters for the data of: (A) Total number of social approaches. (B) Social approach latency. (C) Total social interaction time. All groups (n = 8), Control Subjects-Social Novelty group; ELS and PSZ (n = 8), Maternal Separation-Social Novelty group. ANOVA and Student's t-test was used to compare behavioral data between groups Control and the rest of the group. with Bonferroni correction. Significance for all analyses was defined as $p < 0.05$ (*: P <0.05; **: P <0.01)

Furthermore, on the context social interaction dynamics, the post hoc analysis revealed noteworthy findings. Firstly, when comparing the control group to all other groups ($p < .001$ **Figure 4C**), signifying significant distinctions in social interaction patterns between the control group and the remaining groups. In addition, within the inter-group comparisons, we observed

statistically significant variations, Group 1 and Group 2 displayed a highly significant difference in social interaction ($p < 0.001$), as did Group 2 vs Group 4 ($p < 0.001$). Group 7 vs Group 8 also exhibited a highly significant discrepancy in social interaction ($p = 0.005$).

II. Impact of ELS and PSZ on Neutrophil lymphocytes ratio (NLR) and the Monocyte-Lymphocyte Ratio (MLR)

The ANOVA on the NLR revealed significant differences between the Control group and several experimental groups Control group and group 1 ($p < 0.001$), group 2 ($p = 0.0001$), group 4 ($p < 0.001$), group 5 ($p < 0.001$), group 6 ($p < 0.001$), group 7 ($p < 0.001$), and group 8 ($p < 0.001$) (see **Figure 5A**).

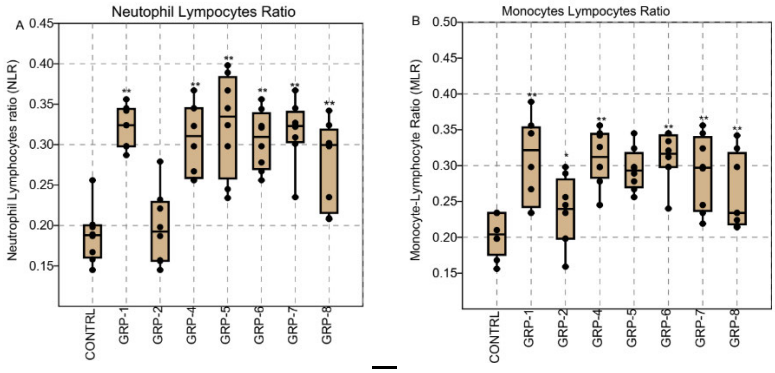

Figure 4-0-3: Boxplots and Jitters for the data of (A) Neutrophil-Lymphocyte Ratio (NLR) and (B) Monocyte-Lymphocyte Ratio (MLR) in different experimental groups (n = 8). Control group vs. experimental groups. ANOVA and Bonferroni-corrected Student's t-test were employed to compare hematological data between the control group and the experimental groups. Significance levels were set at $p < 0.05$ (*: $p < 0.05$; **: $p < 0.01$).

In addition, the post hoc showed significant differences between the control group and the experimental groups on the MLR, group 1 ($p = 0.029$), group 2 ($p = 0.022$), group 4 ($p = 0.003$), group 5 ($p = 0.003$), group 6 ($p = 0.002$), and group 7 ($p = 0.009$ see **Figure 5B)** all exhibited significantly different social interaction patterns compared to the Control group. However, no significant difference was found between the Control group and group 8 ($p = 0.075$).

