Novel Approaches to Modeling Depression in Female and Male Rats:

Highlighting the Significance of Age and Sex Hormones

by

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ABSTRACT

Research demonstrates that chronic stress produces a depressive-like profile in rodents, affecting several domains including, cognition, depressive-like behavior, and anxiety-like behavior. However, chronic stress leads to these outcomes in a sex-dependent manner, as young adult female rodents fail to exhibit impaired cognition and increased depressive and anxiety-like behavior following chronic stress. The primary goal of this dissertation was to reveal novel elements contributing to female susceptibility to stress-induced depressive-like presentations and possible factors that may counteract such outcomes. In chapter 2, novel stress paradigms were investigated to determine whether more robust stressors would lead to spatial memory deficits and elevated anxiety in young adult female and male rats. Results demonstrated that chronic stress impaired spatial memory in males, while the robust stressors failed to impair spatial memory in females. Chapter 3 revealed that both females and males in chapter 2 showed BLA dendritic hypertrophy days following the stressor without hippocampal alterations, with the latter likely due to the passage of time allowing for restructuring. Consequently, chapters 4 through 6 were conducted to investigate whether females would show chronic stress effects at middle-age in ovariectomized (OVX) females because menopause is a period of high vulnerability to cognitive and depressive-like effects. Chapter 4 investigated whether the stress hormone, corticosterone, would impair spatial working

memory and increase the depressive-like profile of OVX, middle-aged female rats, which was confirmed using the radial arm water maze (RAWM), sucrose preference (SP), forced swim test (FST), and elevated plus maze (EPM). Chapter 5 investigated if estradiol (E2) may prevent the negative valence outcomes induced by OVX in middle-aged female rats. However, E2 showed antidepressant properties during FST, but failed to do so in other behavioral tasks. Chapter 6 further explored E2's role in mitigating corticosterone-induced effects on cognition and mood in middle-aged female and male rats, with more pronounced antidepressant effects in females. Notably, this chapter unveiled a novel correlation between spatial memory and anxiety-like behavior in corticosterone-treated female rats. Collectively, these studies delineate a corticosterone-based model of depression in female rodents and introduce a novel approach for analyzing variables across multiple behavioral domains.

DEDICATION

To my cherished friends and beloved family, your unwavering support has been the cornerstone of my belief in making a meaningful impact in this world. To my brothers, Thad and Moe, whose guidance has shaped my understanding of manhood and the pursuit of dreams. Darren, Nick, and Brandon, your steadfast companionship from the outset has been invaluable. And to St. Mark's Lutheran Church, your presence throughout my academic journey has not only made you family but has also imparted invaluable lessons on what it means to be a compassionate and noble individual.

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It's me and you forever Squirrel.

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CHAPTER 1

GENERAL INTRODUCTION

Major Depressive Disorder (MDD) stands as one of the most prevalent neuropsychiatric ailments, affecting millions of individuals globally each year and imposing significant economic and healthcare challenges (Q. Liu et al., 2020). Hallmark symptoms of MDD are (1) depressed mood and/or (2) anhedonia or loss of interest or pleasure. Other MDD symptoms may include, comorbid anxiety, diminished ability to focus, feelings of worthlessness or excessive guilt, insomnia/hypersomnia, psychomotor agitation/retardation, significant weight loss or weight gain and recurrent thoughts of death or suicide. To be diagnosed, an individual must be experiencing significant distress in important areas of functioning with at least one hallmark symptom and a minimum of five symptoms present during the same 2-week period (American Psychiatric Association, 2013). Many therapies are available to treat MDD, but despite the wide variety of treatments, antidepressants are only efficacious in 30%-40% of depressed patients (Blackburn, 2019). Additionally, current antidepressant treatments have multifaceted mechanisms of action, which remain unclear (B. Liu et al., 2017). Moreover, about 50% of people treated relapse after one episode of MDD and that percentage increases further after each additional episode (Beshai et al., 2011; Biesheuvel-Leliefeld et al., 2015; Richards, 2011). The substantial failure rate and frequent instances of relapse in antidepressant treatment underscore the critical need for ongoing research aimed at identifying innovative therapeutic targets.

Women are almost twice as likely than men to suffer from MDD (National Institute of Mental Health, 2017), and show a few timepoints across the lifespan where MDD incidences are particularly noteworthy. Sex differences in heightened vulnerability for MDD are first detected with the onset of puberty, and spike again with pregnancy and the postpartum period. Finally, women show MDD vulnerability during the menopausal transition or the perimenopause phase where ovarian hormones fluctuate before ceasing (H. G. Burger et al., 1995; Santoro et al., 1996). These periods of life have in common the fluctuation of ovarian hormones, which are critical for reproduction, as well as maintenance of neuronal functioning. Clinical research has highlighted the involvement of ovarian hormones, such as 17β-estradiol (E2), in vulnerability to MDD episodes, particularly in aging populations (K. Albert et al., 2020; K. M. Albert & Newhouse, 2019; Newhouse & Albert, 2015). Portions of my dissertation are focused on aging populations and are particularly concerned with focusing on the middle-aged demographic when MDD rates are high in women, especially as they enter menopause.

Stressful life events and the subsequent release of stress glucocorticoid (GC) hormones, such as cortisol in humans and corticosterone (CORT) in rodents, trigger or exacerbate MDD episodes (De Kloet, 2004; De Kloet et al., 2005). Major risk factors for the development of MDD include stressful life events, which are suggested to predispose individuals for biased cognitive and behavioral patterns that are associated with the development of MDD (Caspi et al., 2003; S. Cohen et al., 2007). These biased psychological factors include the tendency to ruminate, irrational beliefs and maladaptive

schemas, which are considered to be factors that play a critical role in MDD pathology (Eberhart et al., 2011; Kircanski et al., 2012; Łosiak et al., 2019). Clinical studies also report that a significant proportion of MDD patients present hypercortisolemia (or elevated cortisol levels) and/or cortisol arrhythmicity (Herbert, 2013; Iob et al., 2020; Sachar & Baron, 1979). In those with remitted MDD, a dexamethasone test can be utilized to ascertain cortisol responsivity and predicts MDD relapse for who show GC hyper-responsivity (Zobel et al., 2001). Further, the cognitive dysfunction that is commonly expressed with MDD patients (Austin et al., 2001; Gotlib & Joormann, 2010; LeMoult & Gotlib, 2019) may be triggered or exacerbated by stress and GCs (Brown et al., 1999; Hammen et al., 2009; Lee et al., 2007). For example, in some studies of depressed patients, memory impairments correlate with elevated systemic cortisol (Egeland et al., 2005; Van Londen et al., 1998). Additionally, limbic brain structures rich in GC receptors are commonly changed in MDD patients compared to controls (Myers et al., 2014). In particular, compared to controls, MDD patients exhibit smaller volumes in brain regions such as the hippocampus (Bremner et al., 2000a; Colla et al., 2007a; Coryell & Young, 2005; MacQueen et al., 2003; Sheline et al., 2003) and medial prefrontal cortex (Coryell & Young, 2005; Frodl et al., 2008; Konarski et al., 2008). In contrast, MDD patients often display smaller amygdala volume compared to controls (M. J. Rubinow et al., 2016; Saleh et al., 2012; van Eijndhoven et al., 2009). Consequently, stress and GCs may trigger MDD, the progression of which may lead to brain-region specific structural alterations.

Animal models can be crucial to understand underlying MDD mechanisms and brain targets, which can help identify novel treatments and therapeutic targets. One useful model to study MDD involves chronic stress in rodents (Nestler & Hyman, 2010; Willner & Mitchell, 2002). Following chronic stress, rodents show behaviors that are depressive-like, such as reduced interest in pleasurable activities and elevated helplessness, outcomes that can be reversed with antidepressant treatment (Willner, 2005). For example, rodents readily consume sweets and chronic stress reduces sucrose consumption as a sign of anhedonia in the sucrose preference task (M. Y. Liu et al., 2018; Willner et al., 1987). In the forced swim test (FST), chronically stressed rodents spend less time actively swimming and more time passively floating to suggest learned helplessness (Cryan et al., 2002; Nestler et al., 2002). In both the SP and FST, classical and fast-acting antidepressant medications can reverse the depressive-like phenotype (Slattery & Cryan, 2012; Willner et al., 1987). Chronic stress also results in impaired cognition. Following chronic stress, rodents show impaired performance on various mPFC-mediated working memory tasks (Gaelle et al., 2019; Hoffman et al., 2011; Mika et al., 2012a; Mizoguchi et al., 2000). In addition to the behavioral impact, chronic stress alters neurons in brain regions underlying MDD (Nestler et al., 2002). In males, chronic stress induces a retraction of dendritic arbors in the mPFC (Garrett & Wellman, 2009; Goldwater et al., 2009; Perez-Cruz et al., 2007) and hippocampus (Conrad, 2006; Conrad et al., 2001; Ortiz et al., 2015), an outcome that increases in complexity when stress ends and a period without stress exposure is allowed (Bloss et al., 2010; Hoffman et al., 2011; Ortiz et al.,

2014; Radley et al., 2005; Sousa et al., 2000b). Chronic stress also decreases brain derived neurotrophic factor (BDNF) in the hippocampus, a protein that is critical for synaptic, morphological and cognitive plasticity (Gourley & Taylor, 2009; Lakshminarasimhan & Chattarji, 2012; Ortiz et al., 2014, 2018). Of note, MDD patients also display decreased BDNF serum levels (Kishi et al., 2018) as well as reduced BDNF levels in the mPFC and hippocampus (Dunham et al., 2009; Dwivedi et al., 2003; Sheldrick et al., 2017; Youssef et al., 2018). Furthermore, chronic stress reduces hippocampal neurogenesis, as well as new cell differentiation and survival (Cameron & Schoenfeld, 2018; Pham et al., 2003; Podgorny & Gulyaeva, 2021; Snyder et al., 2009, 2011). In contrast to the mPFC and hippocampus, chronic stress leads to dendritic hypertrophy within the amygdala that persist through weeks of post-stress recovery (Patel et al., 2018; Peay et al., 2023; Vyas et al., 2002, 2006). Chronic stress in adult rodents produces consistent behavioral and neuronal alterations that align with clinical MDD. Thus, chronic stress in adult rodents is a useful tool in understanding components of MDD neural underpinnings.

Sex differences following chronic stress in rodents for some cognitive functions remain perplexing because the effects are mainly found in males, but not in females. Cognitive fog is a core symptom of MDD in both males and females, and so it may be the case that males and females exhibit different cognitive dysfunction. Moreover, a majority of the preclinical work is performed in males, although this pattern is changing. While women are nearly twice as likely as men to be diagnosed with MDD (Heller, 1993;

Weissman et al., 1993), chronically stressed female rats seem to resist these changes (such as dendritic retraction), as it pertains to the hippocampus (Conrad et al., 2012; L. A. Galea et al., 1997a). Structures such as the amygdala may offer greater insight to the sex differences in neuronal response to stress. Though less is known of the impact of chronic stress on female amygdala neurons, reports suggest that they are less responsive to chronic stress compared to males (Lin et al., 2009). Ovary-intact, young adult females exhibit resilience in the face of chronic stress, future studies should focus on manipulations and age groups that may help identify female vulnerability.

My first study and master's thesis project as a graduate student and presented in Chapter 2 aimed to address the lack of chronic stress effects on spatial ability in female rodents. Chronic restraint stress commonly impairs males on a variety of spatial tasks such as the object placement, Y-Maze and water mazes (Conrad, 2010; Conrad et al., 1996a; Ortiz et al., 2015; Wright & Conrad, 2005), effects often not found in females (Duarte-Guterman et al., 2015; Jaric et al., 2019; V. Luine et al., 2017; McLaughlin et al., 2005). One concern with the chronic restraint paradigm in our lab is that with repeated exposure, animals begin to exhibit blunted stress responses, which produces attenuated serum CORT levels (Babb et al., 2014; Grissom & Bhatnagar, 2009). Some argue that this attenuation reflects an adaptive process that leads to increased predictability and control over the challenge (Grissom & Bhatnagar, 2009). As a result, some believe that as a stressor becomes predictable, it fails to be a stressor (Koolhaas et al., 2011). To overcome this potential issue, one study implemented a modified restraint paradigm over

10 days, which led to potentiated serum CORT levels and also elevated anxiety levels (W. Zhang et al., 2014). Consequently, our lab sought to determine whether this modified chronic stressor may lead to behavioral and morphological changes in both male and female young adult rats when applied to an extended duration to a minimum of three weeks, a time frame that is necessary to produce cognitive deficits (McLaughlin et al., 2007). In a series of studies with multiple variations of chronic stressors (increasing unpredictability, intensity and duration), we found that females still failed to show impaired spatial memory, even though males expressed spatial memory deficits (Peay et al., 2020). It is important to highlight that most of the studies described thus far used young adult, ovary-intact female rodents. Since the clinical literature suggests that the female risk for MDD increases at key timepoints in life, where sex hormones fluctuate, such as pubertal onset, pregnancy, and menopause, then perhaps a strategic use of a key age group in female rats, such as middle age, may be useful to identify differences in the cognitive domain impacted.

Another concern is that chronic stress-induced spatial memory deficits in males are transient and show improvements in the days and weeks following the end of the chronic stressor. Studies using various chronic stress models show that spatial memory begins to improve in the weeks following the end of chronic stress (Hoffman et al., 2011; V. Luine et al., 1994a; Ortiz et al., 2014, 2015; Peay et al., 2020; Sousa et al., 2000b). Moreover, hippocampal dendritic arbors increase in complexity from five to ten days following the termination of the chronic stressors (Conrad et al., 1999). A consistent

finding following chronic stress is that males exhibit spatial memory deficits, which improve with the passage of time after the chronic stressor ends, highlighting the limited opportunity for behavioral assessment. This improvement phenomenon puts constraints on repeated testing and highlights the value of modified stress paradigms that allow for multiple behavioral tests.

A third concern is that chronic stress impacts brain regions and circuitry differently in females compared to males. In addition to the hippocampus, stress-related disorders are associated with structural and functional differences of the mPFC (Bremner et al., 2007; Drevets, 2000; Jin et al., 2020). Research shows that male and female mPFC-mediated learning are equally vulnerable to chronic stress effects (Anderson et al., 2020) and some studies even show that the mPFC in females is more sensitive than in males to chronic stress (Maeng et al., 2010; Maeng & Shors, 2013; Shansky et al., 2006). Furthermore, dendritic arborization in the female mPFC is especially sensitive to chronic stress (Garrett & Wellman, 2009) as well as E2 fluctuations (Gerrits et al., 2006). Given that females are resilient to stress-induced hippocampal changes, but show changes in the mPFC (Duman, 2017; Garrett & Wellman, 2009; Jin et al., 2020), then the mPFC may be important in MDD pathology of females and its function is a target for some of the behavioral tasks used in my dissertation.

Rationale for Dissertation

The overarching goal of this dissertation was to gain a better understanding of female susceptibility to cognitive dysfunction and depressive-like behavior following

chronic stress. Research shows that chronic stress impairs male, but not female spatial memory in young adult rodents and this is underscored by the published results in Chapter 2 (Peay et al., 2020). Chapter 3 investigated the dendritic morphology of two brain regions that are highly susceptible to chronic stress effects, the hippocampus and amygdala, from the male and female rats investigated in Chapter 2, days removed from the stress paradigm. Published results from Chapter 3 demonstrate that chronic stress leads to sustained amygdalar neuron hypertrophy in both male and female rats to show that the stressors impacted female physiology despite the failure to demonstrate spatial memory deficits (Peay et al., 2023). Furthermore, Chapter 3 also showed that chronic stress failed to alter dendritic morphology in CA3 hippocampal neurons of both sexes when 8 days passed following the end of stress (Peay et al., 2023). This finding narrows the window by which stress-induced, hippocampal-mediated effects can be measured, such as spatial reference memory impairment (Ortiz et al., 2014; Ortiz & Conrad, 2018). Studying spatial working memory deficits in females is an area of focus, as clinical research demonstrates spatial and non-spatial working memory deficits in both male and female patients (Altemus et al., 2014; Du et al., 2021; Egeland et al., 2005; Galkin et al., 2020; Nikolin et al., 2021). These results may suggest that young adult, ovary-intact rodents may be less than ideal to understand MDD pathology in females and highlight the limits of using chronic stress combined with a behavioral battery of assessments. Since key ovarian transitions are important as it relates to MDD, chapters 4 through 6 focused on a timepoint that is highly vulnerable, around the age of menopause, or middle age. The

OVX model was implemented as it offers a "blank slate", without endogenous ovarian hormones that may obscure effects from the manipulations (Mennenga & Bimonte-Nelson, 2013; Prakapenka et al., 2018). This arrangement afforded the ability to study behavioral consequences of CORT and E2, both independently and in combination to understand their contributions to cognitive and depressive-like outcomes.

Chapter 4 investigated the depressive-like effects of CORT, including cognition and anxiety, in OVX middle aged female rats. Chronic CORT administration offers many benefits compared to other chronic stress paradigms. One benefit of CORT administration is that it allows for multiple behavioral assessments without disrupting the "stressor." This advantage avoids post-stress modifications of brain plasticity when stressors end and instead, facilitates investigation of these hormone effects over an extended duration. Another advantage of CORT administration is that exogenous CORT provides an opportunity to directly identify how CORT contributes to stress-related change in behavior and neuronal function (Darcet et al., 2014; Magariños et al., 1998; Nacher et al., 2004). In addition, rodents exposed to chronic CORT exhibit robust and highly reproducible depressive-like behaviors and heightened anxiety (Gourley et al., 2008; McEwen, 2017; Nestler et al., 2002; Pazini et al., 2016; Willner, 2005; Xie et al., 2018), with these effects being blocked or reversed with classical antidepressants, such as fluoxetine, or a single dose of fast-acting ketamine (David et al., 2009; Fukumoto et al., 2017; Pazini et al., 2016). Chronic CORT treatment also results in cognitive deficits, such as in spatial working memory performance (V. N. Luine et al., 1993; McLay et al., 1998),

and acquisition of reward-related learning in operant tasks (Olausson et al., 2013). Finally, chronic CORT administration is associated with several neurobiological changes seen in MDD models including disrupted hippocampal neurogenesis, cell proliferation and survival of new cells as well as decreased BDNF in brain regions such as the mPFC and hippocampus (Demuyser et al., 2016; Miller & Hen, 2015; Wong & Herbert, 2006). In the amygdala, CORT treatment results in dendritic hypertrophy and elevates the expression of memory-related genes (Mitra & Sapolsky, 2008; Monsey et al., 2014; Wong & Herbert, 2006). Consequently, CORT exposure in rodents can be a valuable tool to understand characteristics of MDD.