III. Impact of ELS and PSZ on inflammatory biomarkers such as Interleukin 1b & 6, MMP-9 and BDNF

The ANOVA showed significant differences between the Control group and several experimental groups [group 1 ($p < .001$), group 2 ($p < .001$), group 4 ($p = .021$), group 5 ($p = 0.192$), group 6 ($p = 0.065$), and group 7 ($p = 0.009$, **see Figure 6A**]. However, it's worth noting that no significant difference was observed between the Control group vs group 8 ($p = 0.075$), suggesting comparable outcomes between these two groups. On the interleukin 6, the ANOVA

also indicated significant differences in means among the groups but with a larger effect size ($p < 0.001$, $\omega^2 = 0.747$ see **Figure 6B**). Furthermore, ANOVA for MMP-9 plasma levels showed a significant difference in means among the groups ($p < 0.001$, $\omega^2 = 0.747$ See **Figure 6C**). Finally, post hoc comparisons on the BDNF revealed several noteworthy findings (**see Figure 6D)**. First, group 1 vs control group ($p < 0.001$). Similarly, group 2 vs control group ($p < 0.001$). Group 4 vs control group ($p = 0.003$). Group 5 showed a significant difference ($p = 0.05$), indicating variations in outcomes. Group 6 displayed a significant difference ($p = 0.044$) compared to the Control group. Finally, group 7 vs control group. However, group 8 vs control group ($p = 0.997$).

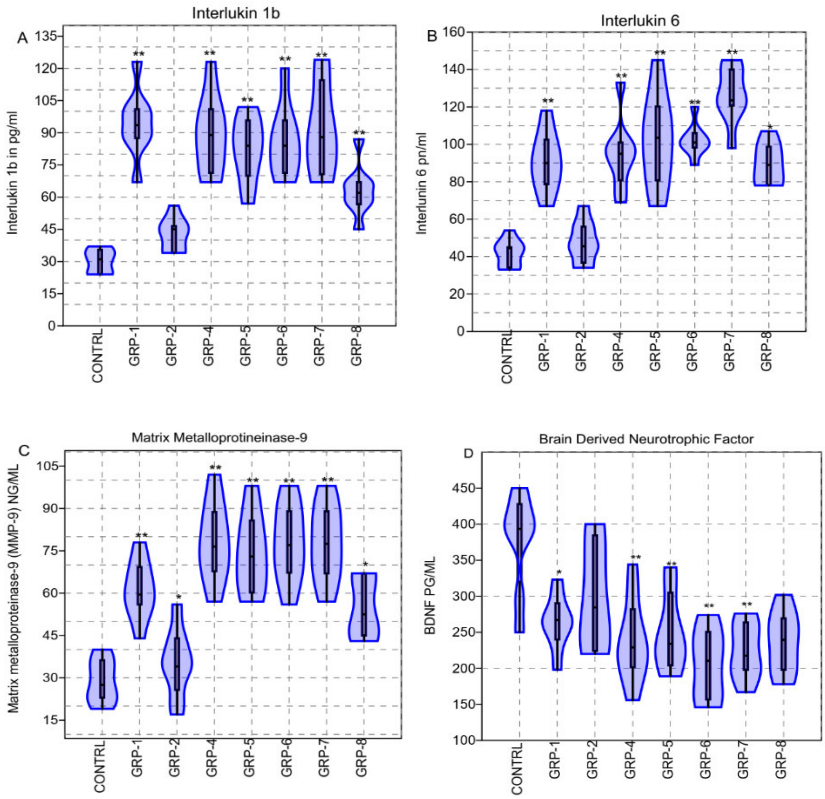

Figure 4-0-4: Plasma biomarker Levels in response to ELS and KSZ (A) Interleukin-1β (IL-1β) levels, (B) Interleukin-6 (IL-6) levels, (C) Matrix Metalloproteinase-9 (MMP-9) levels, and (D) BDNF levels in plasma samples from rats subjected to chronic stress (n = 8) compared to control rats (n = 8). Data are presented as violin boxplots with individual data points (jitters) superimposed. Statistical analyses were performed using ANOVA followed by Bonferroni-corrected Student's t-test to assess differences between the Control group and the Experimental group. Significance levels were set at $p < 0.05$ (*: $p < 0.05$; **: $p < 0.01$).

IV. ELS and PSZ impact on PFC and Hippocampus Astrocytes processes intersections

The ANOVA results on the total astrocytes processes intersections in PFC revealed a significant difference in means among the groups however with a moderate effect size ($p =$

0.001, $\omega^2 = 0.539$ see **Figure 0-4**). Post hoc comparisons on the total astrocytes processes revealed significant differences compared to the Control group ($p = 0.001$). Group 2 displayed significant differences when compared to the control group ($p = 0.001$) vs group 5 ($p = 0.023$). Group 4 exhibited significant differences when compared to the control group ($p < 0.001$) and group 5 ($p = 0.015$), suggesting variations in outcomes. Group 7 showed significant differences compared to the control group ($p < 0.001$), group 5 ($p > 0.05$), and group 8 ($p > 0.05$), indicating distinct outcomes in these comparisons. However, no significant differences were found between the control group vs group 6 ($p = 0.105$) or group 8 ($p > 0.05$). Equally the hippocampal post hoc comparisons on the total astrocytes processes revealed significant difference in means among the treat groups and control ($p = 0.0001$). Moreover, group 7 showed significant differences compared to the control group ($p < 0.001$), group 5 ($p > 0.05$), and group 8 ($p > 0.05$), indicating distinct outcomes in these comparisons.

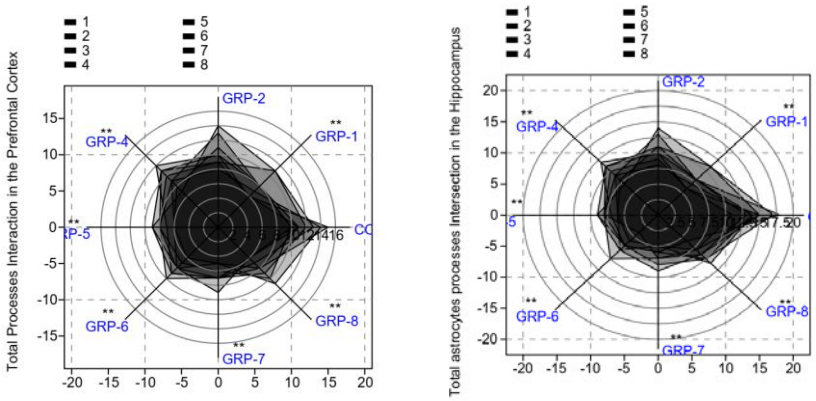

Figure 4-0-5: Radar plots representing total Astrocyte processes morphology (A) Radar plot illustrating the morphological characteristics of Total astrocyte processes in the Prefrontal Cortex. Each axis represents a specific morphological parameter, and the radial lines connect data points for different experimental groups. (B) Radar plot displaying the morphology of total astrocyte processes in the hippocampus. Statistical analyses were performed using ANOVA followed by Bonferroni-corrected student's t-test to assess differences between the control group and the experimental group. Significance levels were set at $p < 0.05$ (*: $p < 0.05$; **: $p < 0.01$).

4. Discussion

The immune system is a vital mechanism of surveillance and defense, unlike the nervous system. The development of the immune system is not completed at birth, but rather continues throughout childhood, environmental stimulation in childhood years can have profound effects on the immune system. The findings of this study revealed that stressors such as MS, GS, and KSZ or their interactive nature shapes vulnerability to develop social interaction, anxiety and depressive like outcomes through inflammatory damages and astrocyte processes pruning in SD rats later in life. Consistent with the study findings, ELS or KSZ such as MS has been