Chapter 5 explored the potential of E2 to ameliorate negative valence outcomes in OVX middle-aged female rats. E2 has long been suspected to modulate MDD vulnerability in women (K. M. Albert & Newhouse, 2019). Estrogen therapy generally reduces the severe fluctuations in mood during perimenopause (Altshuler et al., 2001; De Novaes Soares et al., 2001; Payne, 2003) and clinical studies show that E2 may even act as an antidepressant (De Novaes Soares et al., 2001). E2 modulates systems implicated in the pathophysiology of MDD and these include neurotransmitter deficiency, neuroplasticity, inflammation and stress responsivity (D. R. Rubinow et al., 2015). Furthermore, E2 regulates synthesis, metabolism and the receptor trafficking of classical neurotransmitters implicated in MDD such as serotonin, dopamine and norepinephrine (Alyea & Watson, 2009; Herbison et al., 2000; D. R. Rubinow et al., 1998). Additionally, E2 regulates both basal and stimulated HPA axis activity and acts opposite to stress in

stimulating BDNF (Figueiredo et al., 2002; Roca et al., 2003; Srivastava et al., 2013; Weiser & Handa, 2009). Chronic E2 treatment prevents stress induced hippocampal CA3 dendritic retraction in OVX rats and elevations in E2 are associated with increased BDNF levels in both the hippocampus and mPFC (Cavus & Duman, 2003; Gibbs, 1998; Karisetty et al., 2017; V. Luine & Frankfurt, 2013). E2 rapidly triggers cell signaling and epigenetic processes to produce downstream alterations in gene expression, local protein synthesis, synaptic physiology, and dendritic morphology (Akama & McEwen, 2003; Frick & Kim, 2018; Phan et al., 2012; Z. Zhao et al., 2010). In parallel, the natural, cyclic endogenous fluctuations of E2 levels in preclinical models, correspond with similar alterations in depressive-like behavior. When E2 levels are high in the proestrus phase of the female rodent estrous cycle, depressive-like behavior is minimal. When the endogenous source of E2 is removed via OVX in rodents, depressive-like behavior is increased, which can be reversed by the subsequent administration of E2 (Bowman et al., 2002; C. A. Frye & Walf, 2004; V. N. Luine et al., 1998; Walf & Frye, 2005, 2006). Furthermore, in models of postpartum depression when E2 levels are low, depressive-like behavior is increased (L. A. M. Galea et al., 2001). Altogether, the literature supports that idea that E2 has antidepressant properties and so studying possible interactions between CORT and E2 may provide insight into potential new therapeutic targets in the face of a stressful challenge.

Apart from E2's beneficial effects on mood, E2 can also improve cognition under certain circumstances. E2 is the most potent and prevalent circulating estrogen and

regulates the function of the mPFC, hippocampus, amygdala and other regions that facilitate memory-consolidation processes (Taxier et al., 2020a). Research demonstrates that systemic E2 administration in young OVX rodents enhances spatial working memory in water maze tasks (Bimonte & Denenberg, 1999; Daniel et al., 1997). Moreover, systemic E2 also enhances spatial reference memory in maze and object exploration tasks (Gresack & Frick, 2006; V. Luine & Frankfurt, 2020; V. N. Luine et al., 1998; McLaughlin et al., 2008). Researchers also found that the effects of E2 vary by brain region, with benefits in spatial working memory observed following infusion of E2 into both the mPFC as well as the hippocampus (Frick, 2009). In addition to impacting spatial and working memory, E2 is also a potent modulator of social memory, a crucial ability in social species, such as rats (Ferguson et al., 2002). Multiple studies demonstrate that both natural and exogenous E2 significantly improve social recognition memory (Gabor et al., 2012; Hlinak, 1993; Karlsson et al., 2016; Phan et al., 2012; Sánchez-Andrade & Kendrick, 2011; Spiteri & Ågmo, 2009; Tang et al., 2005). Aging models are valuable in understanding the neurobiological impacts of E2. In middle-aged, but not aged (20-month-old or older) OVX rodents, systemic E2 administration also enhances spatial working and recognition memory (Daniel et al., 2006; Markowska & Savonenko, 2002; Prakapenka et al., 2018; Singh et al., 1994; Talboom et al., 2008; Vaucher et al., 2002). The fact that middle-aged, but not senior, rodents can benefit from E2 treatment, highlights the critical window for E2 to facilitate cognition and further validates the use of middle-aged female rats.

Chapter 6 aimed to investigate the adverse effects of CORT on depressive profile, and the benefits that E2 treatment may offer, in gonadectomized (GDX), middle aged female and male rats. The goal of this investigation was to determine whether CORT and E2 have interactive effects on domains of depressive-profile, including cognition, depressive-like behavior and anxiety in middle-aged female and male rodents without their gonadally-derived sex hormones. The removal of gonadal hormones enables investigation to directly assess the effects of CORT and E2 in a model that is a "blank slate" (Mennenga & Bimonte-Nelson, 2013; Prakapenka et al., 2018). Cognition was assessed with RAWM spatial working memory, depressive-like behavior was assessed with SP and social exploration testing, and anxiety profile was measured with defensive marble bury and EPM assessments. In addition to traditional analyses, Spearman correlations were implanted in order to gain further insight to relationships between behavioral variables in the various depressive domains including cognition, depressive-like behavior and anxiety-like behavior.

CHAPTER 2

This chapter was published in Behavioural Brain Research in 2020 and is titled:

CHRONIC UNPREDICTABLE INTERMITTENT RESTRAINT STRESS DISRUPTS SPATIAL MEMORY IN MALE, BUT NOT FEMALE RATS

ABSTRACT

Chronic stress leads to sex-dependent outcomes on spatial memory by producing deficits in males, but not in females. Recently it was reported that compared to daily restraint, intermittent restraint (IR) produced more robust stress and anxiety responses in male rats. Whether IR would be sufficiently robust to impair hippocampal-dependent spatial memory in both male and female rats was investigated. IR involved mixing restraint with non-restraint days over weeks before assessing spatial memory and anxiety profile on the radial arm water maze, object placement, novel object recognition, Y-maze, open field and novelty suppressed feeding. Experiments 1 and 2 used Sprague-Dawley male rats only and determined that IR for 6hrs/d (IR6), but not 2hrs/d, impaired spatial memory and that task order was important. In experiment 3, IR6 was extended for 6wks before spatial memory testing commenced using both sexes. Unexpectedly, an extended IR6 paradigm failed to impair spatial memory in either sex, suggesting that by 6wks IR6 may have become predictable. In experiment 4, an unpredictable IR (UIR) paradigm was implemented, in which restraint duration (30 or 60-min) combined with orbital shaking, time of day, and the days off from UIR were varied. UIR impaired spatial memory in males, but not in females. Together with other reports, these findings support the interpretation that chronic stress negatively impairs hippocampal-dependent function in males but not in females. We interpret these findings to show that females are more resilient to chronic stress than are males as it pertains to spatial ability.

1. Introduction

Major Depressive Disorder (MDD) affects more than 300 million people worldwide and is the leading cause of global disability (American Psychiatric Association, 2013; WHO, 2017). Despite the wide variety of interventions, approximately a third of MDD patients fail to improve with treatment (M. B. Keller, 2005; Souery et al., 1999), emphasizing the need to identify novel mechanisms for potentially new therapeutic targets. Chronic stress in preclinical research is commonly used to study depressive-like symptoms (Nestler & Hyman, 2010; Willner, 1991; Willner & Mitchell, 2002) and the respective changes in cognitive function (De Kloet et al., 2005; M. F. Marin et al., 2011). While no one animal model produces all symptoms of MDD, together they can offer new novel insights (Lapiz-Bluhm et al., 2008).

Chronic restraint is a common manipulation in rodents, but also carries some caveats. Restraint is relatively cost-effective, has readily available materials for construction, and is straightforward to implement. Restraint also produces fairly consistent outcomes across animals, which is not always the case for paradigms that require two animals to engage, such as with social defeat (Koolhaas et al., 1997; Martinez et al., 1998). Some caveats include the concern that restraint stress is not ecologically relevant (Koolhaas et al., 2006); however, this is less of an issue when the goal is to induce neurobiological changes in certain limbic structures, such as the hippocampus, before initiating behavioral assessments. In addition, chronic restraint is a homotypic (i.e., a repeat of the same type of) stressor, leading to adrenal response habituation in

which the stress steroid, corticosterone, levels in the blood become less pronounced than compared to the first restraint exposure (Babb et al., 2014; Grissom & Bhatnagar, 2009; Koolhaas et al., 2011). Again, this is less of a concern because the muted corticosterone response aligns with adrenal dysregulation found in patients with MDD (Grissom et al., 2007; Grissom & Bhatnagar, 2009; Jean Kant et al., 1985; Marti & Armario, 1997; Pitman et al., 1988; Stamp & Herbert, 1999). Consequently, restraint stress in rodents produces a subset of outcomes that align with those found in MDD, especially as they pertain to the hippocampus and stress responsivity.

A puzzling outcome following chronic daily restraint is the sex differences observed in spatial ability. In male rodents, chronic restraint stress compromises the hippocampus and impairs hippocampal-dependent spatial learning and memory (Conrad, 2010; Conrad et al., 1996b; Kleen et al., 2006; V. Luine et al., 1994a; Sunanda et al., 2000; Wright & Conrad, 2005, 2008). In contrast, female rodents fail to show hippocampal-dependent memory deficits following chronic restraint (Conrad et al., 2003; Kitraki et al., 2004; V. Luine, 2002; V. Luine et al., 2017) or even other chronic stressors (Ortiz et al., 2015; Wei et al., 2014; Yuen et al., 2016). Instead, female rodents almost seem to be resilient in the face of chronic stress, and may even show improved spatial ability in the Morris Water Maze, Y-maze and Radial arm water maze (RAWM), (Beck & Luine, 2002; Bowman et al., 2001, 2003; Conrad et al., 2003, 2012; Kitraki et al., 2004; McFadden et al., 2011a; Ortiz et al., 2015). The concern is that in humans, women are nearly twice as likely as men to be diagnosed with MDD, even when accounting for

willingness to seek out help (Heller, 1993; Weissman et al., 1993). Consequently, one aim is to identify a chronic stress paradigm that leads to cognitive deficits in female rodents.

When characterizing the behavioral phenotype in animal models, obtaining several behavior measures is helpful. For that reason, behavioral batteries using multiple tests can be advantageous in order to examine different aspects of the spatial memory domain and cognitive abilities. This requires measuring cognition over multiple days, but the timeline of the daily restraint paradigm is restrictive because spatial memory deficits begin to improve in the days and weeks after chronic stress has ended (Hoffman et al., 2011; V. Luine et al., 1994b; Sousa et al., 2000a). Given the limited window of time to capture cognitive deficits following chronic restraint, identifying a paradigm that allows for multiple cognitive assessments during this window may be of great benefit.

Recently, Zhang and colleagues (2014) found that a modified restraint model using a work-week design, produced stress and anxiety responses that were greater than that observed with chronic daily restraint for the same duration. Specifically, in male Sprague-Dawley rats, restraint for 20 minutes/day in a hemi-cylinder for 5 days, followed by two days off, and then with restraint for two more days produced robust effects on stress responses, body weight gain and anxiety levels than compared to restraint daily for the same period. This raised the question as to whether the robust nature of this interrupted restraint stress paradigm may be useful in producing more substantial effects on spatial memory in both male and female rats.

While this interrupted restraint paradigm has the potential to be a more robust stressor than daily restraint, many questions remain as it pertains to the way it is used to assess spatial memory. The goal of the current series of experiments was to use a modified version of the interrupted restraint paradigm described by Zhang et al., (2014), which we termed intermittent restraint (IR), on hippocampal function in both male and female rodents. First, it was unclear whether an extended duration of three weeks (instead of nine days as used by Zhang et al., (W. Zhang et al., 2014)) would have similar potentiating effects on impairing hippocampal function in males when compared to the daily restraint paradigm. Second, Zhang and colleagues (2014) used plastic hemi-cylinders to restrain rats for just 20 min each day; however, past work from our team found that daily restraint in wire mesh for 2 hours/day for three weeks failed to impair spatial memory in males (McLaughlin et al., 2007). Consequently, experiment 1 included intermittent restraint for two-hours (IR2) and six-hours (IR6), as well as the traditional daily restraint paradigm for six hours/day (DR6) and compared outcomes on spatial ability in male rats. In addition, the IR may produce a more robust deficit on spatial memory and so a behavioral battery was incorporated in the event that spatial memory impairments persisted beyond the few days after restraint ended. Hence, experiments 1 and 2 used a behavioral battery in male rats, with experiment 2 testing from the least to most aversive task. Experiment 3 tested both male and female rats using an extended IR6 paradigm for a minimum of 6 weeks. Experiment 4 implemented an unpredictable intermittent restraint (UIR) paradigm in both sexes, with behavioral testing occurring on

days when UIR was not used. We tested the hypothesis that IR and/or UIR would produce spatial memory deficits in both male and female rats and that these deficits would be long-lasting.

2. Materials and methods

2.1. Subjects.

Arizona State University Institutional Animal Care and Use Committee approved the procedures, which align with the Guide for the Care and Use of Laboratory Animals. Male and Female Sprague-Dawley (Charles River Laboratories, Hollister, CA, USA) rats weighing approximately 200-225 grams upon arrival were pair housed in standard laboratory cages (21-22 °C, corncob bedding). Male and female rats were housed in same-sex housing units and were tested on separate days or in different rooms. Except where noted below, animals were allowed food and water *ad libitum*. Animals were housed on a reverse 12:12 light cycle; lights off at 07:00. All procedures occurred during the dark phase of the light cycle.

2.2. Chronic stress procedure

Rats were chronically stressed by wire mesh restraint. Restrainers were constructed from wire mesh (19 cm diameter × 26.5 cm long for males, 16.5 cm diameter x 26.5 cm long for females, aluminum screen wire Model #3001120, Lowes) with the cut

edges and ends sealed with Plasti Dip (Performix #075815116024). Once rats were placed in the restraint, the ends were secured with black binder clips (Staples Inc., Framingham, MA, USA). Animals were upgraded to larger wire-mesh restrainers as they grew (21.5 cm diameter x 29 cm long for males, 19 cm circumference x 26.5 cm long for females). Control rats (CON) were always housed in a chamber separate than the stressed rats in order to reduce the likelihood of communication through odor, sounds and sight. To maintain similar handling procedures and access to food and water across groups, CON rats were handled daily, and food restricted for the same duration as the restrained rats. Body weights were measured weekly for all groups. For experiments 1-3, restraint occurred between the hours of 09:00 and 15:00 of the dark phase of the light cycle. In experiment 4, restraint occurred between the hours of 07:00 and 21:00. One to three days following the last behavioral testing day in Experiments 1 and 4, rats were euthanized using isoflurane and rapidly decapitated. Adrenal glands, thymus and uterus were excised and weighed for a secondary measure of stressor effectiveness.

2.3. Treatment conditions

Experiment 1: Effects of three weeks IR2 and IR6 on spatial memory in male rats. One stress group was restrained for 6h/day for 21 consecutive days (daily restraint, 6-hours/d, DR6), another was restrained for 6h/day in an interrupted pattern: 5 days restrained then two days without restraint over a period of 23 days (Intermittent restraint, 6-hours/d, IR6) and the third was restrained for 2h/day over a period of 23 days (IR2).

The sum total of restraint days for DR and IR was 21 and 17 days, respectively. CON rats were not restrained. Each group, n=12 for a total of 48 rats.

Experiment 2: Effects of three weeks IR6 on a behavioral battery, ordered with the least aversive task first and using male rats. Three treatment conditions were used: CON (n= 14), IR6 (n= 12) and DR6 (n=12). DR6 and IR6 restraint were described in experiment 1.

Experiment 3: Effects of an extended IR6 paradigm on spatial memory in male and female rats. IR6 was used on half the male and female rats and was performed for 6 weeks before the first spatial task was implemented. Details are listed in experiment 1. Each group (n= 12) for a total of 48 rats. One female rat died from unrelated complications during the experiment, and so her behavior was included up until that point.

Experiment 4: Effect of unpredictable intermittent restraint (UIR) on spatial memory in male and female rats. The stressor was changed to be unpredictable and robust, termed "Unpredictable Intermittent Restraint" or UIR. Restraint occurred for 30 or 60 min, while on an orbital shaker (120 rpm) and at different times of day (ranging from 7:00 to 21:00) and for different consecutive day lengths (2-6 days) before one or two days off without a stressor. There were four groups (n= 12) for a total of 48 rats.

2.4. Behavioral tests

In order to minimize potential behavioral outcomes that may arise from rats being exposed to different investigators, worn/unlaundered t-shirts were located in the testing room out of the rodent's view when inside the apparatus (Sorge et al., 2014). Fans were placed in the room to provide white noise and to disperse odors. Four different testing rooms were used, and curtains drawn as needed to ensure that testing environments and cues differed across tasks. All behavior was recorded by cameras (GoPro Hero3) mounted on the ceiling. Unless otherwise noted, three home cages of pair-housed rats were carted from the animal colony and placed in an adjacent room until it was time for testing. *An investigator retrieved the home-cage from the adjacent room and brought it to the testing room.* The investigator remained behind a curtain during trials, out of sight of the rats, but could view the rats on a live video feed via a computer monitor.

2.4.1. Y-maze

The Y-maze is a task that requires hippocampal function and spatial memory to navigate (Conrad et al., 1996b; Wright & Conrad, 2005). Y-maze testing occurred over two days to accommodate the large number of rats (20-24 rats tested on each day, counterbalanced for treatment).

2.4.1.1. Y-maze apparatus.

Two Y-maze apparatuses were located in the same room and were constructed of black Plexiglas. Three identical and symmetrical arms (58.4 cm long × 20.3 cm wide x 38.1 cm height) radiated from the center. The sides were tall enough that the rats could not jump out of the maze. Outside the maze, large, explicit cues (painted shapes on the walls and furniture in the room) were located on the walls and around the room in order to encourage the use of extra-maze cues. The light intensity at the floor of the maze was 80-90 lux for the duration of testing. No explicit cues were present inside of the maze. Corncob rat bedding covered the maze floor to about 3 cm thick.

2.4.1.2. Y-maze procedure

Cage mates were tested simultaneously, in side-by-side Y-mazes. For trial 1, a rat was placed in one arm, which was then designated the "start" arm for that rat. Another arm was blocked with black Plexiglas, so the rat was able to explore the start and the other open arm, called the "other" arm. Rats were given 15 min to explore the maze and two accessible arms before they were removed, returned to their home cage and brought back to the animal colony. After each Y-maze exposure, the bedding in the maze was mixed to dissipate the odors before the next set of rats were tested. At the end of trial 1 and before the start of trial 2, the two Y-mazes were swapped so that rats would be tested in a new Y-maze, but in the exact same position as before to further reduce the likelihood that they would use intra-maze cues.

Trial 2 began after a 4-hr intertrial interval (ITI). Rats were brought back to the testing room as before and now the previously blocked arm, the "novel" arm, was open to investigation. Rats started in the same arm as in trial 1 and were given 5 minutes to explore. The start, other, and novel arms were counter-balanced across groups but held constant for a given rat.

2.4.1.3. Y-maze *quantification*

Behavior was quantified at a later date by an investigator who was blind to the treatments and novel/other arm identities. The dependent variables measured in trial 2 were the number of entries made (entry) and time spent in each arm (dwell) during each minute. An entry was defined as the forelimbs crossing from the middle of the maze into an arm entrance. The first two minutes of exploration during testing were used for analysis because rats habituate quickly to the Y-maze (Dellu et al., 1992). The start arm was not included in the analysis because the rats were placed there at the beginning of the trial, causing an inherent bias compared to the novel and other arms. Entry and dwell data were converted into percentages for all three arms (novel, start, and other), with chance being equal to 33.3%. Discrimination performance for the novel arm compared to the other arm was calculated by subtracting the percentage of entries into the other arm from the percentage of entries into the novel arm. Dwell was calculated similarly for percentage of time spent in the arms. For simplicity, only entry data are shown in the figures. Chance for the discrimination index would be 50% and with a preference for the novel arm being a value greater than 50%.

2.4.2. Open Field (OF)

OF acclimates rats to the environment in which they are to be tested on subsequent days. Moreover, OF can serve as a measure of locomotor activity and anxiety-like behavior (Prut & Belzung, 2003; Seibenhener & Wooten, 2015).

2.4.2.1. OF apparatus

The OF apparatus consisted of two side-by-side black square fields (96.5 cm x 96.5 cm) with high walls (38.1 cm height) to prevent escape and yet permitting the rats to see the extra-maze cues around the walls and room (painted geometric shapes, shelving). The light intensity at the floor of the field was 150-160 lux for the duration of OF testing and during the following testing days.

2.4.2.2. OF procedure

An investigator carted three pairs of rats from the home colony room and placed in an adjacent room until OF testing. In the OF test, rats were tested in pairs with their cage mate in an adjacent OF apparatus. Rats were allowed to explore for 10 min and then removed and returned to the animal colony. After each trial, an investigator wiped the arena with paper towels and Lime/Sea Salt Scented, Method All-Purpose cleaner.

2.4.2.3. OF *quantification*

Behavior was quantified at a later date by an investigator who was unaware of the treatment identities. The 10-min open field trial was quantified in two, 5-min blocks,

utilizing a 4 x 4 grid. The first 5-min block was utilized for analyses because rats readily habituate to OF testing (Brenes et al., 2009; Walsh & Cummins, 1976), but the full ten minutes provided rats with time to acclimate to the arena and room. Peripheral crossings were quantified as the front two paws crossing a line on the periphery of the grid. Central crossings were quantified as the front two paws crossing a center gridline. An anxiety index was calculated as used in our past work (Nishimura et al., 2017):

$$1 - \frac{(\text{Center crossings} / \text{Total crossings}) + (\text{Time in center}/300)}{2}$$

Total locomotor activity was scored as the number of line crossings (front two paws crossing any line).

2.4.3. Novel Object Recognition (NOR) and Object Placement (OP)

NOR can assess a type of memory that does not necessarily require the hippocampus (Balderas et al., 2008; Barker & Warburton, 2011; Mumby, 2002) and was used to provide minimal cognitive challenge with just 1 minute inter-trial-interval (ITI). Experiment 4 also implemented a 1-hour ITI NOR test as an added measure of cognitive ability. OP testing assesses hippocampal-mediated spatial memory in which rats use the spatial context to detect familiar objects in new locations (Ennaceur et al., 1997; Mumby, 2002; Nishimura et al., 2017; Spanswick & Sutherland, 2010). A 1-hour ITI in OP has been shown to be effective in assessing hippocampal mediated deficits (de Bruin et al.,

2011; Pitsikas et al., 2007). For this reasoning, a 1-hour ITI was utilized for OP testing in the experiments in this study.

2.4.3.1. NOR and OP apparatus

NOR and OP occurred in the same OF arena in which rats were previously acclimated and in a testing room under similar conditions. When shifting from one task to the next, the OF remained in the same location, but vinyl curtains were used to obscure/change the cues in the room.

The objects were sufficiently large so that rats could not climb or topple them and were made of ceramic, metal, or glass for easy cleaning. Each object had at least four duplicates so that they could be swapped quickly. The objects included a plastic red opaque rectangle (24 cm high x 10 cm x 8 cm), a tall, slender opaque green glass bottle (36 cm high, 9 cm wide), and a tall, slender gold rectangle attached to a heavy rectangular base (23 cm high, 6 cm wide). To ensure object stability, the objects were either filled with sand (if hollow) or secured to a heavy aluminum base. Before each trial, the objects and arena were cleaned with Method All-Purpose Cleaner. Different scented cleaners were used for OP and NOR, but the same scent was used within a session.

2.4.3.2. NOR and OP procedure

All rats were tested on the same day with pairs of cage mates being tested simultaneously in adjacent fields. During trial 1, the arena contained two identical objects. A rat was placed in a corner of the arena, away from the objects and allowed to explore for 3 min. After the trial ended, the rat was removed and returned to its home

cage with its cage mate. The arena and objects were wiped clean. After the designated ITI, rats were returned to the arena in trial 2 at the same starting location as before, but with the following differences. In the NOR, both objects were replaced, but one object was identical to the object used in trial 1 and the other was exchanged for an object that was unique. These objects were placed in the same location as was used in trial 1. In the OP, the objects were replaced with new, but identical objects to those used in trial 1; however, one object was moved to a novel location and the other remained in the same location as in trial 1. In trial 2, rats were allowed to explore the objects in for 3 min. The start locations of the rats and the object locations were counter-balanced across groups but held constant for a given rat.

2.4.3.3. NOR and OP quantification

Behavior was quantified at a later date by an investigator who was unaware of the treatment identities. Exploration was defined as the rat facing the object within 3 cm and attentively interacting with the object. Rats were excluded from analyses if they failed to explore both objects in trial 1 or explored the objects for less than 10 second total in either trial 1 or 2. An object exploration index was calculated as reported elsewhere (Nishimura et al., 2017):

$$\frac{\text{Time spent exploring novel object (NOR) or location (OP) in trial 2}}{\text{Total amount of time spent exploring both objects in trial 2}}$$

2.4.4. Radial Arm Water Maze (RAWM)

RAWM testing was conducted because of its use in measuring spatial ability in rodents (Diamond et al., 1999; Hoffman et al., 2011; Ortiz et al., 2014) and in assessing spatial learning and memory following chronic stress (Hoffman et al., 2011; Ortiz et al., 2014, 2015, 2018).

2.4.4.1. RAWM apparatus

Two RAWM mazes and rooms were used. The RAWM was constructed of black polypropylene, with eight symmetrical arms (27.9 cm long × 12.7 cm wide) originating from a circular center (48 cm diameter). The maze was filled with water and allowed to equilibrate to room temperature ranging from 20 to 22 °C. Black powder tempera paint was added to the water until its opacity was sufficient to conceal a black rubber platform placed in one of the eight arms. The testing rooms provided several prominent extra-maze cues including the door to the room, shelves, heat lamps, and cues made of black and white construction paper located on the walls.

2.4.4.2. RAWM procedure

Groups were counterbalanced between the two testing rooms and experimenters. Rats were tested in squads of 6-8 (e.g. two rats from each experimental group). Once a rat finished a trial, the other rats in the squad completed the given trial before the first rat in the squad began the next trial. Testing occurred over three days with 8 trials occurring on each of the first 2 days and a single retention trial occurring on the third day (17 total trials). Each trial consisted of releasing a rat into an arm (start arm) that did not contain

the platform, the start arm was also never directly across from the platform arm to increase the navigational demand of the task. For each rat, the start arm location varied across trials, while the platform arm remained in the same location. Once a rat reached the hidden platform, it was permitted to remain on the platform for 15 s to visualize the room spatially before being returned to its testing cage in the same room, under a heat lamp. If a rat failed to find the platform within 3 min, the experimenter used a pole to guide the rat to the hidden platform. After each trial, any debris was skimmed and removed with a net and the water stirred to reduce the likelihood of rats using non-spatial cues.

2.4.4.3. RAWM quantification

The investigator recorded arm entrances during the behavioral testing and then arm entries were quantified at a later date. An entrance was recorded when the tip of the rat's nose crossed a mark on the outside of the maze (about 22 cm into the arm). Reference memory errors were considered the number of first-time entries into arms that did not contain the platform within a given trial (first entries into the start arm were also quantified as reference memory errors). Working memory errors were considered the number of repeat entries into an arm that did not contain the platform within a given trial (i.e. repeat entries into an arm where a reference memory error was previously committed in the same trial).

2.4.5. Novelty Suppressed Feeding (NSF)

Anxiety-like behavior was assessed using a test to examine how readily rats will eat food in a novel environment (Gould et al., 2015; Snyder et al., 2011). Slow latency to approach the food and begin eating may be indicative of high anxiety.

2.4.5.1. NSF apparatus

NSF occurred in a novel testing room, but using the same dual-field apparatus as implemented for the OF, OP and NOR. The OF arena was brightly lit (170-180 lux) and located in a novel testing room without obvious spatial cues. Before each rat was tested, the arena was cleaned with 70% isopropyl alcohol.

2.4.5.2. NSF procedure

Twenty-four hours prior to NSF, rats were food deprived, but with unlimited access to drinking water. On the day of testing, *rats were carted, two pairs at a time in their home cages, to a holding room prior to testing. Then, an investigator retrieved the cage and brought it to the NSF testing arena.* In the center of the arena there was a pile of standard rodent chow. The rat was placed in one corner of the field, and the amount of time it took the rat to approach the food was recorded. If the rat did not approach the food after 8 min, the testing was terminated, and the animal was given a score of 480 sec. When completed, the animals were returned to the animal colony and placed individually in their cages for 10-minutes. In each cage was a pre-weighed piece of rat chow with water still available. The amount of food consumed during the home-cage feeding was measured to assess the motivation of the rats to eat in a familiar environment. Littermates

were re-united after the end of the home-cage eating assessment and food was provided *ad libitum*.

2.4.6. Elevated Platform (EP) stressor

When implemented seven days following chronic stress, novel acute stressors may increase activation in corticolimbic structures in female rats (Moench et al., 2019; Moench & Wellman, 2017). The impact of this novel stressor was assessed on spatial ability at the end of the fourth experiment. Seven days following the last UIR day, rats that previously underwent UIR were exposed to this EP stressor and then spatial memory using the Y-maze was tested one day after the EP stressor concluded.

2.4.6.1. EP Apparatus

An elevated platform (12 cm x 12 cm, elevated 90 cm from ground) was used as a unique stressor. EP occurred in a novel testing room.

2.4.6.2. EP procedure

The UIR rats were transported in pairs in their home cages and the rat pairs were tested simultaneously in two separate rooms. Rats were placed on the top of the EP for 30 minutes. In the event that a rat fell, an investigator immediately returned it to the platform. The EP was performed seven days after the end of UIR, based upon a study that found cognitive deficits using a similar acute stressor seven days after the end of restraint in female rats (Moench et al., 2019). One day after the EP stressor, all rats (UIR and CON), were tested in the Y-maze as described previously.

2.5. Statistical analyses

Data were analyzed using SPSS (v. 23). Analysis of variance (ANOVA) was used to analyze parametric data. Body weights were analyzed as a change in body weight from the start of the study to the end of the study, as the experimental timelines varied. Fisher's LSD post hoc tests were used when ANOVA reached significance. In some cases, planned comparisons were performed. Parametric data were represented as means ± S.E.M. For object tests and the Y-Maze, Wilcoxon Signed Rank tests were used on nonparametric data and represented as medians and quartiles. Statistical significance was defined when p-values were equal to or less than 0.05.

3. Results

3.1. Experiment 1: Effects of three weeks IR2 and IR6 on spatial memory in male rats

Two IR paradigms using 2 and 6 hours of restraint were compared with the 6 hours of daily restraint paradigm on several spatial tasks (Fig. 2.1A). Rats were tested first on the RAWM because of consistent chronic stress effects from past work (Hoffman et al., 2011; Ortiz et al., 2015), and because the most robust chronic stress effects occur in RM assessment (Hoffman et al., 2011; Ortiz et al., 2015).

As expected, all groups acquired the task rapidly, as shown by decreased errors as trials progressed (Day 1, $F_{7,301} = 13.515$, $p < 0.05$; Day 2 $F_{7,301} = 8.224$, $p < 0.05$), with no

significant main effect of group or interaction (Fig. 2.1B). Surprisingly, on Day 3 when a single retention trial was given, no significant effects were observed (Fig. 2.1C). However, the data showed high variability and so a subsequent analysis was performed comparing IR6 with CON, as we expected IR6 to be most impaired (McLaughlin et al., 2007) and errors from training day 1 and 2 were used as a covariate to reduce the variance and revealed a significant effect. IR6 made more errors than did CON on the retention test ($p < 0.05$ for group, using T1 and T17 as the within-subjects variable).

The next tests performed used an appetitive incentive in the OP, NOR and Y-maze. Unexpectedly, many of the rats failed to sufficiently explore OP and NOR, despite acclimation to the OF under similar parameters used with success in past studies in which RAWM testing was followed by object testing (Ortiz et al., 2018). In the OP, 50% of the rats failed to meet criteria and this was distributed similarly across the experimental groups (n = 6/all groups). Distributions of the OP index from individual rats were plotted (Fig. 2.1D). While the subject number is low and with insufficient power, it is notable that the group with an OP index distributed around chance levels is IR6. In the NOR with a 1-min ITI and minimal cognitive load, the subject number ranged from six (CON), eight (IR6, IR2) and nine (DR6). Wilcoxon paired analysis revealed that CON spent more time with the novel object than they did with the familiar object ($p < 0.05$, Fig. 2.1E). The NOR index from the other groups did not reach statistical significance, even though they had more rats than did the CON ($p > 0.1$ for DR6, IR6, IR2). On the Y-maze, which was performed six and seven days after the end of IR, nearly all rats explored the arms in

trial 2 with just one rat in each in CON and DR6 failing to leave the start arm. Wilcoxon paired tests showed that rats in most treatment conditions entered and spent more time in the novel arm compared to the other arm over the first two minutes for CON ($p < 0.05$), DR6 ($p < 0.05$), and IR6 ($p < 0.05$). IR2 failed to show a significant preference for the novel arm and performed at chance levels (Fig. 2.1F).

To determine whether anxiety or motor ability may have impacted performance, additional assessments were performed on the OF, OP and Y-maze. An anxiety index was calculated to determine whether the groups differed in anxiety profile regardless of locomotor activity. In the OF, the anxiety index was high and similar for all groups (greater than 90%, Fig. 2.1G). Consequently, anxiety profile was unlikely to explain differences in performance among groups. However, the groups demonstrated heightened anxiety overall, perhaps from the prior day exposures on the RAWM. This may also explain the lack of investigation for many of the rats on the OP, which requires motivation to explore. For the total time spent exploring objects during trial 2 in OP, an ANOVA revealed significant differences between groups on Trial 2 ($F_{1,3} = 3.56$, $p < 0.05$). LSD post-hoc tests showed that IR2 spent more time exploring both objects than the rest of the groups during OP ($p < 0.05$ compared to CON, DR6, IR6, Fig. 2.1H, note, no group differences were found on NOR for trial 2, data not shown). No other statistical differences were found. On the Y-maze, all groups entered a similar number of arms over the first two minutes, ranging from 6.3 ± 0.6 for CON to 7.8 ± 0.4 for IR6 (Fig. 2.1I). These OP data suggest that motor or motivation may have contributed to the IR2 group's

spatial profile on OP, but a lack of an effect on the total entries of the Y-maze suggest that motor/motivation was unlikely to contribute to spatial ability in the Y-maze. Importantly, CON and IR6 showed similar motor/motivational ability and suggests that they are similarly motivated.

In summary, patterns were observed to suggest that IR6 may have exhibited impaired spatial memory on the RAWM compared to CON, but that performance on the OP and NOR may have been obscured by high anxiety. In addition, spatial memory was displayed on the Y-maze by days 6 and 7 from the CON, DR6 and IR6, but not IR2. Given the high anxiety metric and the exploration differences on OP, the behavior for IR2 was harder to interpret than performance observed earlier on RAWM. These findings suggest that IR6 may have compromised spatial ability on the RAWM, but that this effect was not long lasting.

3.2. Experiment 2: Effects of three weeks IR6 on a behavioral battery, ordered with the least aversive task first and using male rats

The effects of the IR6 and DR6 on a behavioral battery were compared, but with the testing order starting with the least aversive task first (Y-maze) and ending with the most aversive task (RAWM, Fig. 2.2A).

On the first two days after restraint ended, rats were tested on the Y-maze. CON rats demonstrated spatial memory, whereas DR6 and IR6 did not (Fig. 2.2B). For the CON rats, Wilcoxon Signed Rank Tests indicated a significantly greater number of

entries in the novel arm than in the other arm ($p < 0.05$) as well as significantly more time spent in the novel arm ($p < 0.05$, data not shown). For the DR6 and IR6 rats, the Wilcoxon analyses failed to reveal a significant difference for entries made (or time spent, data not shown) in the novel and other arms.

The rats were tested in the NOR on the third day after the end of restraint. Since rats had just 1-min ITI, all groups were expected to recognize and spend more time with the novel object compared to the familiar object. A Wilcoxon Signed Rank Test showed that CON and IR6 rats explored the novel object significantly more than the familiar object ($p < 0.05$), an effect that was not found with the DR6 rats (Fig. 2.2C).

OP occurred on the fourth day after the end of restraint. Wilcoxon Signed Rank Tests were performed to determine whether each group explored the object in the novel location more than the object in the same location after a 1-hr delay (Fig. 2.2D). The CON rats spent significantly more time with the object in the novel location than the object in the same location ($p < 0.05$). DR6 rats performed at chance by exploring both objects similarly. Interestingly, IR6 rats explored the object in the same location more than the new location ($p < 0.05$). Additional analysis was performed to compare across groups using a 1-way ANOVA for the OP discrimination index, revealing a significant effect ($F_{2,31} = 6.200$, $p < 0.05$, Power = 0.860, Fig. 2.2D). LSD post-hoc analyses found a significant difference between the OP discrimination index for CON and IR6 rats, with CON rats having a greater OP discrimination index than did IR6 ($p < 0.05$, Fig. 2.2D).

RAWM testing began on the fifth day following the end of restraint and occurred over three days. RAWM testing has typically revealed differences in performance between chronically stress male rats and non-stressed controls (Ortiz et al., 2015, 2018). During acquisition on days 1 and 2, all three groups made fewer first time entry errors as trials proceeded (Fig. 2.2E). A repeated measures ANOVA for groups across the 8 trials on day 1 showed a significant effect of trial on first time entry errors ($F_{7,259} = 8.838$, $p < 0.05$). By day 2, a repeated measures ANOVA for groups across the 8 trials did not show a significant effect of trial on first time entry errors to suggest that the groups reached a plateau. However, when these trials were analyzed in bins of 2 trials (e.g., a repeated measure of four bins), a significant effect of bin was observed with rats making fewest errors during the last bin compared to the first ($F_{3,111} = 3.537$, $p < 0.05$). There were no other significant effects on either day 1 or 2. On the third day, a one-way ANOVA for first time entry errors was not significant to reveal that rats were making similar number of first-time entry errors (Fig. 2.2F).