reported to reduce social behaviour, increase anxiety and depressive like outcomes through social interaction behaviour in rats (Bondar et al., 2018; Walker et al., 2023). The reduced social interaction , increased anxiety and depressive like responses could be due to dysregulation of the immune system in rodents (Hayley et al., 2021; Wilson et al., 2002). Stress and psychopathological factors can trigger a chronic state of low-grade inflammation (Patki et al., 2013). In light of this, several studies have subsequently investigated the association between early-life stress and later immune functioning in rodents and non-human primates (Kautz et al., 2023). Assessments of immune function encompasses a diverse array of indicators, spanning from naturally occurring pro-inflammatory cytokines in the bloodstream to immune cell proliferation triggered by antigens in the spleen, to the expression levels of genes associated with inflammation in the brain, and the composition of the gut microbiota (Bower & Kuhlman, 2023; Straub & Cutolo, 2018; Tosevski & Milovancevic, 2006). Understandably, due to this combination of various study designs and measurement methods, the findings have yielded inconsistent outcomes concerning the connections between ELS and the immune system (Danese & J Lewis, 2017; Graham et al., 2006).

Consistent with substantial evidence demonstrating a decline in social interaction and an elevation in anxiety resulting from the influence of ELS and KSZ, there is also notable research indicating alterations in immune cell proliferation, particularly concerning the NLR and MLR (Demir et al., 2015; Korkmaz et al., 2016). We also observed a significant increase in the NLR and MLR in the groups exposed to ELS, KSZ, or both, in comparison to the control group, except for Group 2 (exposed to prenatal stress only). Additionally, Group 8 (comprising normal parents and KSZ pups) exhibited a statistically significant difference in both NLR and MLR when compared to Group 7 (consisting of schizophrenia parents and pups). The current findings align with previous research on neuropsychiatric conditions, specifically regarding NLR, PLR, and MLR. For instance, one study suggested that individuals with Alzheimer's disease exhibited elevated NLR compared to healthy controls (HC) (Kuyumcu et al., 2012), although this outcome appears to be more closely associated with age. Similarly, other investigations have reported increased NLR, PLR, and MLR values in individuals with Schizophrenia when compared to HC (O'Brien & Price, 2013; Özdin et al., 2017). Additionally, a meta-analysis revealed that individuals diagnosed with MDD displayed higher NLR levels compared to HC (Mazza et al., 2018). In cases of chronic stress or exposure to psychopathological factors such as ELS or PSZ like aforementioned, the balance between these cell types may be disrupted. An elevated NLR is often associated with chronic inflammation and stress (Özdin et al., 2017). Moreover, in line with a recent systematic review and meta-

analysis focusing on individuals with MDD in China, it was observed that individuals with depression had elevated NLR, PLR, and MLR when compared to individuals without the condition (Cheng et al., 2022).

In addition to immune cells, pro-inflammatory biomarkers have been a significant topic of discussion as potential contributors to impaired social interaction and pre-onset anxiety in the context of MDD. This has been an ongoing focus of research and discussion in the field of psychiatry for some time now (Kumar et al., 2021; Miłkowska et al., 2017a, 2017b; Na et al., 2014). Pro-inflammatory cytokines are predominantly produced by activated macrophages and are involved in the up-regulation of inflammatory reactions. There is abundant evidence that certain pro-inflammatory cytokines such as IL-1β, IL-6, and TNF-α are affected by ELS and PSZ, and likely the first to causes anxiety and depression before the onset of MDD (Ruiz et al., 2022). In a manner akin to this research, we observed a significant increase in the IL-1β and IL-6 across the treatment groups compared to the control. Although the ontogenesis of the immune system starts well before birth there was no significant difference between the control and group 2 (prenatal stress). Several separate studies have reported the impact of ELS and PSZ on the aforementioned interleukins. Research has linked ELS to a more pronounced increase in circulating IL-6 levels when individuals are subjected to tasks like delivering a speech about their job qualifications and performing mental arithmetic in front of impassive evaluators (Carpenter et al., 2010)(Janusek et al., 2017). Furthermore, a recent meta-analysis indicated that individuals with a history of childhood trauma exhibit notably elevated levels of circulating IL-6, TNF-α, and C-reactive protein (CRP), even in the absence of specific laboratory stressors, although not necessarily in the absence of the ordinary stressors encountered in daily adult life (Baumeister et al., 2016). In addition to the two pro-inflammatory biomarkers, in the current study we observed significant increase in the MMP-9 in the groups exposed to ELS and KSZ compared to the control. Indeed, elevated MMP-9 levels are often associated with inflammation. In cases of chronic stress or psychopathological exposure, MMP-9 levels may increase, potentially affecting neural plasticity and contributing to reduced social interaction and anxiety (Li et al., 2022). Furthermore, MMP-9, BDNF plays a pivotal role in neuronal survival, growth, and neurotransmitter regulation. It actively contributes to shaping neuronal plasticity, a fundamental factor for learning and memory processes. In the current study, we observed a significant reduction in BDNF levels in the groups exposed to ELS and KSZ rats compared to the control group. Notably, positive parenting interventions led to elevated BDNF levels, as evidenced by the significant differences