To determine whether anxiety or motor ability may have impacted performance, additional assessments were performed on the OF, OP and Y-maze. A one-way ANOVA performed on anxiety index in the OF revealed significant differences ($F_{2,33} = 6.644$, $p < 0.05$, Power = 0.980, Fig. 2.2G). LSD post-hoc analyses showed that DR6 and IR6 rats expressed a higher anxiety profile than did CON ($p < 0.05$). To determine whether locomotor activity or motivation to explore the Y-maze differed across groups, total entries (sum of entries into Novel, Start and Other arms over minutes 1 and 2) were

analyzed using a one-way ANOVA. No significant differences were detected (Fig. 2.2H). The total number of entries averaged 8.1 ± 0.6 for CON, 7.5 ± 0.6 for DR6 and 7.5 ± 0.5 for IR6. Therefore, differences in spatial memory in the Y-maze were unlikely due to motivation to explore. For the OP, the total time spent exploring the objects was compared with a 1-way ANOVA and revealed no significant effects. The total time exploring objects (in seconds) averaged 31.7 ± 3.6 for CON, 28.1 ± 3.6 for DR6 and 28.8 ± 3.2 for IR6 (Fig. 2.2I).

In summary, changing the task order helped with behavioral assessment as nearly all rats explored the mazes throughout the behavioral battery. In the first task using the Y-maze, both IR6 and DR6 showed impaired spatial memory at a time when the CON rats exhibited spatial memory by entering the novel arm more than they did the other arm. As testing continued in different mazes over days, IR6 and DR6 began to show the potential to demonstrate spatial ability. In the OP performed on day 4 after stress, CON showed a better OP discrimination Index than did IR6, but IR6 may have avoided the moved object. On the NOR when cognitive load was minimal with 1-min ITI, CON and IR6 preferred the novel object over the familiar one, but IR2 performed at chance. By the time they were tested on the RAWM, the last task of the session, all rats acquired it and performed similarly. Motor abilities were unlikely to explain the spatial memory differences observed in the beginning on the Y-maze and OP, and although anxiety profiles were elevated for both DR6 and IR6, the rats explored similarly and well during the low cognitive load test. We conclude that a 6-hour IR paradigm may lead to impaired

hippocampal-dependent spatial ability with comparably robust deficits as found with DR6. However, these effects failed to be long-lasting, as groups performed similarly by days five and seven.

3.3. Experiment 3: Effects of an extended IR6 paradigm on spatial memory in male and female rats

The effects of an extended 6-hr IR paradigm were explored in both male and female rats by increasing the stress period from three to six weeks before the first behavioral test, as longer periods of restraint resulted in more robust spatial memory deficits on the RAWM in male rats (Hutchinson et al., 2012). After six weeks of IR6, rats were tested on the Y-maze and then an additional three weeks of IR6 was implement before a second Y-maze test. Thereafter, behavioral assessments on different tasks occurred weekly (Fig. 2.3A).

In the first Y-Maze following 6-wks of IR, all groups (CON-M, IR6-M, CON-F, IR6-F) entered (or spent more time in, data not shown) the novel arm than the other arm to reflect intact spatial ability (Fig. 2.3B). Wilcoxon Signed Rank Tests revealed that rats entered (and/or spent more time in, data not shown) the novel arm than the other arm ($p < 0.05$, CON-M, IR6-M, CON-R, IR6-F). A two-way ANOVA across treatment conditions for the entry index did not show a significant effect.

The rats were given another three weeks of IR and then tested again in the Y-maze in a different room. After 9-wks of IR, the rats still showed spatial ability (Fig.

2.3C). Wilcoxon Signed Rank Tests indicated a significantly greater number of entries (and/or time spent) in the novel arm than in the other arm for CON-M, IR6-M, CON-F, and IR6-F ($p < 0.05$). A two-way ANOVA revealed no significant effects on %entry index across groups.

After another week of IR, the rats were tested on the OP, which occurred during the 10^{th} week of restraint. Wilcoxon Signed Rank Tests were performed to determine whether each group explored the object in the novel location more than the object in the same location. Unexpectedly, no significant differences were detected: all groups, including the controls (CON-M, IR6-M, CON-F and IR6-F), explored the objects in the novel and same location similarly. A two-way ANOVA also found no significant differences across groups for OP discrimination (Fig. 2.3D).

After another week of IRS, the rats were tested in the NOR during the 11^{th} week of restraint. Since rats had just 1-min ITI with a minimum cognitive load, all groups were expected to recognize and spend more time with the novel object compared to the familiar object. Wilcoxon Signed Rank Tests showed that all groups explored the novel object significantly more than the familiar object ($p < 0.05$, Fig. 2.3E). A two-way ANOVA found no significant differences across groups in NOR discrimination.

To determine whether anxiety or motor ability may have impacted performance, additional assessments were performed on the OF, OP and Y-maze. A two-way ANOVA performed on anxiety index in the OF revealed a significant interaction of stress and sex ($F_{1,43} = 3.827$, $p = 0.05$, Power = 0.481, Fig. 2.3F). LSD post-hoc analyses showed that

IR6-F rats expressed a reduced anxiety profile compared to CON-F ($p < 0.05$), CON-M ($p < 0.05$), and IR6-M ($p < 0.05$, Fig. 2.3F). To determine whether locomotor activity or motivation to explore the Y-maze differed across groups, total entries (sum of entries into novel, start and other arms over minutes 1 and 2) were analyzed using two-way ANOVAs. No significant differences were detected in the first Y-maze after 6-weeks of IR6 to suggest that the groups were similarly motivated to explore (data not shown). The total number of entries averaged 7.8 ± 0.8 for CON-M, 8.2 ± 0.7 for IR6-M, 9.8 ± 0.6 for CON-F and 8.9 ± 0.7 for IR6-F. For the Y-maze after 9-weeks of IR6, a two-way ANOVA revealed a significant effect of sex with female rats making more total entries than male rats ($F_{1,42} = 10.205$, $p < 0.05$, Power = 0.877, Fig. 2.3G) with no other significant effects. The total number of entries averaged 7.5 ± 0.8 for CON-M, 7.7 ± 1.0 for IR6-M, 10.4 ± 0.8 for CON-F and 9.9 ± 0.6 for IR6-F. In OP, the total time spent exploring the objects was compared with a two-way ANOVA and revealed a significant effect of sex with male rats spending more time exploring objects compared to female rats ($F_{1,39} = 4.228$, $p < 0.05$, Power = 0.518, Fig. 2.3H) with no other significant effects. The total time exploring objects (in seconds) averaged 48.0 ± 6.5 for CON-M, 56.7 ± 4.2 for IR6-M, 48.4 ± 6.5 for CON-F and 54.3 ± 6.1 for IR6-F.

In summary, an extended IR paradigm for six and even nine weeks failed to lead to impaired spatial memory. Both IR6-M and IR6-F showed spatial memory on the Y-maze by entering (and/or spending more time in) the novel arm than the other arm. OP behavior was less clear as all groups, including controls, failed to discriminate and spent

similar amounts of time exploring both objects. On the NOR when cognitive load was minimal, all groups discriminated and preferred the novel object over the familiar one. We conclude that when the IR paradigm was extended beyond three weeks to six or nine weeks, IR failed to lead to impaired spatial memory, perhaps due to the paradigm becoming predictable.

3.4. Experiment 4: Effects of UIR on spatial ability in male and female rats.

In experiments 1 and 2, IR led to impaired spatial memory at three weeks, but experiment 3 showed that when IR was extended to six or nine weeks, spatial memory deficits were not detected to suggest that IR may have become predictable. Under predictable circumstances, chronic stress responses becomes less robust (L. A. Galea et al., 1997b; Koolhaas et al., 2011). Consequently, IR was modified as an unpredictable intermittent restraint (UIR) and spatial memory was explored in both male and female rats. The UIR paradigm involved varying the time of day which restraint occurred as well as the duration of restraint (either 30 min or 1 hr), with restraint repeating once a day for a period of 2 to 6 consecutive days before a 1- or 2-day restraint hiatus. Moreover, restraint occurred on an orbital shaker to increase the robustness of the restraint with a shorter duration. After a 26-day UIR period (reflecting 21 restraint days), behavioral testing began and occurred weekly on days without restraint with UIR continuing the day after behavioral testing. As a final assessment, a robust heterotypic stressor was performed at the end of UIR because it produced sex differences in set-shifting ability

(Moench et al., 2019). However, its effect on spatial ability is unknown. A timeline of the experiment is shown in figure 2.4A.

In the first spatial task, sex differences were observed in the Y-maze (Fig. 2.4B). In the males, Wilcoxon Signed Rank Tests indicated a significantly greater number of entries and time spent in the novel arm than in the other arm for the controls (CON-M, $p < 0.05$), but not in UIR-M. In the females, a tendency to enter and spend more time in the novel arm more than the other arm was found in UIR-F ($p < 0.10$, Fig. 2.4B), but not in CON-F. A two-way ANOVA did not reveal any significant effects on the %entry index (or %dwell index). Motivation to explore was unlikely to have impacted performance, as total entries on the Y-maze (sum of entries into Novel, Start and Other arms over minutes 1 and 2) were statistically similar (Fig. 2.4C). The total number of entries averaged 10.2 ± 0.9 for CON-M, 9.3 ± 0.8 for UIR-M, 9.3 ± 0.8 for CON-F and 9.6 ± 0.8 for UIR-F. Therefore, differences in spatial memory in the Y-maze were unlikely due to motivational differences to explore.

A second Y-maze test was performed seven days after the last UIR session concluded and one day after an acute 30-min EP stressor, as this manipulation has been shown to impair prefrontal cortex-mediated function (Moench et al., 2019). However, no differences among groups were detected on the post-EP Y-maze from a two-way ANOVA and all groups demonstrated spatial memory (Fig. 2.4D). Wilcoxon Signed Rank Tests indicated that all groups significantly entered (and/or spent more time in) the novel arm than in the other arm ($p < 0.05$).

OP occurred twice in this experiment, during the 2nd and 5th weeks of behavioral testing. In both OP tasks (1-hr ITI), none of the groups showed a significant preference for one object over the other and explored both objects similarly (Wilcoxon Signed Rank, Fig. 2.4E for the first task, data not shown for the second task). Moreover, a two-way ANOVA for the OP indexes did not reveal any significant effects.

The rats were tested in two versions of the NOR (1-min and 1-hr ITI) during the 3rd week of behavioral testing. Wilcoxon Signed Rank Tests showed that all groups discriminated and explored the novel object significantly more than the familiar object under both conditions (ITI 1-min, $p < 0.05$, Fig. 2.4F; 1-hr ITI $p < 0.05$, Fig. 2.4G). A two-way ANOVA did not show any significant effects among groups for the NOR index in either task.

To determine whether anxiety may have impacted performance, the OF and NSF were used. In the OF, a two-way ANOVA performed on the anxiety index did not reveal any significant differences among groups, although there was a tendency for females to have a higher anxiety index than in males ($F_{1,44} = 3.265$, $p < 0.10$, Fig. 2.4H). In the NSF, there were no significant differences across groups in latency to approach food (Fig. 2.4I) and home cage feeding was statistically similar. Together, the OF and NSF data suggest that the UIR groups had similar overall anxiety profiles as the CON groups. Although females may have had a higher anxiety index, this does not explain why UIR-F may have differed from CON-F.

In OP1 and OP2, the total time spent exploring the objects in trial 2 was compared with a two-way ANOVA and revealed a significant effect of sex in OP1 ($F_{1,40} = 5.338$, $p < 0.05$, Power = 0.616, Fig. 2.4J) and OP2 ($F_{1,41} = 16.641$, $p < 0.05$, Power = 0.978, data not shown), with no other significant effects. The total time exploring objects (in seconds) averaged for OP1: 29.3 ± 2.5 for CON-M, 23.6 ± 2.6 for UIR-M, 32.5 ± 4.5 for CON-F and 38.9 ± 5.3 for UIR-F (Fig. 2.4J) and for OP2: 27.8 ± 5.1 for CON-M, 24.4 ± 3.8 for UIR-M, 37.8 ± 4.8 for CON-F and 43.5 ± 3.5 for UIR-F. While females spent more time with the objects than did males, all rats performed similarly and at chance on the OP.

In summary, the UIR paradigm was effective in leading to impaired spatial memory in male, but not female rats. In the first spatial assessment, CON-M entered the novel arm more than the other arm, while UIR-M performed at chance, which could not be explained by motor differences or anxiety profile. In contrast to males, UIR in females did not result in spatial memory deficits and may have even been beneficial, as UIR-F rats showed improved discrimination compared to CON-F, with more novel arm exploration than the other arm. Motor abilities are unlikely to explain the spatial memory differences observed in the first Y-maze and lack of an effect in OP. We did not find any deficits in spatial memory in the Y-maze following the EP stressor, suggesting rats had either recovered from deficits by that time point and/or the EP procedure did not interfere with spatial ability. We conclude that a UIR paradigm may lead to impaired

hippocampal-dependent spatial ability with robust deficits in male rats that fail to present in female rats.

3.5. Physiological Measures

In all four experiments, IR or UIR attenuated body weight gain compared to the same-sex controls (Table 1). Follow-up post-hoc tests from a significant one-way ANOVA in experiment 1 ($F_{3,44} = 16.357$, $p < 0.05$), showed that DR6, IR6, IR2 gained less weight compared to CON over three weeks ($p < 0.05$) and that, DR6 gained the least body weight compared to IR6 and IR2 ($p < 0.05$). IR6 and IR2 gained similar amounts of weight. In experiment 2, follow-up from a significant one-way ANOVA ($F_{3,44} = 1153.777$, $p < 0.05$), showed that DR6 and IR6 gained less weight than CON ($p < 0.05$) and that DR6 gained significantly less body weight than did IR6 ($p < 0.05$). In experiment 3 and 4, two-way ANOVAs were performed for stress and sex with both revealing significant effects of stress (Exp 3, $F_{1,43} = 86.088$, $p < 0.05$; Exp 4, $F_{1,44} = 59.523$, $p < 0.05$), sex (Exp 3, $F_{1,43} = 454.343$, $p < 0.05$; Exp 4, $F_{1,44} = 436.739$, $p < 0.05$), and interaction (Exp 3, $F_{1,43} = 11.888$, $p < 0.05$, Exp 4, $F_{1,44} = 6.359$, $p < 0.05$). Post-hoc tests from the interaction revealed that while the stressors attenuated body weight gain in both sexes ($p < 0.05$), males showed more robust effects than found with females.

In experiment 4, thymus, adrenal and uterine weights were analyzed as an additional measure of stressor effectiveness (Table 2). A two-way ANOVA for thymus weight revealed a significant effect of stress ($F_{1,44} = 10.982$, $p < 0.05$) and sex ($F_{1,44} = 27.557$, $p < 0.05$) with no significant interaction. UIR increased thymus weight in both

males and females with males showing heavier thymus. A two-way ANOVA for adrenal gland weights revealed a significant effect of sex ($F_{1,43} = 15.090$, $p < 0.05$), with no significant stress or interaction. Males had larger adrenals than females, as would be expected. Uterine weights in females were not statistically different.

4. Discussion

The current study investigated whether an IR paradigm could be extended to study chronic restraint effects on spatial memory deficits in both male and female rats. We report that IR may be useful to investigate spatial ability in male rats within a relatively brief period, such as the three weeks of IR, but not after an extended IR duration of six or nine weeks. Moreover, when spatial memory deficits were detected in male rats, the effects of IR were transient because spatial memory deficits begin to improve within a few days after restraint ended. When IR continued for an extended duration for up to six weeks, IR male and female rats failed to demonstrate spatial memory impairments, suggesting that the IR paradigm may have become predictable. A modified version of IR that was made to be unpredictable (UIR) through restraining rats at different number of consecutive days restrained (2 to 6 days), changing the time of day restrained, and mixing up the duration of restraint (30 or 60 min) when combined with gentle shaking. The outcome showed that UIR males were impaired on spatial ability, whereas UIR females were not. These experiments introduce UIR as an effective chronic

stressor to investigate spatial memory deficits in males and highlight sex differences in how chronic restraint impacts spatial ability.

An important outcome of these experiments is the corroboration of sex differences in how chronic stress alters spatial memory. UIR impaired spatial memory in the Y-maze of male rats without impairing spatial memory of female rats. For the stressed females (Fig. 2.4B), they showed a non-significant tendency ($p<0.1$) to prefer the novel arm, while their same-sex controls failed to reach this p-value. The results are consistent with the findings of others documenting that males show deficits in spatial ability (Bowman et al., 2003; Conrad et al., 1996b, 2012; Hoffman et al., 2011; Kleen et al., 2006; V. Luine, 2002; V. Luine et al., 1994b; Moosavi et al., 2007; Nishimura et al., 2017; Ortiz et al., 2015; Sandi et al., 2003; Wright & Conrad, 2005), but not in females. For the females more specifically, an extensive literature shows that chronic stress either fails to alter spatial memory (Bowman et al., 2002; Bowman & Kelly, 2012; Ortiz et al., 2015) or even enhances it (Beck & Luine, 2002; Bisagno et al., 2004; Kitraki et al., 2004). Chronic stress may also alter female motivation, as we found that stressed female rats delayed their exploration of the novel arm exploration in the Y-maze (Conrad et al., 2003), though this did not occur in the current experiments. It is unlikely that the stressed females perceived the UIR differently than males, as physiological measures validated UIR effectiveness, consistent other reports on chronic stress in rodents (Bhatnagar & Dallman, 1998; Conrad et al., 2001, 2012; L. A. Galea et al., 1997a; McFadden et al., 2011b; McKittrick et al., 2000). Once possible explanation as to why stressed females

were less vulnerable to spatial memory deficits than stressed males may be that chronic stress altered a different cognitive function in females than was investigated in the current study. For example, chronic stress impaired cognitive flexibility in females using a set-shifting task (Grafe et al., 2017), which requires the prefrontal cortex (Birrell & Brown, 2000). Or perhaps the type of stressor could be important, as heterotypic stressors, defined as a novel stressor unique from prior stressors, impaired set-shifting in females but not males (Moench et al., 2019). Altogether, we interpret our results in the context of how the control females performed and published reports that UIR did not impair spatial ability of female rats and corroborates the literature highlighting the sex differences in chronic stress-induced spatial memory effects.

The original intent of using the IR paradigm was to determine whether the potentiated effects on stress responsiveness and anxiety reported previously (W. Zhang et al., 2014) could be extended to spatial memory. While experiment 1 showed some evidence for three weeks of IR to lead to more robust spatial memory deficits than observed for DR (Fig. 2.1C), this was not always observed. In experiment 2, DR6 and IR6 males performed poorly and similarly on the Y-maze. On the OP task performed on day 4 after chronic stress ended in experiment 2, DR6 males performed at chance, but IR6 males recognized the novel arm by showing avoidance behavior, which is consistent with chronic stress facilitating neurocircuitries that favor habits rather than flexible behaviors (Dias-Ferreira et al., 2009). Then for experiment 3 when IR was extended to six and even nine weeks, no spatial memory deficits were detected in either sex. The

longer paradigm was used because our past study showed that that five weeks of chronic daily restraint resulted in more robust spatial memory deficits in males than compared to a three week exposure (Hutchinson et al., 2012). The lack of an effect with six- and nine-weeks of IR to impair spatial memory in the current study was unlikely attributed to stressor effectiveness because IR attenuated body weight gain, a reliable measure of chronic stress in rodents (Bollinger et al., 2016; Henckens et al., 2015; M. T. Marin et al., 2007; Martí et al., 1994; Retana-Márquez et al., 2003). Perhaps the consistent five day exposure and two days off from restraint led to a muted stress response, a phenomenon documented to occur with repeated exposures to the same stressor (Viau & Sawchenko, 2002), and as such, could have increased predictability (Grissom & Bhatnagar, 2009) to thereby make IR less stressful (Koolhaas et al., 2011). While this interpretation does not explain why DR would lead to more severe spatial memory deficits when extended from three to five weeks (Hutchinson et al., 2012), this may apply to the IR paradigm, perhaps by making it more tolerable by having predicted days off from restraint. Therefore, IR may produce potentiated stress responses and anxiety after 1-1/2 weeks, see (W. Zhang et al., 2014), but its effects on producing robust spatial memory deficits are mixed after 3 weeks and certainly ineffective at leading to impairments after 6 weeks.

A consistent theme following chronic stress is that males show spatial memory deficits, which improve in the days after the chronic stressor ends. In one of the first studies to investigate this phenomenon, 4 weeks of chronic stress impaired spatial learning on the Morris Water Maze task, with these deficits improving after one month

passed from the end of the stressor (Sousa et al., 2000a). Later, our lab found that 3 weeks of chronic restraint hindered spatial memory on the RAWM, an effect that improved with the passage of time (Hoffman et al., 2011). Our current experiments add to the literature and suggest that the timeframe from when spatial memory deficits improve following the end of these chronic restraint paradigms is shorter, within the range of four to seven days. These findings are consistent with the changes in hippocampal CA3 apical dendritic arbors following chronic stress: chronic stress leads to CA3 apical dendritic retraction (Conrad, 2006; Conrad et al., 1999; L. A. Galea et al., 1997a; McKittrick et al., 2000; Ortiz et al., 2014; Wright et al., 2006), and these dendritic arbors become more complex within four to ten days after chronic stress has ended (Conrad et al., 1999).

Another consideration for the relatively fast spatial memory improvement is that the rats may have benefited from the repeated behavioral assessments. For example, environmental enrichment counteracts chronic stress-induced learning and memory deficits (Cui et al., 2006; Hutchinson et al., 2012; Ilin & Richter-Levin, 2009; Wright & Conrad, 2008). Aspects of the cognitive assessments implemented in this study, such as the opportunity to explore objects and environments, could be perceived as enriching and may have similar effects as environmental enrichment in rats. For instance, CA3 synaptic density recovers rapidly after two days of water maze training, suggesting that learning may promote synaptic plasticity and counteract the chronic stress effects (Sandi et al., 2003). Another interpretation is that the rats were able to transfer information from one

testing situation to another (Winocur & Gilbert, 1984; Winocur & Mills, 1970; Winocur & Salzen, 1968), but this likelihood was minimized by using unique testing rooms and contexts for each cognitive task. Whether spatial memory deficits improved from repeated testing or from the passage of time is unclear, but it is important to note that the phenomenon was found in all three of the restraint paradigms (IR, UIR and DR) and could not be explained by motor or motivational issues.

The UIR paradigm was introduced after concluding experiment 3 because the ability to behaviorally test both control and stress rodents on full days without disrupting the stress manipulation would be of great benefit in experiments that require multiple assessments. Moreover, the issue that spatial memory improves in as little as 5 days after the chronic stress manipulation has ended makes an intermittent stress paradigm valuable when repeated behavioral testing is desired. Experiment 4 demonstrated that UIR was effective at producing spatial memory deficits in males, an outcome that would be expected from the literature (Conrad et al., 2003; Kleen et al., 2006; V. Luine et al., 1994b; Ortiz et al., 2015; Rahman et al., 2016; Riaz et al., 2015; Sunanda et al., 2000). Consequently, UIR provides a way to obtain multiple behavioral measures without disrupting the chronic stress paradigm.

An unforeseen outcome was the failure of the majority of control rats across groups to explore the moved object more than the unmoved object during OP testing in experiments 3 (Fig. 2.3D) and 4 (Fig. 2.4E). We incorporated the OP into our behavioral battery based upon our own success (and others) with using it. Our past work using

young adult Sprague-Dawley female rats revealed robust effects using OP in non-stressed rats (McLaughlin et al., 2008) and after chronic stress (Conrad et al., 2012), as have others (Bisagno et al., 2003; Bowman et al., 2003; Frick & Gresack, 2003). Similar robust effects on OP were reported with male rats by us and others (V. Luine et al., 1994b; Nishimura et al., 2017; Ortiz et al., 2018). However, our lab recently discovered that middle-aged female rats failed to explore during object testing even with repeated exposure (Koebele, Nishimura, et al., 2020a), bringing some questions about OP reliability as it pertains to females. As we reflect on the tests used in the current work, experiments 3 and 4 started with Y-maze testing, which differed from experiment 1. Testing on the Y-maze first may have had implications on willingness to explore in OP later because rats were able to seek out novelty in the Y-maze using thigmotaxis but were unable to explore objects in the open field unless they ventured away from the wall. Additional differences that would have encouraged exploration on the Y-maze was that Y-maze testing occurred with bedding material and lower light intensity (lux = 80-90) than used for OP (lux = 150-160), which was done to enhance visualization of spatial cues and differentiate it from other tasks. In comparison with other reports that had success with OP, the OP was the only task used (Beck & Luine, 2002; Conrad et al., 2012; V. Luine et al., 1994b; McCormick et al., 2010; Nishimura et al., 2017) or a different type of task preceded it (Koebele, Nishimura, et al., 2020a; Ortiz et al., 2018), but we have not found an example in which OP followed Y-maze testing. Consequently,

it is possible that the controls may have been less willing to explore or pay attention to the objects in the OP so soon after being exposed to the Y-maze.

We also observed that the behavioral testing order impacted performance. In experiment 1, when the RAWM occurred first, followed without breaks in daily testing by the OP and NOR, half of the rats failed to explore despite being presented with an OF arena for acclimation. In experiment 2, when the Y-maze occurred first, subsequent object exploration was greatly increased, ranging from 83% to 100% participation across treatment conditions. Other reports documented order effects and one found that mice explored less in the open field and the Y-Maze when a behavioral battery preceded them, but how a behavioral battery impacted performance on the Morris Water Maze was less obvious (Võikar et al., 2004). When aversive tasks, such as the Morris Water Maze, precede comparatively less aversive tasks, such as OF, mice exhibited reduced locomotion (Blokland et al., 2012; McIlwain et al., 2001). Taken together, the current series of studies corroborate the literature that if multiple behavioral tasks are to be used to assess chronic stress effects on spatial ability, then perhaps testing should start from the least to the most aversive paradigm, except in the case that the Y-maze is used and have the Y-maze follow tests that require open field exploration, such as OP.

In conclusion, the results from the present set of experiments show important sex differences in how chronic stress alters spatial ability and introduce UIR as a useful paradigm to probe these effects when repeated behavioral measures are needed. We report that UIR led to spatial memory deficits in male, but not female rats. The

overwhelming evidence from the current study and others suggest that chronic stress affects male and female rats differently. Chronic stress impairs hippocampal function in male rats, as evidenced by poor spatial ability, but fails to impair spatial ability in female rats. Instead, other studies suggest chronic stress may alter the vulnerability of females to cognitive arousal and related attentional tasks (Bangasser et al., 2016). Future studies should continue to probe the types of respective cognitive vulnerabilities exhibited by males and females in response to chronic stress.

CHAPTER 3

This chapter was published in Neuroscience Letters in 2023 and is titled:

CHRONIC STRESS LEADS TO PERSISTENT AND CONTRASTING STELLATE NEURON DENDRITIC HYPERTROPHY IN THE AMYGDALA OF MALE AND FEMALE RATS

Abstract

In males, chronic stress enhances dendritic complexity in the amygdala, a region important in emotion regulation. An amygdalar subregion, the basolateral amygdala (BLA), receives input from structures, including the hippocampus and prefrontal cortex to coordinate emotional learning and memory. This study quantified changes in dendritic complexity of BLA stellate neurons ten days after a novel and unpredictable chronic stress paradigm ended in both male and female rats. In addition, dendritic complexity of hippocampal neurons in male rats was assessed at a similar timepoint. Following Golgi processing, stressed male and female rats showed enhanced BLA dendritic complexity: increased arborization occurred near the soma in males and distally in females. As the brain was sampled ten days after chronic stress ended, BLA dendritic hypertrophy persisted in both sexes after the stressor had ended. For the hippocampus, CA3 apical dendritic complexity was similar for control and stressed males when assessed eight days after stress ended to suggest that any stress-induced changes had resolved. These results show persistent enhancement of BLA dendritic arborization in both sexes following chronic stress, reveal sex differences in how BLA hypertrophy manifests, and suggest a putative neurobiological substrate by which chronic stress may create a vulnerable phenotype for emotional dysfunction.

Introduction

Chronic stress leads to robust changes in brain structure and function with important sex differences. The hippocampus receives much attention because of its sensitivity to stressors and its involvement with mental health, with cognitive disruption and decreased hippocampal volume being key features in depression and Alzheimer's disease (Aschenbrenner et al., 2018; Berger et al., 2020; Bremner et al., 2000b; Colla et al., 2007b). Furthermore, chronic stress corresponds with smaller hippocampi (Belleau et al., 2019; McEwen & Akil, 2020; Sheline et al., 2019) and dendritic atrophy (Ortiz et al., 2018; Ortiz & Conrad, 2018; Seewoo et al., 2020), especially within the CA3 region of males (Conrad et al., 2017). In females, ovarian hormones may interact with stress to alter hippocampal neuronal structure and function (McLaughlin et al., 2010; Yagi & Galea, 2019), such as protecting hippocampal dendritic complexity and spine synapses (Baka et al., 2017; Conrad et al., 2012; Huzian et al., 2021; McLaughlin et al., 2010). Thus, chronic stress and ovarian hormones may interact to influence the hippocampus and other stress-sensitive brain regions in both males and females.

One interpretation as to why chronic stress may have failed to impact hippocampal structure and function in females as observed in males may involve stressor robustness and/or predictability (M. T. Marin et al., 2007). Consequently, we used an intermittent restraint (IR) design that enhanced stress responses and anxiety in males (W. Zhang et al., 2014), but modified to enhance stressor robustness and unpredictability, termed Unpredictable Intermittent Restraint (UIR) to determine whether UIR females

would exhibit impaired spatial ability (Peay et al., 2020). Despite changes to increase robustness and unpredictability, UIR-exposed females showed resilience in their spatial ability. Thus, stressor robustness and unpredictability were unlikely to explain the sex differences in hippocampal function following chronic stress.

The amygdala is another brain region sensitive to stress and part of the limbic system important in emotional memory and regulation (Andrewes & Jenkins, 2019; Denny et al., 2015; McEwen et al., 2016). Further, the amygdala is implicated in affective disorders such as depression and anxiety (Hamilton et al., 2008; W. Z. Liu et al., 2020). Following chronic stress, amygdalar volume increases (Kuo et al., 2012) and dendritic arbors proliferate (Roozendaal et al., 2009; Vyas et al., 2002), especially within the basolateral amygdala (BLA) (Padival et al., 2013; Patel et al., 2018; Vyas et al., 2002). Moreover, BLA dendritic hypertrophy persists for at least a month after stressors end (Hoffman et al., 2014), which contrasts to the transient dendritic atrophy within the hippocampus that disappears within 10 days (Conrad et al., 1999; Hoffman et al., 2011). To date, the effects of chronic stress on BLA dendritic hypertrophy are reported from male rodents, but whether similar changes occur in females are unknown. To determine whether female BLA neurons are sensitive to the UIR manipulation, brain tissue was quantified for BLA dendritic complexity from males and females from a prior experiment in which spatial ability and anxiety-like behaviors were assessed and then published (Peay et al., 2020). Further, the hippocampus was sampled in a separate set of rats to

assess a novel timepoint for changes in CA3 dendritic arborization at eight days from the end of stress, a timepoint that was previously unexplored.

Methods

Subjects

Arizona State University Institutional Animal Care and Use Committee approved the procedures, which align with the Guide for the Care and Use of Laboratory Animals. Male and female Sprague-Dawley rats (Charles River Laboratories, Hollister, CA, USA), weighing approximately 200-225 grams upon arrival were pair housed in same-sex housing units in standard laboratory cages (21-22 °C, corncob bedding). Rats were housed on a reverse 12:12 light cycle (lights off at 07:00) and the sexes were tested on separate days or in different rooms. All procedures occurred during the dark phase of the light cycle. Rats were allowed food and water *ad libitum* except where noted. The behavior from these rats was published (Peay et al., 2020), and the brains from those rats utilized for this report.

Chronic Stress Procedures (Peay et al., 2020)

Rats were chronically stressed using wire mesh restrainers, secured with clips, see (Peay et al., 2020). Control (CON) rats for both sexes were housed in separate chambers than the stressed rats to reduce the likelihood of odor, sound, and sight communication. To maintain similar handling procedures and access to food and water, CON rats were handled daily, and food restricted for the same duration as the restrained rats. Body weights were measured weekly.

Unpredictable Intermittent Restraint (UIR): The restraint was made to be unpredictable, brief, and robust, termed, "Unpredictable Intermittent Restraint" (UIR) (Peay et al., 2020). Restraint occurred for 30 or 60 min while on an orbital shaker (120 rpm) and occurred at different times of day (between 7:00 and 21:00) and for different consecutive day lengths (2-6 days) before a day or two off without a stressor (Fig. 3.1A). UIR occurred for 26 days prior to the start of behavioral testing and then another 21 days total between behavioral testing sessions.

Intermittent Restraint (IR): The IR experiments were used to optimize restraint parameters based upon an interrupted pattern (W. Zhang et al., 2014), at a duration that produces hippocampal dendritic retraction and impaired spatial ability in male Sprague Dawley rats (McLaughlin et al., 2007). The IR groups were restrained in wire mesh (2- or 6-hr/d) in an interrupted pattern: 5 days restrained then two days without restraint over a period of 23 days (2-hr/d (IR2) or 6-hr/d (IR6)). The IR groups were compared to the traditional restraint process (6hr/d for 21 consecutive days, daily restraint, DR6) and to a non-restrained control (CON). Restraint started at 09:00 and ended at 15:00. The sum of restraint days for DR and IR was 21 and 15 days, respectively (Fig. 3.2A).

Slide preparation

Brains were collected 10 days (UIR) and 8 days (IR) following the end of stress and stained (FD Rapid Golgi-Stain) using procedures as described (Hoffman et al., 2011), with a few exceptions (120 μm thickness; sectioned between Bregma -2.12 and -4.52).

The rat numbers were coded to ensure neuronal tracing and quantification were completed blind to the experimental conditions.

Stellate neurons in the BLA and pyramidal neurons in the hippocampal CA3 region were selected for dendritic arborization quantification. Stellate neurons express round somas, with dendrites extending radially, while pyramidal neurons express triangular somas with a prominent apical dendrite opposite to thinner basal dendrites. Selected neurons had: 1) cell body and dendrites fully impregnated and untruncated, 2) cell relatively isolated, and 3) were within the BLA or CA3 region as appropriate. Neurons were manually traced using an Olympus BX51 microscope with a camera lucida attachment (200x or 400x magnification) for the BLA and hippocampus, respectively. A separate investigator checked each neuron to ensure criteria were met and accuracy of dendritic arbor drawings. The number of cells per rat ranged from 2-12 and the number of rats per group ranged from 5-12. Dendritic arborization was quantified by counting the total number of dendritic bifurcations or branches from the apical and basal shafts (separately if a hippocampal neuron) and using Sholl analysis, to assess dendritic complexity as a function of distance from the soma (Sholl, 1953). For the hippocampal pyramidal cells, data were averaged across same cell type for short-shafted neurons and long-shafted neurons and then averaged together for one value for each rat and then analyzed across rats for each experimental group (Conrad et al., 2017).

Statistical Analysis

Data were analyzed using SPSS (Version 28). Analyses of Variance (ANOVAs) were performed after confirming the appropriateness with Levene's test for homogeneity of variance. Fisher's LSD post-hoc tests were used when $p < 0.05$.

Results

BLA dendritic hypertrophy persists following UIR in both sexes

BLA stellate neurons in male and female UIR rats showed amplified dendritic branching complexity compared to same-sex controls (Fig 3.1B). An ANOVA using Stress and Sex as independent variables for total dendritic branch bifurcations in the BLA reached a significant effect of Stress ($F_{1,23} = 8.511$, $p = 0.009$, observed power = 0.792), with no other significant effects. UIR rats showed a greater number of bifurcations than did CON and the effect was similar for both males and females.

The Sholl analysis of dendritic intersections at 20-µm intervals from the BLA stellate soma supported the total branch point assessment, with sex differences. A mixed factor ANOVA for Stress and Sex across distances from the soma for dendritic intersections reached significance for Stress ($F_{1,20} = 4.403$, $p = 0.049$, observed power = 0.515) and a 3-way interaction for Stress, Sex, and Distance from the soma ($F_{7,140} = 4.482$, $p < 0.001$, observed power = 0.991). Males and females were then analyzed separately to parse out the 3-way interaction. A two-way ANOVA for Stress and distance from the soma for the number of dendritic intersections revealed significant interactions

for males ($F_{7,77}$ = 3.142, p = 0.006, observed power = 0.929) and females ($F_{7,63}$ = 2.304, p = 0.037, observed power = 0.804), indicating that UIR altered the dendritic intersections at specific distances from the soma in both sexes (Fig. 3.1C-E). One-way ANOVA analyses for dendritic intersections at 20-µm intervals from the soma revealed that in males, UIR enhanced dendritic arborizations at distances close to the soma (40-µm, $F_{1,12}$ = 9.532, p = 0.010, observed power = 0.802; and 60-µm, $F_{1,12}$ = 9.445, p = 0.011, observed power = 0.799). Whereas for females, UIR enhanced dendritic arborizations at distal locations from the soma (140-µm, $F_{1,10}$ = 5.140, p = 0.050, observed power = 0.525; and did not reach significance at 100-µm, $F_{1,10}$ = 3.515, p = 0.094 and 120-µm, $F_{1,10}$ = 4.159, p = 0.072). Analyses at other distances did not reach significance as well.