between group 8 (comprising normal parents and KSZ pups) and group 7 (comprising KSZ parents and their offspring).

Originally considered an important regulator of early neuron development and survival (Barde *et al*., 1982), BDNF has more recently been implicated in various processes within the mature brain, including synaptic plasticity (Park and Poo, 2013). Earlier studies have shown that BDNF mRNA is upregulated by stimulation paradigms that induce long-term potentiation (LTP) in regions such as the hippocampus and prefrontal cortex. Historically, the presynaptic effects of BDNF have been attributed to its modulation of vesicular glutamate release efficiency in mammalian synapses, including those in the hippocampus (Tyler *et al*., 2002). Finally, as a results of low BDNF in the brain in the current study we also observed significant reduction in astrocytes processes in the hippocampus and PFC between the groups exposed to ELS and KSZ compared to the control, with also significant differences between the group 8 and 7. While there is no direct, singular relationship between these MMP-9, BDNF and astrocytes processes, they are all components of a broader network of factors that can influence social interaction in various ways. While BDNF has been linked to learning and memory processes, which are integral to social interaction. It is possible that BDNF levels and activity could influence an individual's ability to learn and remember social cues, leading to changes in social interaction. Abnormal MMP-9 activity has been associated with neuropsychiatric disorders, some of which involve deficits in social interaction, anxiety and depression. By and large as shown by the current study, changes in astrocyte function could potentially impact the neural circuits involved in social interaction, learning and memory.

Conclusions

In conclusion, the present study sheds light on the intricate web of interactions between early life stress, schizophrenia, neuro-inflammation, and their collective impact on depressive like outcomes in a rat model. These study findings emphasize the multifaceted nature of these factors and their potential role in shaping neurodevelopmental outcomes. This study underscores the importance of considering early life experiences as significant contributors to the risk and manifestation of psychiatric conditions like schizophrenia. Early life stress may serve as a critical trigger, potentiating the development of depressive like outcomes often associated with this complex disorder. Moreover, the involvement of neuro-inflammation as a mediating factor underscores the need for a more comprehensive understanding of the immune-brain interface in the context of mental health.

While the study findings provide valuable insights into the interplay between these factors, it is crucial to acknowledge the limitations of this rat model and the inherent complexities of translating these findings to human populations. Nevertheless, this work highlights the need for further investigation into the mechanisms by which early life stress and neuro-inflammation contribute to the heterogeneity of schizophrenia symptomatology, including depression.

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CHAPTER FIVE

SYNTHESIS

Early life experiences have a significant impact on children's neural, behavioural, and psychological development, with long-term consequences in a variety of domains (Bischoff et al., 2019; Mpofana et al., 2016). Throughout the lifespan, these experiences have a negative impact on neural plasticity and as a result, alter their behaviour and psychological processes. To date, research has shown the etiology of psychopathology of ELS and PSZ. However, as far as our current knowledge extends, no study has documented the synergistic effects of ELS and PSZ on the motor, memory and cognitive functioning, let alone attempt to model this phenomenon in rodents. Given the multifaceted nature of the impact of ELS, it becomes imperative to account for factors operating at various levels and throughout different stages of development. In light of this, this study focused on the distinct effects of ELS and PSZ on motor, memory and cognitive functioning in a rat model. In this study the researchers observed behavioral, molecular, and cellular alterations associated with the impact of ELS and KSZ in SD rats later in life.