Eight days after IR ended, CA3 dendritic complexity was similar across groups

Eight days after stressor termination, the stressors had no effect on hippocampal dendritic complexity. Hippocampal CA3 neurons from M-CON, M-IR6, M-IR2 and M-DR6 showed similar dendritic branching in both the apical and basal regions (not shown), an effect supported by the Sholl analysis (Fig. 3.2).

Discussion

Our study determined whether a previously implemented unpredictable and robust restraint paradigm would produce long-lasting changes in the dendritic arborization of the BLA and CA3 hippocampal regions. Our results are the first to show that UIR enhanced

BLA dendritic arborization in both male and female rats with unique sex differences. Male BLA neurons exhibited enhanced dendritic arborization proximal to the soma, whereas female BLA neurons expressed enhanced dendritic arborization distal to the soma. For the IR paradigm used in males only, dendritic complexity was similar across the treatments to suggest that hippocampal dendritic atrophy was transient as CA3 dendritic atrophy may improve as soon as eight to ten days from a stressor ending (Conrad et al., 1999, 2017; Leuner & Gould, 2010; Leuner & Shors, 2013; Ortiz et al., 2018). These results show contrasting effects between the brain regions (BLA and hippocampus) for the effects on dendritic complexity (hypertrophy versus atrophy) and in duration (persistent and transient).

BLA dendritic hypertrophy persisted for 10 days following the end of chronic stress, though the hippocampal CA3 dendrites showed no signs of a stress effect at 8 days. This demonstrates that chronic stress has long-lasting changes in the BLA pertaining to dendritic structure. In the rodent BLA, chronic stress often results in prolonged enhanced dendritic arborization (Padival et al., 2013; Patel et al., 2018; Vyas et al., 2002). The effects of a stressor can be observed in the BLA within just one day (Hoffman et al., 2017) and may last a month or longer (Denny et al., 2015; Hoffman et al., 2017; McEwen et al., 2016; Vyas et al., 2004). The sensitivity, quick response, and long-lasting effects of stress on the BLA dendrites highlight a potential neural substrate that may contribute to emotional dysfunction, such as depression.

The failure for chronic stress to alter hippocampal CA3 dendritic arbors was unlikely from the stressor being ineffective. The IR paradigm disrupted body weight gain (CON: 123.8g ± 9.8, IR6: 73.9g ± 5.4, IR2: 93.4g ± 8.0) and impaired spatial memory, outcomes that are common features in chronically stressed male rodents (Conrad, 2006; Conrad et al., 1996a; Hoffman et al., 2011; McLaughlin et al., 2007; Ortiz et al., 2014). Moreover, the spatial memory deficits occurred in the first few days of testing, when hippocampal CA3 dendritic atrophy is known to exist (Conrad et al., 1999; McLaughlin et al., 2007). Then spatial memory improved or recovered within a week following the end of the IR, corresponding to the approximate time that brain tissue was processed, and yet significant effects were absent. Consequently, the timeline for spatial memory impairment and subsequent improvement corresponded with the time that CA3 hippocampal dendritic atrophy likely existed and then had time to increase branching following stressor termination (Conrad et al., 1999, 2017; Hoffman et al., 2011; V. Luine et al., 1994a; Ortiz et al., 2018). One potential limitation is the absence of CA3 hippocampal neuron morphology quantification following UIR. However, CA3 dendritic branching in UIR rats was likely similar as their CON counterparts, because they had a ten day delay between UIR and brain processing, and spatial memory was unaltered by then (Peay et al., 2020).

UIR stress led to dendritic hypertrophy in stellate BLA neurons in both male and females, but with regional differences: dendritic hypertrophy occurred closest to the soma in males and distally from the soma in females. Sex differences following gut microbial

alteration or with age on dendritic complexity were reported on the pyramidal neurons within the BLA: males showed dendritic hypertrophy at distal regions from the soma, while females exhibit hypertrophy at proximal locations (Geary et al., 2021; M. J. Rubinow et al., 2009). Evidence suggests that gonadal hormones, specifically estradiol and neuroactive progestogens such as allopregnanolone, can influence structure and function of the BLA (Price & McCool, 2022; Yang & Wang, 2017). Consequently, the neurons within BLA show complex dendritic plasticity to organismal experience with chronic stress being one of these modulators.

Dendritic changes in BLA stellate neurons likely have functional repercussions. Stellate neurons are part of a local excitatory circuit (Beitchman et al., 2020; Hartmann et al., 2017) with inhibitory control on GABAergic neurons, which in turn influence the central amygdala (CeA), a key BLA output (Pare & Duvarci, 2012; X. Zhang et al., 2018). The CeA targets brain circuits involved in physiological and behavioral responses to stress, including anxiety and fear expression (Duvarci & Pare, 2014; X. Zhang et al., 2018). Regions controlling the BLA include the dorsal hippocampus and medial prefrontal cortex, mPFC (Guthman et al., 2020; Lau et al., 2017; Maren & Fanselow, 1995; McGarry & Carter, 2016). Under baseline conditions, the mPFC and dorsal hippocampus can suppress BLA activity, while chronic stress reduces the mPFC and dorsal hippocampus' control of the BLA (Colyn et al., 2019; Lau et al., 2017). Consequently, changes in BLA dendritic architecture may reflect how specific afferents may influence synaptic integration and alter BLA output (Andreasen & Lambert, 1998;

Blume et al., 2019). The current results extend findings on how male and female rats may exhibit vulnerability to chronic stress in emotional functioning, but with subtle differences with implications that have yet to be deciphered.

CHAPTER 4

CORTICOSTERONE DISRUPTS WORKING MEMORY AND ELEVATES DEPRESSIVE AND ANXIETY-LIKE BEHAVIOR IN MIDDLE-AGED, OVARIECTOMIZED FEMALE RATS

Introduction

Major depressive disorder (MDD) is a complex ailment that affects 8.4% of the United States adult population (*Major Depression*, 2020; SAMHSA, 2020). MDD is one of the main contributors to the global burden of disease and is the current leading cause of disability as measured by years lived with disability (Ledford, 2014; Planchez et al., 2019). MDD is characterized by at least two weeks presence of core symptoms, either 1) depressed mood and/or 2) a loss of interest or pleasure, also known as anhedonia. The core symptoms are accompanied by a combination of various symptoms that cause distress and impair social, occupational and domestic functioning such as, weight loss or gain, diminished ability to think or concentrate, social withdrawal, fatigue or loss of energy nearly every day, feelings of worthlessness or excessive guilt, and recurrent suicidal ideation (American Psychiatric Association, 2013; Perini et al., 2019; Villarroel & Terlizzi, 2020). Moreover, individuals often present with other mental disorders such as heightened anxiety, or drug and alcohol abuse (Steffen et al., 2020). The variation in MDD symptomology and presence of psychiatric comorbidity, create a challenging disorder to diagnose and treat.

Sex differences in MDD occurrence emerge after the onset of puberty, when females begin to show increased incidence compared to males. In humans, increased vulnerability to MDD in females continues through life and only declines after menopause, when menses cease for at least 12 months (K. M. Albert & Newhouse, 2019; Altemus et al., 2014; Herson & Kulkarni, 2022). Females show increases in occurrence

of MDD during life events that involve hormonal fluctuation such as the post-partum period as well as during middle-age, prior to the onset of menopause (K. Albert et al., 2020; Duman, 2017). Studies show that the menopausal transition produces increased vulnerability to both depressive symptoms as well as new onset MDD in females with no prior history of affective disorders (Schmidt & Rubinow, 2009; Willi & Ehlert, 2019). Therefore, female ovarian hormones may provide insight onto MDD symptomology and perhaps treatment.

The etiology of MDD is far from resolve, though stress, and specifically chronic stress, is commonly associated with the development of MDD. The onset of MDD is linked to altered activity of the hypothalamic-pituitary-adrenal (HPA) axis, which is central to the stress response. HPA axis activation eventually leads to the release of glucocorticoids, such as cortisol, from the adrenal cortex, which profoundly impacts in the brain. For example, glucocorticoids influence neuronal proliferation and memory acquisition (Herbert, 2013; Herbert et al., 2006; Pariante & Lightman, 2008). Individuals with MDD often present with elevated cortisol levels as well as disrupted cortisol rhythmicity (Qin et al., 2019; Staufenbiel et al., 2013). Consequently, MDD and the stress response are intertwined and warrant further study.

Rodent models can provide an opportunity to investigate underlying mechanisms of MDD but understanding the processes in both sexes is important. In our past work, we used chronic stress to produce increased depressive-like behavior, impaired spatial memory, and heightened anxiety in young adult male rats (Conrad, 2010; McLaughlin et

al., 2007; Wright & Conrad, 2005). However, gonadally-intact, young adult females are often resilient to many of these impacts, even with modified stress paradigms (Bowman & Kelly, 2012; Hodes & Epperson, 2019; Huynh et al., 2011; Kitraki et al., 2004; Ortiz et al., 2015; Peay et al., 2020). These research findings in gonadally-intact, young adult rodents emphasize the need to study other models that may be susceptible to chronic stress. For example, ovariectomized (OVX) and aged rodents show increased depressive-like behavior compared to intact and young adult counterparts (Kiss et al., 2012; Ter Horst et al., 2009). Studies also show that OVX and aged rodents show spatial memory deficits in tasks such as the radial arm water maze (Kiss et al., 2012; Koebele et al., 2017; Koebele, Mennenga, et al., 2020; Koebele, Nishimura, et al., 2020b; McLaughlin et al., 2010; Shansky et al., 2006). Studying the impacts of stress, hormonal manipulations, and aging rodents may provide insight into the sex differences of MDD prevalence.

While many rodent models of MDD are available, exogenous corticosterone (CORT) exposure provides an opportunity to mimic HPA axis hyperactivity that occurs with chronic stress and then to study the subsequent behavioral outcomes. In adult male rodents, CORT treatment increases depressive-like behavior and leads to spatial memory deficits (Demuyser et al., 2016; Ding et al., 2018; Lui et al., 2017; V. N. Luine et al., 1993; Marks et al., 2009; McLay et al., 1998; Xie et al., 2018). Many chronic stress paradigms require rigid hours, during which behavioral testing cannot be conducted. In addition, the impacts of chronic stress on spatial ability often change and improve in the days following the end of the stressor, which limits the window to study potential

cognitive deficits, if present (Hoffman et al., 2011; Ortiz & Conrad, 2018). In comparison, CORT treatment can be implemented efficiently via injection or even in drinking water, expanding the testing window to allow testing to occur during injections. Specifically, CORT treatment administered via drinking water offers many advantages over other routes of CORT administration because drinking water administration reduces the stress of injection and results in a consistent diurnal elevated CORT pattern similar to stress exposure (Bartels et al., 2021; Gasparini et al., 2016). In summation, exogenous CORT treatment provides many benefits to the experimenter, as it can be implemented easily without disrupting opportunities to test the rodents on a variety of behavioral measures.

In this study, we tested whether CORT treatment would result in depressive-like behavior, heightened anxiety, and impaired working memory in female middle-aged OVX rats. Many studies only focus on either depressive or cognitive outcomes. The multiple domains of MDD makes it critical to understand as many of these as possible, including, depressive-like, anxiety-like, and cognitive aspects. This study utilizes a behavioral battery in order to assess aspects of depressive-like behavior such as anhedonia, social withdrawal, behavioral despair and heightened anxiety as well as possible impacts in working memory and learning. We utilized female middle-aged, OVX rodents as this model provides a "blank slate" to study CORT's influences at a timepoint that individuals are considered vulnerable to depressive-like impacts.

Methods

Subjects

Arizona State University Institutional Animal Care and Use Committee approved the procedures, which align with the Guide for the Care and Use of Laboratory Animals. 24 Middle-aged (11-12 months) female Fischer-344-cdf rats (National Institute on Aging, Charles River Laboratories, Raleigh, NC, USA) were pair housed in standard laboratory cages (21-22 °C). Except where noted below, animals were allowed food and water *ad libitum*. Animals were housed on a reverse 12:12 light cycle; lights off at 07:00. All procedures occurred during the dark phase of the light cycle. Four of the rats were reserved as conspecific strangers for social exploration testing. Following sucrose preference testing, one of the rats in the vehicle group died, thus the total number of animals was 19 for the subsequent tasks.

Ovariectomy (OVX) Surgery

All rats were OVX after habituating to the animal colony room. Rats were injected with Meloxicam (1 mg/kg) and Buprenorphine (0.2 mg/kg) to prophylactically treat pain and then anesthetized with isoflurane. The rats were laid prone, and the dorsal surgical sites were shaved and prepared with antiseptic surgical scrub alternating with 70% ethanol three times prior to incision. For each side, a single snip was made and then gently opened to 1.0 to 1.5 cm long dorsolateral incision was made in the skin and peritoneum caudal to the last rib. The ovary and uterine horn tip were ligated with dissolvable Vicryl suture (Stoelting Co.) and removed with scissors. The muscle was then

closed with dissolvable suture, Marcaine was applied, and then the skin closed with wound clips (George Teimann & Co.). Post-surgical care included treating the surgical site with antibiotic ointment, and injections of Meloxicam (1 mg/kg) and Buprenorphine (0.2 mg/kg) daily for two days. Remaining staples were removed 10-14 days after surgery.

Corticosterone (CORT) Treatment

CORT treatment began 7 days after OVX to give the rats time to recover following surgery and treatment continued throughout tissue collection. Ten rats were placed on CORT treatments and their typical drinking water was replaced with a 400ug/ml CORT solution in 2.4% ethanol. CORT solution was prepared by first preparing a CORT stock solution, by dissolving 8.3g of CORT powder (MilliporeSigma) into 500ml of 100% ethanol. The CORT stock was then diluted to 400ug/ml with reverse osmosis (RO) water and red food coloring (McCormick & Co. inc.) was added to label the final solution and distinguish it from the vehicle solution and to ensure that personnel involved with husbandry would avoid topping off the bottles with RO water. VEH rats had their typical drinking water replaced with a 2.4% ethanol with RO water solution that was colored green to signify the VEH solution. CORT and VEH treatments continued from onset through tissue collection day.

Behavior Assessment

After 4 weeks of CORT exposure, the rats began a battery of behavioral tasks. An illustration of the manipulations and testing order are shown (Fig. 4.1). The task order

was as follows: radial arm water maze (RAWM) training, sucrose preference (SP), social exploration, defensive marble burying, novelty suppressed feeding (NSF), elevated plus maze (EPM), RAWM test, visible platform, forced swim test (FST). To reduce potential behavioral effects that could result from rats being exposed to various experimenters, unwashed t-shirts were placed in the testing room, concealed from the rat's view during testing (Sorge et al., 2014). To provide white noise and ensure the dispersion of odors, fans were placed in testing rooms.

Radial arm water maze (RAWM)

The RAWM apparatus was made with black Plexiglas and consisted of a circular arena (48 cm diameter) with eight identically sized and spaced arms (27.9 cm long X 12.7 cm wide) radiating symmetrically from the center. The apparatus was filled with water (18-20 °C) rendered opaque with non-toxic black paint and was in a room with salient extra-maze cues on the surrounding walls. Four rubber platforms (10 cm diameter) served as an escape from the maze and were placed 2.5-3 cm under the water at the ends in four of the eight arms. The assigned platform locations remained consistent throughout testing for a given rat but were counterbalanced within and across treatment groups. In addition, platform location criteria required that platforms not be placed in the starting arm, nor immediately across from a starting arm, and that a maximum of two platforms could be placed in adjacent arms.

Training occurred over 12 consecutive days and consisted of 4 trials per day. Rats were brought to the testing room in individual testing cages via cart in squads of 9 or 10

with testing order counterbalanced across groups. In each trial, the rat was given 3 minutes to locate a platform and the experimenter recorded each arm entry throughout the trial. The rat was given 15 seconds to remain on the platform before being removed from the maze and returned to its individual heated testing cage for a 30 second interval. During the inter-trial-interval, the located platform was removed, any floating debris discarded, and the water stirred to distribute any potential olfactory cues. The removal of the platform after it was located on each trial required the rat to locate one of the remaining platforms and avoid entering an arm once the platform was found within a given day. This procedure was repeated until the end of trial 4 when all platforms were located, and testing concluded for that rat for the day. Throughout the process, the experimenter was visible to the rats during testing and stood in the same position while the rats were tested.

Testing occurred weeks later and involved similar procedures as described for training, but with two changes. Rats readily acquired the task and so, 4 consecutive days consisting of 4 trials/day were given to assess retention. On the fourth and final day of testing, a 2-hour delay was implemented between trials 2 and 3 to create a high working memory load evaluate working memory retention following a delay (Bernaud et al., 2022; Braden et al., 2017; Koebele et al., 2019).

Arm entries were quantified when the rat's nose crossed 11 cm into the arm, which was marked outside of the arm, visible to the experimenter and concealed to the test rat. Entries into arms without platforms were categorized as one of three error types.

Working memory correct errors (WMC), were entries into arms that previously contained a platform within a day, but no longer contained a platform as it was previously located (trials 2-4 only). Reference memory errors (RM) were first time entries into arms that never contained a platform. Working memory incorrect errors (WMI) were repeat entries into arms that never contained a platform. Total errors were the sum of all three error types (WMC + RM + WMI) on each trial. The final testing day was analyzed separately as there was an additional memory challenge with the 2-hour delay between trials 2 and 3.

Sucrose Preference (SP) Testing

SP was utilized to measure hedonic profile by exposing the rats to a free choice between a highly palatable sucrose solution or their standard drinking water. A reduced preference for the sucrose solution served as an indicator of anhedonia (M. Y. Liu et al., 2018; Willner, 2005; Willner et al., 1987). This study utilized an extended SP assessment without the need for food or water deprivation procedures (Najjar et al., 2018; Taliaz et al., 2010). Rats were single housed and habituated to two drinking bottles, one 2% sucrose in their treatment water and one their typical treatment water and food was provided *ad libitum* for 3 days. After 3 days, the amount of sucrose solution and typical water consumed was recorded. Thereafter the rats were habituated to 1% sucrose and typical water were used to assess sucrose preference for 7 days with the test days as the final 3 days.

An SP index was calculated (amount of sucrose drink consumed compared to total amount of sucrose and water consumed) over the four 2% sucrose habituation days as well as the final three 1% sucrose test days and reported as a percentage. Rodent models of depression show reduced SP index, an effect that is validated with antidepressant treatment (Willner, 2005). The bottle locations were counter-balanced and swapped daily to avoid location preference.

Social Exploration

The social exploration assessment was utilized as a measure of anxiety and depressive-like behavior, as social withdrawal is an element of depression (American Psychiatric Association, 2013; File & Seth, 2003; Goñi-Balentziaga et al., 2018; Hackenberg et al., 2021). Social exploration testing was conducted in a 3-chamber apparatus composed of 3 identical clear-plastic chambers (32 liters), connected by 2 PVC tubes (9cm length, 11cm diameter) to provide access to each of the chambers. Each apparatus chamber had a plastic lid with holes and objects and animals were confined in 64-ounce Glad food storage containers (The Clorox Co.) with lids and holes punctured throughout the top of the container. Testing was recorded with an overhead GoPro camera for later quantification.

The test rat was acclimated to each of the chambers individually, with access to the other chambers restricted. Next, the rat was acclimated to the entire 3-chamber apparatus without restricted access. On the first test trial, one end chamber contained a stranger female conspecific (i.e., rats have never seen each other before) in a confined

box. The rats were able to see, hear, and smell each other, but without physical interactions. The other end chamber contained an inanimate plastic object, confined similarly as for the stranger rat. The test rat was placed in the center chamber and could explore all three chambers for 10 min. Sociability-index was computed as a percentage based on the time spent in the chamber with the stranger vs. object for the 10 min trial. Immediately after the first test trial social novelty preference was assessed. The arena was cleaned with French Lavender scented, Method All-Purpose cleaner, and the original stranger was placed in a new container and into one end of the arena. On the opposite side of the original stranger, a novel stranger was placed in an identical container and placed on the opposite side as the original stranger rat. The test rat was placed in the center chamber and could explore all three chambers for 10 min. Social Novelty-index was computed as a percentage based on the time spent in the chamber with the novel rat vs. familiar rat for 10 min. Time spent in each of the side chambers was later quantified and time spent in the center chamber calculated as the overall time (10-min) with the time in each side chamber subtracted.

Defensive Marble Burying

The defensive marble burying task was implemented to evaluate active (burying) or passive (freezing or immobility) coping responses which relate to anxiety-like behavior (De Boer & Koolhaas, 2003; de Brouwer et al., 2019). Rats were tested simultaneously in squads of 7-8 in standard home cages isolated by sound-attenuating cabinets (Coulbourn, E10-23, 78.7cm W x 53.3 cm D x 50.8 cm H) or custom

(Melamine: 63.5 cm W x 61.0 cm D x 71.1 cm H). Rats were positioned in a standard home cage with a deep 5cm later of bedding and allowed to freely explore for 10 minutes. The subject was then placed in their home cage as 4 marbles were placed in a line along one end of the cage and on the surface of the bedding. Furthermore, pilot work demonstrated that middle-aged OVX rats sometimes hoarded the marbles (unpublished), thus the aversive nature of the task was increased (Ho et al., 2002; Ku et al., 2016; O'Connor et al., 2016) by topping each marble with 500ul Tabasco hot sauce (McIlhenny Co.). The subject was then placed in the test cage and allowed to explore for 15 minutes while being recorded. After 15 minutes, the subject was removed from the test cage and the number of marbles buried by at least 2/3 were counted.

Marble burying behavior was recorded using an overhead GoPro camera for later quantification. Behaviors quantified include, marble investigation, marble burying, grooming and immobility. Marble investigation was defined as the rat facing and/or interacting with a marble within 2 cm, but not actively burying the object, increased marble investigation was considered a lower anxiety-like profile. Marble burying was considered an active anxiety-like behavior and was defined as actively moving the bedding in the direction of the marbles with either forepaws or hindlimbs. Grooming was considered a passive anxiety-like behavior and was defined as licking body or actively moving forepaws on face/body. Immobility was also considered a passive anxiety-like behavior and was defined as the absence of active movement, grooming, or interaction with the environment. The first 10 minutes of the test were reported as the behavior in the

last 5 minutes was indistinct between groups as the rats had acclimated to the environment.

Novelty Suppressed Feeding (NSF)

NSF was utilized to assess anxiety profile by measuring rats' willingness to consume food in a novel environment (Blasco-Serra et al., 2017; Bodnoff et al., 1988; David et al., 2009; Gould et al., 2015; Snyder et al., 2011). NSF occurred in an open field arena located in a novel, brightly lit (170-180 lux) testing room without obvious spatial cues. The arena consisted of a black square field (96.5cm X 96.5cm) with high walls (38.1cm) to prevent escape.

Before each rat was tested, any previous chow was discarded, the arena was cleaned with Lime/Sea Salt scented, Method All-Purpose cleaner, and new chow was placed in the arena. Twenty-four hours prior to NSF, rats were food deprived, but with unlimited access to drinking water. On the day of testing, rats were carted, two pairs at a time in their home cages, to a holding room prior to testing. Then, an investigator retrieved the cage and brought it to the NSF testing arena. In the center of the arena there was a small pile of standard rodent chow (4-5 pieces). The rat was placed in one corner of the field, and the amount of time it took the rat to approach and nearly eat the food was recorded. If the rat did not approach the food after 8 min, the testing was terminated, and the animal was given a score of 480 sec. Latency to approach the food in eating was used as a measure of anxiety, with higher latency indicating an elevated anxiety profile. When completed, the animals were returned to the animal colony and placed individually in

their cages for 10-minutes. In each cage was a pre-weighed piece of rat chow with water still available. The amount of food consumed during the home-cage feeding was measured to assess the motivation of the rats to eat in a familiar environment. Littermates were re-united after the end of the home-cage eating assessment and food was provided *ad libitum*.

Elevated Plus Maze (EPM)

EPM was utilized as a measure of anxiety-like behavior in by differentiating time spent exploring either open and unprotected or closed and protected contexts (Handley & Mithani, 1984; Knight et al., 2021). The EPM apparatus consisted of open arms and opposing arms (50 cm long, 10 cm wide) containing 30 cm high opaque walls, crossed in the middle perpendicularly to each other, and a center area. The open arms of the EPM also contained a small Plexiglas lip (0.5 mm) to reduce the likelihood that the rats would fall. The maze was surrounded by curtains to obscure the surrounding environment and experimenter while the rat was in the maze. Lighting during testing was ambient (130-150 lux).

On the day of EPM testing, rats were transported in their home cage via cart from the colony room to an adjacent room to habituate for a minimum of 30 minutes prior to testing. Rats were brought to the testing room in their home cage in alternating pairs of VEH and CORT rats. Each rat was tested individually, first being placed in an open arm, facing the center area, and given 5 minutes to explore the EPM before being returned to its home cage. Cage mates started in opposing open arms, which were counterbalanced

across trials. The EPM was cleaned thoroughly using 70% isopropyl alcohol between each rat.

EPM performance was recorded using an overhead GoPro camera for later quantification. Open and closed arm entries were defined as the front two paws entering the arm, and open arm time began the moment the forelimbs entered the open arm and ended upon exit. Entries and time spent in the closed arms indicated anxiety-like behavior. An anxiety index was calculated using an equation which unifies all EPM factors into one ratio (Huynh et al., 2011); anxiety index values range from 0 to 1, with higher values indicating increased anxiety profiles.

$$Anxiety\ index = 1 - \left[\frac{(open\ arm\ time \div total\ time) + (open\ arm\ entries \div total\ entries)}{2} \right]$$

Visible Platform (VP)

The VP was used to assess each rat's motivational, visual, and/or motor proficiency. The apparatus was a 100 x 60 cm rectangular tub filled with clear water. A visible rubber platform (10 cm in diameter) was placed in one of three marked locations in the tub and sat approximately 5 cm above the surface of the water. Opaque curtains surrounded the maze in a circular fashion to remove extra-maze spatial cues. Lighting in the room was ambient during testing (120-130 lux).

Rats were tested in squads of 9-10 and completed 6 trials within the testing day. After transporting the squads from the colony room via cart to the testing room, cages were placed under a heat lamp. The experimenter brought each rat into the curtained area

and dropped the rat off at a constant start location in the tub. The platform was placed in one of three possible marked locations along the opposite wall of the start location (left, center and right), exposing the rat to each platform location twice across the 6 trials. Each subject began in the same start location and had 90 seconds to locate the platform, once located the rat remained on the platform for 15 seconds before being removed from the curtained area and placed back in its heated testing cage for a 10-minute inter-trial-interval and their squad mates completed their trial. The squad testing was completed when the final rat concluded its 6th trial. The experimenter recorded the latency (seconds) to locate the platform on each trial.

Forced Swim Test (FST)

The FST is commonly used to assess depressive-like behavior in rodents and thought to reflect behavioral despair (Huynh et al., 2011; Lino-De-Oliveira et al., 2005). The apparatus consisted of a clear, cylindrical Plexiglas tank (45 cm height, 20 cm diameter) filled with water (28◦ C) to about 30 cm, such that when floating, a rat could did not touch the bottom of the tank. 4 tanks were utilized at a time and vision to nearby tanks and experimenters was obscured with partitions. Behavior was recorded with side and overhead GoPro cameras to be quantified later.

FST consisted of a two-day protocol with one trial occurring each day. Rats were tested simultaneously in squads of 4, which were counterbalanced between groups. On the first day of testing, squads were transported via cart one at a time into an adjacent room to habituate for 30 minutes prior to home cages being carried to a novel testing

room. Each rat was placed into individual tanks for 10 min and then immediately removed and placed under a heat lamp for 1-hour prior to being returned to the housing colony. The FST tanks were rinsed between rats and refilled with fresh water for each rat (24 ◦C). On day 2 of testing, each rat was placed back into the tank for 5 min and behavior was recorded with a GoPro for later quantification. After testing, each rat was again placed under a heat lamp prior to being returned to the housing colony.

FST behaviors quantified included time spent swimming, climbing and immobility. Swimming was defined as paw movement underwater as well as diving behavior. Climbing was defined as actively pawing at the walls of the apparatus with paws breaching the water surface. Immobility was defined as floating or the lack of movement. Rats that spend less time swimming and climbing, and more time immobile are considered to have elevated depressive-like profiles (Detke et al., 1995; Huynh et al., 2011; Lino-De-Oliveira et al., 2005; Slattery & Cryan, 2012).

Euthanasia

Rats were euthanized the day following testing. Following decapitation, adrenal glands and thymus were excised and weighed a secondary measures of treatment effectiveness (Hara et al., 1981).

Statistical Analysis

The statistical software package, SPSS (Version 28) was used to analyze the data. When two groups were compared, student's t-tests were utilized with Cohen's d serving

as a measure of effect size (J. Cohen, 2013; Lakens, 2013). For analyses with more than one dependent measure, a mixed factors ANOVA was performed with partial eta-squared (η_p^2) serving as a measure of effect size (Keppel, 1991; Lakens, 2013; Richardson, 2011). Levene's test for homogeneity of variance was used to ensure that the statistical analysis did not violate the ANOVA assumption of homogeneity between groups. Data were represented as means (*M)* $\pm$ S.E.M. Statistical significance was defined when *p*-values were equal to or less than 0.05.

Results

Cognitive Assessment with the RAWM

During training, total memory errors, which included the sum of WMC, WMI and RM errors, revealed that both groups successfully learned the task, as demonstrated by distinct learning curves with errors decreasing as training days progressed. A repeated measures ANOVA for Treatment (VEH, CORT) across days 1-12 for total errors revealed a significant repeated effect of Day ($F(11,198) = 11.428$, $p < 0.001$, $\eta_p^2 = 0.388$) with no treatment interaction. Regardless of treatment, rats made fewer errors on day 5 (7.1 ± 0.93) than day 1 (12.6 ± 1.43, $t(19) = 3.298$, $p = 0.004$, $d = 0.737$) and fewer errors on day 10 (4.1 ± 0.70) compared to day 5 ($t(19) = 2.984$, $p = 0.008$, $d = 0.667$). Both treatment groups acquired the RAWM similarly (Fig. 4.2B). Error types were analyzed separately as well and there were no significant group differences.

How well the rats retained the RAWM training information was assessed about a month after the initial training to reveal that CORT-treated rats made more errors when the working memory load was especially high. Testing consisted of 3 days of RAWM, followed by a fourth day of testing that included a delay between trials 2 and 3. A repeated measures ANOVA for Treatment (VEH, CORT) across days 1-3 for total errors revealed no significant effects. Both treatment groups displayed similar RAWM memory performance during the first 3 days of RAWM testing (Fig. 4.2C). Error types were analyzed separately to reveal no signs of group differences. Day 4 included a delay between trials 2 and 3 thus Day 4 was analyzed separately. A t-test was significant ($t(17) = -2.634$, $p = 0.017$, $d = -1.210$), with CORT treated rats making more total errors on Day 4 (6.50 $\pm$ 1.32) than VEH rats (2.67, $\pm$ 0.44, Fig. 4.2C). Day 4 was investigated further by analyzing the total errors made across the four trials. An omnibus repeated-measures ANOVA for treatment across trials 1-4 during Day 4 revealed a significant repeated effect of trial ($F(3,51) = 25.024$, $p < 0.001$, $\eta_p^2 = 0.595$) and a significant trial by treatment interaction ($F(3,51) = 5.140$, $p = 0.004$, $\eta_p^2 = 0.232$). As trials progressed, rats made significantly more errors (Fig. 4.2D), with LSD post-hoc tests showing that the CORT-treated rats (3.90, $\pm$ 1.97) making more errors on trial 4 than compared to VEH-treated rats (1.56, $\pm$ 1.24, $p= 0.017$). Day 4 also revealed a significant effect with error type. Specifically, an omnibus repeated-measures ANOVA for treatment across trials 2-4 during Day 4 for WMC errors revealed a significant repeated effect by trial ($F(2,34) = 22.137$, $p < 0.001$, $\eta_p^2 = 0.566$) and a significant trial by treatment interaction ($F(2,34) =$

3.860, p = 0.031, η_p^2 = 0.185). WMC errors increased as trials progressed and by Trial 4, LSD post-hoc tests showed that CORT-treated rats (2.50 ± 0.32) made more WMC errors than did VEH-treated rats (1.11± 0.34, p = 0.008, Fig. 4.2E). WMI and RM errors revealed no significant group differences.

Visible Platform (VP)

Both VEH and CORT treated rats readily escaped to the VP and performed similarly to support that visual, motor, or motivational aspects were unlikely to influence RAWM behavior. Regardless of treatment, rats took less time to escape the maze as trials progressed, as a repeated measures ANOVA for group across trials (6) revealed a significant repeated effect of trial (F(5,85) = 3.214, p = 0.010, η_p^2 = 0.159) without a significant effect of treatment or interaction. Rats took less time to escape by the last trial (6.61 ± 0.79) than the first (12.45 ± 1.75).

Depressive-like Behavior

SP Testing

For the 1% sucrose test phase over the final three days of testing, CORT treatment significantly reduced 1% SP without altering the total volume of liquid consumed (Fig. 4.3B-C). CORT treated rats drank significantly less of the 1% sucrose compared to VEH treated (CORT 66.2 ± 6.5, VEH 85.4 ± 3.3, Fig. 4.3B), as shown by student's t-test ($t(18) = 7.933$, $p < 0.001$, $d = 3.645$). Groups showed similar appetite to drink as there were no differences in liquid consumption per day during the same time (CORT = 17.1 ml/day ± 2.0; VEH = 17.4 ml/day ± 2.3, Fig. 4.3C). The SP index was also assessed during the

three days of acclimation when rats were first single-housed and exposed to two bottles with one containing 2% sucrose (Fig. 4.3D-E). CORT-treated rats drank less sucrose ($t(18) = 5.149$, $p < 0.001$, $d = 2.303$, CORT (76.4 $\pm$ 6.4), VEH (88.1 $\pm$ 3.2) Fig. 4.3D), as well as less overall (i.e., total volume of both water and 2% sucrose, $t(18) = 5.350$, $p < 0.001$, $d = 2.393$, CORT (24.6 ml/day $\pm$ 4.5), VEH (35.8 ml/day $\pm$ 4.5) Fig. 4.3E). Thus, CORT-treated rats showed differences in SP after acclimation, but the SP effects during its introduction may have been confounded from reduced consumption overall.

Forced Swim Test (FST)

VEH and CORT-treated rats differed on two measures of the FST. Compared to VEH, CORT-treated rats spent more time immobile ($t(17) = -2.481$, $p = 0.024$, $d = -1.140$; CORT = 196.7 $\pm$ 44.0 seconds versus VEH = 149.70 $\pm$ 43.80 seconds, Fig. 4.8B) and less time climbing ($t(17) = 2.276$, $p = 0.036$, $d = 1.046$; CORT = 86.9 $\pm$ 43.8 seconds versus VEH = 129.3 $\pm$ 36.60 seconds, Fig. 4.8C). The swimming measure was unaffected as CORT and VEH rats spent a similar amount of time swimming during FST (CORT = 16.4 s $\pm$ 2.7; VEH = 21.0 s $\pm$ 2.9, Fig. 4.8D).

Social Exploration

The first trial of social exploration assessed sociability, measuring the duration rats chose to spend with a novel conspecific or an inanimate object. To compare across the two groups, the sociability index was statically similar ($t(17) = 0.819$, $p = 0.424$, $d = 0.376$, 4.4B) to suggest the groups were statistically similar. Separate analyses using individual paired t-tests were performed to determine whether each treatment showed a

preference for a novel conspecific or an inanimate object. The VEH-treated rats spent more time with the novel conspecific (conspecific 287.9 s ± 61.0 s) than compared to the inanimate object (79.8 ± 37.7 s, t(8) = 2.653, p = 0.029, d = 0.884, Fig. 4.4C), whereas the CORT-treated rats showed no significant preference (time with novel conspecific 216.9 ±66.4 s ± 44.9 s vs inanimate object) to suggest that CORT-treatment reduced sociability.

The second trial of social exploration assessed whether rats would prefer a new conspecific over the previous conspecific used in trial 1 as a measure of social novelty. When analyzing the social novelty index between the two groups, the social novelty index was statistically similar ($t(17) = 0.822$, $p = 0.423$, $d = 0.377$, 4.4D) to suggest that the groups behaved similarly. Separate analyses were performed to determine whether each treatment showed a preference for the new conspecific compared to the previously exposed con specific, Individual paired t-tests for each group showed no significant difference in time spent with a new unfamiliar conspecific compared to the previously exposed conspecific (VEH, time with novel conspecific 231.6 s ± 81.7 s vs inanimate object 150.2 ± 75.0 s: CORT, time with novel conspecific 186.8 s ± 82.8 s vs inanimate object 170.3 ± 53.9 s, Fig. 4.4E). Overall exploratory activity was similar between groups as groups spent similar amounts of time exploring the side compartments during sociability ($t(17) = 0.465$, $p = 0.648$, $d = 0.214$) as well as the social novelty testing ($t(17) = 1.047$, $p = 0.310$, $d = 0.481$).