The hippocampus and prefrontal cortex are pivotal domains that exert significant influence over cognitive and motor functioning. The hippocampus, renowned for its role in memory and spatial navigation, aids in memory formation, spatial mapping, and the ability to differentiate between similar memories (Preston & Eichenbaum, 2013; Tavares & Tort, 2022). In contrast, the prefrontal cortex, often referred to as the brain's "executive center," governs higher-order cognitive processes and motor control. As previously discussed, considering the intricate and multifaceted nature of ELS and KSZ, particularly their potential interactive effects on offspring, this study is designed to delve into this complexity. To begin with, the primary objective of this research was to examine the combined influence of ELS and KSZ on a range of behavioral and cognitive aspects in (SD) rats. Specifically, we investigated parameters such as locomotor activity, anxiety-related behaviors, exploratory tendencies, and spatial memory in these animal subjects. Findings from this study showed the enduring effects of ELS and KSZ on psychomotor retardation and increased anxiety-like behavior. In addition, we unveiled impairments in spatial memory in the ELS group and the group subjected to both ELS and parental psychopathology. Taken together, a significant reduction in the volume of GFAP+ astrocytes were observed as a result of exposure to ELS. Notably, this adverse effect was ameliorated in the presence of positive parental mental health. Anxiety and depression are often considered hallmark symptoms towards the development of mental illness (Perini et al., 2019).

In addition to the previously mentioned objectives, this research delved into the influence of ELS and KSZ or their combined effects on social interaction, social preference, and anxiety-related behaviors in a rat model. These study findings reveal a significant decrease in hedonic and increase in anxiety like responses from ELS and KSZ in rats. Interestingly, when normal parental mental health was present, we observed a reversal in the previously noted decline in GFAP+ astrocytes and reduced anxiety levels. Therefore, this suggests a potential protective role of normal parental mental health in mitigating depressive like symptoms and enhancing PFC astrocyte functionality. The study provides empirical evidence supporting the hypothesis that exposure to ELS and the presence of KSZ significantly impact the manifestation of anxiety and depression.

Experimental studies have linked ELS and KSZ to immune activation (IA), resulting in cognitive decline and, eventually, the pathogenesis of anxiety and depression. Neuro-inflammatory responses resulting from these impacts can have a long-lasting effect on the cognitive domain of the brain, potentially leading to structural abnormalities and behavioral alterations in offspring (Creutzberg et al., 2021; Lumertz et al., 2022). To investigate the pathogenesis of anxiety and depression, the researchers explored the complex, multifaceted interactions between ELS, KSZ, and neuro-inflammation and their collective effect on social interaction, locomotor function, and spatial memory. These study findings revealed that differential ELS and KSZ in rats significantly impaired social interaction and spatial memory. Additionally, there was a decreased intersection of GFAP astrocyte processes in the cortex. Furthermore, differential reductions were observed in the NLR, IL-6, MMP-9, and IFN-g were altered, indicating an inflammatory response.

In conclusion, this research endeavors to offer fresh perspectives on the correlation between ELS and parental psychopathology, specifically schizophrenia, on cognitive and motor outcomes. Notably, it is observed that schizophrenic offspring exhibit improved outcomes with positive parenting. These findings shed light on the complex interplay between parental psychopathology and offspring's outcomes, emphasizing the need for further research in this area.

To the best of our knowledge, this is the first study to have novel insight into a study modelling parental psychopathology in rodents as a model to mimic human disorders. Therefore, the evidence produced here lays the groundwork for further investigations on point to point impact of ELS or Parental mental illness on other domains and brain areas.

Recommendations and Future Studies

The following recommendations are made to understand further the impact of ELS and PSZ on cognitive and motor functioning.

1. Longitudinal Studies: Conduct long-term longitudinal studies that track individuals exposed to ELS and individuals with a family history of PSZ from childhood into adulthood.
2. Multidisciplinary Approach: Encourage interdisciplinary research that combines neuroscience, psychology, genetics, and epidemiology to assess the impact of ELS and PSZ comprehensively.
3. Neuroimaging: Utilize advanced neuroimaging techniques, such as functional MRI (fMRI) and diffusion tensor imaging (DTI), to investigate structural and functional brain changes associated with ELS and PSZ.
4. Explore the genetic and epigenetic factors that may mediate or moderate the impact of ELS and PSZ on cognitive and motor functioning.

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APPENDICES

1. **Addendum A: Ethical approval letter**
2. **Addendum B: Online Animal Ethics Welfare course**

Addendum A: Ethics approval letter

UNIVERSITY OF KWAZULU-NATAL
INYUVESI YAKWAZULU-NATALI

26 January 2022

Mr Fredrick Otieno Oginga (221017678)
School of Laboratory Medicine & Medical Sciences
Westville Campus

Dear Mr Oginga,

Protocol reference number: AREC/00003119/2021
Project title: Differential Effects of Early Life Stress and Schizophrenia On Development of Impulse Control Disorder.

Full Approval – Research Application

With regard to your revised application received on 26 January 2022, the Animal Research Ethics Committee has accepted the documents submitted and **FULL APPROVAL** for the protocol has been granted.

Please note: You are required to provide a bi-annual report to AREC on all animal births/deaths for monitoring purposes.

Please note: There must be adherence to national and institutional COVID-19 regulations and guidelines at all times. Researchers will be personally responsible and liable for non-adherence to national regulations. If in doubt, please contact the Research Ethics Chair and/or the University Dean of Research for advice.

Please note: Any Veterinary and Para-Veterinary procedures must be conducted by a SAVC registered VET or SAVC authorized person.

Any alteration/s to the approved research protocol, i.e Title of Project, Location of the Study, Research Approach and Methods must be reviewed and approved through the amendment/modification prior to its implementation. In case you have further queries, please quote the above reference number.

Please note: Research data should be securely stored in the discipline/department for a period of 5 years.

The ethical clearance certificate is only valid for a period of one year from the date of issue. Renewal for the study must be applied for before 25 January 2023.

I take this opportunity of wishing you everything of the best with your study.

Yours faithfully

[redacted]

Dr Sanil D Singh, BVSc, MS, PhD
Chair: Animal Research Ethics Committee

/kr

cc Supervisor: Dr Thabisile Mpofana and Dr Khethelo Richman Xulu
cc BRU Manager: Dr Jaca

Animal Research Ethics Committee (AREC)
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Addendum B: Online Animal Ethics Welfare course

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Biomedical Resource Unit

June 28, 2021

Dear Dr SD Singh
Chair: Animal Research Ethics Committee
c/o School of LMMS

RE: ATTENDANCE OF LAS COURSE

This letter certifies that Fredrick Oginga: 221017678 has completed the online Animal Ethics and Welfare course.

The 80 % pass rate was successfully achieved. The course covers the **following** topics:

- Animal Ethics and experimentation
- Biosafety
- Environmental enrichment
- Research in marine, wildlife and farm animal circumstances
- Permit regulations
- Drug usage
- Statistics in animal studies
- ARRIVE guidelines

The student may be allowed to initiate research if the relevant practical procedures were done to a level of competency that was signed off by the veterinarian in charge.

Kind Regards

[redacted]

Dr L A Bester
Course Coordinator

28/6/21
Date

To Reduce Replace and Refine Animal Research