Anxiety-like Assessments

Marble Burying

The first 10 minutes of the single-trial marble burying task were analyzed for differences in marble investigation, active burying, grooming and immobility to show that CORT treatment elevated anxiety by one of the measures (Fig. 4.5). CORT treated rats spent less time investigating marbles ($t(17) = 2195$, $p = 0.042$, $d = 1.009$, CORT 22.8 s $\pm$ 40.0; VEH 52.5 s $\pm$ 27.6, Fig. 4.5C). For other assessments, no statistical differences were found for time spent actively burying (CORT = 4.47 s $\pm$ 8.88; VEH = 7.36 s $\pm$ 11.62, Fig. 4.5B), grooming (CORT = 132.41 s $\pm$ 75.44; VEH = 86.20 s $\pm$ = 84.08, Fig. 4.5D), and being immobile (CORT = 211.77 s $\pm$ 105.30; VEH = 171.54 s $\pm$ 114.28, Fig. 4.5 E).

Novelty Suppressed Feeding (NSF)

Both treatment conditions performed statistically similarly on the NSF. Anxiety-like behavior, as measured by latency to approach food (CORT = 68.7.10 s $\pm$ 40.10; VEH = 47.78 s $\pm$ 37.43), did not differ between groups (Fig. 4.6B). Eating behavior in a familiar environment was also similar as groups consumed similar amounts during home cage feeding (CORT = 1.13 g $\pm$ 0.16; VEH = 1.12 g $\pm$ 0.08, Fig. 4.6C).

Elevated Plus Maze (EPM)

CORT rats showed an elevated anxiety profile during EPM testing as CORT-treated rats had a significantly higher anxiety index compared to VEH rats (CORT = 0.69 $\pm$ 0.13; VEH = 0.44 $\pm$ 0.33, $t(17) = -2.150$, $p = 0.046$, $d = -0.988$, Fig. 4.7B). Overall

maze exploration was similar between groups as there were no significant differences in total arm entries (CORT = 5.10 ± 1.60; VEH = 4.22 ± 2.44, Fig. 4.7C).

Physiological Metrics

CORT treatment altered body weight gain during the experiment, as well as adrenal and thymus weights. A repeated-measures ANOVA for treatment (CORT, VEH) by week revealed a significant effect of week ($F(12,204) = 113.789$, $p < 0.001$, $\eta_p^2 = 0.870$) and a treatment by week interaction ($F(12,204) = 3.295$, $p < 0.001$, $\eta_p^2 = 0.162$). Across weeks, regardless of group, rats showed weight gain, weighing less on week 1 (212.62 g ± 2.39) than week 13 (241.13 g ± 3.33, $p < 0.001$, Fig. 4.9A). When weeks were analyzed independently, all weeks were statistically similar aside from week 5 ($t(18) = 2.363$, $p = 0.030$, $d = 1.057$), where CORT treated rats weighed significantly less (219.20 g ± 9.62) than VEH rats (231.00 g ± 12.52, Fig. 4.9A). Adrenal and thymus weights were analyzed (per 100 g body weight) as an additional measure of CORT effectiveness and corroborate the body weight metrics. CORT treatment produced a significant decrease in adrenal weights ($t(17) = 3.146$, $p = 0.006$, $d = 1.445$; CORT = 15.31 ± 1.55 mg / 100 g body weight: VEH = 21.50 ± 1.15 mg/ 100 g body weight, Fig. 4.9B) as well as thymus weights ($t(17) = 4.106$, $p < 0.001$, $d = 1.445$; CORT = 26.66 ± 1.24 mg/ 100 g body weight; VEH = 39.24 ± 2.93 mg/ 100 g body weight, 4.9C).

Discussion

The current study implemented CORT treatment via drinking water to investigate whether CORT would intensify depressive-like symptoms including anhedonia, social exploration, learned helplessness, spatial learning and memory, and anxiety profile in middle-aged, female ovariectomized (OVX) rats. We report that CORT treatment significantly amplified several key features of depressive-like symptoms, and these include immobility in the FST, sucrose consumption in the SP task and social preference in a social exploration test, impaired spatial working memory at the highest working memory load in the RAWM, and anxiety profile from marble investigation and EPM, with no effect on NSF. Changes in body weight, and atrophy of the adrenal and thymus confirmed CORT's effectiveness, as they all displayed attenuation (Gala & Westphal, 1965; Karten et al., 1999; Watanabe et al., 1992; Waters & McCormick, 2011). Overall, the results present a model of depressive-like behavior in female rodents across a wide variety of behavioral metrics.

The current experiment is innovative in that it provides multiple measures of CORT-induced depressive profile and anxiety-like outcomes during a highly vulnerable age for females (middle-age) and without endogenous estrogens. Very few studies have investigated depressive-like behavior, anxiety, and cognition within the same subjects and in middle-aged OVX female rats. For the traditional depressive-like tests, the SP and FST were used (Detke et al., 1995; Lino-De-Oliveira et al., 2005; M. Y. Liu et al., 2018; Willner & Mitchell, 2002). The SP test was unique in that food deprivation was avoided

by the prolonged exposure and acclimation process from the 2% sucrose concentration to the 1% sucrose concentration. Had just the 2% SP been used; the results would have been hard to interpret because CORT decreased both the 2% SP Index and the total amount of liquid consumed from the water and sucrose bottles. However, the extended process allowed acclimation without food deprivation, leading to CORT decreasing 1% SP without altering the total volume of liquid consumed. For the FST, CORT elevated 2 out of 3 depressive-like metrics, as CORT increased immobility and decreased climbing without altering active swimming time. The social exploration assessment was implemented as an additional measure of depressive-like behavior as social withdrawal is a key component of MDD (Girard et al., 2014; Teo et al., 2020). In trial 1 of the social exploration experiment, CORT treated rats failed to show preference for a conspecific while VEH treated rats showed a social preference. The social exploration results indicate that CORT treatment decreased social behavior in this study. Together, CORT-increased depressive-like behaviors, anhedonia, behavioral despair and social withdrawal, as similar group differences were detected during SP, FST and social exploration.

As depressed females show high comorbidity with anxiety (Altemus et al., 2014; Anxiety and Depression Association of America, 2017), several anxiety measures were incorporated using two passive (avoidance) tasks (EPM, NSF) and one task with both passive and active aspects (defensive marble bury).These three assessments were incorporated as rodent models of anxiety show significant behavioral effects as response to anxiolytics during these tasks (Barfield et al., 2013; Blasco-Serra et al., 2017; Cryan &

Sweeney, 2011; de Brouwer et al., 2019; Knight et al., 2021; Lecorps et al., 2016; Rodgers & Dalvi, 1997; Thomas et al., 2009). Both the EPM and marble bury showed significant and reliable effects, which contrasted with the NSF task. In middle-aged female rats, CORT reduced the anxiety index on the EPM and decreased the time spent investigating marbles, with no significant effect on the NSF in OVX. The NSF relies upon the challenge of exploring a novel environment for a food reward after a period of food deprivation. One interpretation is that the NSF was insufficiently challenging to differentiate between the groups in this study. Moreover, small details of the NSF, such as lighting, could obscure results (Blasco-Serra et al., 2017; Burn, 2008; Cryan & Sweeney, 2011). Another possibility for our null effect in NSF is that the food deprivation requirement may have masked the ability to detect CORT-induced effects in OVX rats (Gale & Sclafani, 1977; Richard et al., 2017; Smith et al., 2022). In summary, this study provides two complementary anxiety tasks that use avoidance (of the open arms on the EPM) and active behaviors (investigating marbles and marble burying) as metrics for anxiety, while suggesting that NSF may be less optimal for assessing anxiety in middle-aged OVX females.

Another important feature of this study was the inclusion of cognitive assessment, by utilizing the RAWM. Cognitive fog is now included as s symptom of depression in the DSM version 5 (American Psychiatric Association, 2013). Moreover, females with MDD commonly report issues involving attention, executive function and working memory which often persist, even during remission of other depressive symptoms (Perini et al.,

2019). Consequently, the resistance of cognitive MDD symptoms underscores that cognition may offer a unique opportunity to study MDD etiology. The current study demonstrated that CORT treatment in middle-aged OVX female rats produced working memory disruptions, highlighting the relevance of this model for investigating the neurobiological underpinnings of mood disorders in females. On the RAWM, CORT-treated rats made more total errors when working memory load was highest during a delay. The importance of this finding centers around a plethora of studies showing sex-specific effects following chronic stress on spatial learning and memory. Specifically, chronically stressed male rats show impaired spatial learning and memory (Conrad et al., 1996a; Mika et al., 2012a; Nishimura et al., 2017; Ortiz et al., 2014), while chronically stressed female rats commonly show no effects and even enhanced spatial memory (Kitraki et al., 2004; McLaughlin et al., 2005, 2010; Ortiz et al., 2015; Peay et al., 2020). What is unique about the current study is the implementation of a working memory component for spatial navigation: this study revealed that chronic CORT treated females may be more susceptible to impaired working memory than to alterations in spatial "reference" memory. Although prior work used young, gonadally intact adult females (Bowman & Kelly, 2012; Ma et al., 2019; Peay et al., 2020; Wright & Conrad, 2005), subsequent studies may need to investigate whether age and gonadal status contribute to these effects as well.

Cognitive disorders related to MDD, such as attention, executive function and working memory, are notable as they often persist even during remission of other

depressive symptoms (Perini et al., 2019). Unlike human reports, chronic stress in animal models often produces spatial memory deficits, which then change and even can improve within days following the termination of the chronic stressor (Hoffman et al., 2011; Ortiz et al., 2015; Ortiz & Conrad, 2018; Peay et al., 2020). Unlike stress effects on hippocampal-mediated spatial ability, prefrontal cortex-mediated working memory effects may persist in the weeks and perhaps months following the stressor ending (Arnsten, 2009; Musazzi et al., 2015; Shansky & Morrison, 2009), although some studies may differ (McEwen & Morrison, 2013; Wellman et al., 2020; Wellman & Moench, 2019). Specifically, numerous studies document that lasting stress-evoked spatial working memory deficits on T-maze delayed-alternation as well as radial arm maze tasks (Arnsten, 2015; Gaelle et al., 2019; Mika et al., 2012b; Musazzi et al., 2015; Riaz et al., 2015). This study is the first to demonstrate that the stress hormone, CORT, can produce a depressive-like phenotype that is co-expressed with a clear cognitive dysfunction related to working memory in middle-aged OVX female rats.

Another unique feature of this study was the use of chronic CORT to produce a depressive-like phenotype instead of using chronic stress. Animal models of depression often use chronic stress manipulations (Chiba et al., 2012; D'Aquila et al., 1994; Duman et al., 2016; Fogaça & Duman, 2019; Gobinath et al., 2015; Newhouse & Albert, 2015; Peay et al., 2020; Willner et al., 1992; Xu et al., 2017). Moreover, depression and stress hormones are highly related as many clinical research highlights connections between cortisol levels and stress exposure and depressive symptomology (Caspi et al., 2003;

Egeland et al., 2005; Herbert, 2013; Staufenbiel et al., 2013). We chose to use chronic CORT exposure for several reasons, including past studies that show robust depressive-like effects following CORT treatment (Ding et al., 2018; Gregus et al., 2005; Nickle et al., 2020; Xie et al., 2018). CORT treatment also enables flexible behavioral assessment, for longer durations, over multiple days, as it only requires one daily injection.

Traditional chronic stress models involve manipulations which nearly encompass a light or dark cycle, providing minimal opportunities to assess behavior when the stressor is in effect (Conrad, 2010; Kitraki et al., 2004; Peay et al., 2020; W. Zhang et al., 2014). Additionally, many chronic stress paradigms often end before behavioral assessment, limiting the number of days to capture stress effects before brain changes occur (Goldwater et al., 2009; Hoffman et al., 2011; Ortiz et al., 2015; Ortiz & Conrad, 2018). Another benefit of using chronic CORT treatment over chronic stress manipulation is that CORT leads to consistent exposure to the stress steroid, without attenuation that may occur when the stressor remains consistent and predictable (Brummelte & Galea, 2010; Gregus et al., 2005; Y. Zhao et al., 2008). While an attenuated stress response can be a natural response to some forms of chronic stress, this may make it difficult to directly assess the effects of chronically elevated CORT over the course of an experiment. Consequently, we demonstrate that this CORT manipulation produces a depressive-like phenotype with comorbidity for high anxiety and poor working memory when taxed at a high working memory load.

This study included a social exploration component because depression often results in social withdrawal (Girard et al., 2014; Teo et al., 2020). For the current study, CORT treated rats showed a lack of social interest as they failed to show a preference for a novel conspecific while vehicle treated rats spent more time with a conspecific. Typically, rats spend more time with a conspecific over an inanimate object and more time with novel conspecifics than familiar ones (File & Seth, 2003; Hackenberg et al., 2021; Markham & Juraska, 2007). CORT treated rats exhibited a lack of preference for a conspecific versus and inanimate object, which is a sign of CORT induced social withdrawal, an important aspect of depressive behavior (Stanton et al., 2019). Conversely, neither group of aged females showed a preference during the social novelty trial which adds to previous literature demonstrating a lack of novelty preference and recognition in middle-aged rats. For instance, our lab previously reported that middle-aged females (OVX with or without E2) failed to investigate novelty in a short-term memory task, which contrasted with studies demonstrating that young adult OVX females exposed to stress or E2 showed robust novelty preference (Conrad et al., 2012; Koebele, Nishimura, et al., 2020b). Our work contributes to the growing idea that novelty investigation observed in young adult rats may change with age can be detected by middle-age. Moreover, other components could impact novelty exploration, such as testing protocols (Confined versus free interaction) or housing conditions (pair-housed) that may accentuate sex differences in social behavior (Vetter-O'Hagen & Spear, 2012).

Nevertheless, the current study adds to the literature showing a lack of novelty investigation in middle-aged female rats.

In this study, CORT administration successfully produced depressive-like behavior in middle-aged, OVX female rats using three well-described tasks (SP, FST, Social exploration), and showed comorbidity with increased anxiety profile in two tasks (EPM, marble bury), and impaired spatial working memory in the RAWM when memory load was taxed and failed to significantly alter performance during RAWM training and NSF. These findings emphasize the importance of using several measures of depressive or anxiety-like behavior. The current findings represent a critical progression in identifying female vulnerability to stress-induced depressive-like outcomes and cognitive impairment that aligns with DSM-5. One of the issues encountered with past work studying chronic stress in females was a lack of cognitive detriment when females were to be nearly two-fold more likely to express depressive symptomology than were males (K. M. Albert & Newhouse, 2019; Altemus et al., 2014; V. Luine et al., 2017; Najjar et al., 2018; University of Texas Health Science Center at Houston & Jalnapurkar, 2018). Although depressive symptoms can vary greatly from one individual to another, the consistent failure to find an effect on impaired spatial ability in chronically stressed females may center around the type of memory domain (reference vs working memory) and perhaps the age of the subject (young adult and middle-age). The conclusions are supported by a variety of behavioral measures that show a vulnerable timepoint in life for females to face the potentially detrimental effects of CORT and stress.

CHAPTER 5

CHRONIC ESTRADIOL TREATMENT IMPROVES NEGATIVE VALENCE IN OVARIECTOMIZED, MIDDLE-AGED FEMALE RATS

Introduction

Depression is at least twice as common in females as compared to males (Seedat et al., 2009; Willi & Ehlert, 2019). A variety of well-documented risk factors are associated with the increased prevalence of depression and for females, depression onset is closely correlated with hormonal state (P. R. Albert, 2015; Altemus et al., 2014; Altshuler et al., 2001). Furthermore, anxiety disorders are highly comorbid with depression, especially in the female population (Altemus et al., 2014; Anxiety and Depression Association of America, 2017; Jalnapurkar et al., 2018; Kalin, 2020). The menopausal transition at middle-age represents the passage from reproductive to non-reproductive life and is one period that is tied to increased depression onset in females (Schmidt & Rubinow, 2009; Soares, 2017). During this period, ovarian hormone levels fluctuate in an erratic manner, including estrogen and progesterone, which eventually settle at a lower concentration than typical (H. Burger et al., 2007). This coincides with physical symptoms such as hot flashes and night sweats and also symptoms that overlap with depression such as mood changes, difficulty concentrating and increased anxiety (J. L. Gordon et al., 2019; J. L. Gordon, Rubinow, et al., 2016a; Soares, 2017). Depression in middle-aged women warrants significant consideration as it is a prevalent and complex ailment.

In rodents, chronic stress is commonly used as a model for depressive and anxiety-like behavior. In male rodents, chronic stress leads to depressive and anxiety-like behaviors in tasks such as sucrose preference (SP), elevated plus maze (EPM) and open

field (OF) (Bondi et al., 2008; D'Aquila et al., 1994; Du Preez et al., 2020; Gregus et al., 2005; Huynh et al., 2011; M. Y. Liu et al., 2018; Seewoo et al., 2020; Willner et al., 1987). However, female rodents often fail to exhibit similar depressive and anxiety-like outcomes as observed in male rodent models (Gaspar et al., 2021; Hodes & Epperson, 2019; Huynh et al., 2011; Huzian et al., 2021; Knight et al., 2021). As females in the clinic exhibit higher rates of depression and anxiety, it is critical to model depressive-like outcomes in both males and females to further elucidate the different etiologies of depressive symptoms in males and females. Rodent models involving hormonal manipulations such as ovariectomy (OVX) and aging may provide a useful tool to study depressive-like behavior in females. Numerous studies illustrate that when compared to sham, OVX rats exhibit depressive-like behaviors such as social withdrawal and in tasks such as the FST, OF and EPM (C. A. Frye & Walf, 2004; Grigoryan, 2022; Hlinak, 1993; Markham & Juraska, 2007). Furthermore, aging female rodents exhibit increased depressive-like behavior such as social withdrawal, in addition to increased anxiety in assessments such as the EPM and OF (Boyer et al., 2019; Nolte et al., 2019). Therefore, OVX and aging rodent models may facilitate further understanding of female predisposition to depression and anxiety diagnoses.

Female rodent estrogens are well-documented to offer antidepressant potential, including cognitive benefits, especially in OVX middle-aged rats. For example, treatment with estrogens alleviate depressive and anxiety-like behaviors in tasks such as social exploration and open field in OVX females (Benmansour et al., 2016; L. A. M. Galea et

al., 2001; Garcia et al., 2017; Hlinak, 1993; Renczés et al., 2020; Spiteri & Ågmo, 2009). Impaired cognition is a common indication of depression and is included in the DSM-V (American Psychiatric Association, 2013). One particular estrogen, Estradiol (E2), provides benefits in many cognitive domains, including reference and working memory (Bimonte & Denenberg, 1999; Frick et al., 2018; Koebele, Nishimura, et al., 2020a). Less clear however, is whether E2 offers benefits that coincide with decreased depressive and anxiety profiles. Therefore, further research focusing on the antidepressant nature of E2 will further elucidate female predisposition to depression, enabling discovery of innovative treatments that take advantage of the neurobiological underpinnings of E2.

The current study aimed to further elucidate the impacts of E2 on depressive-like behavior and anxiety in OVX middle-aged rats. The behavioral battery was critical in this study in order to investigate affective behavior involving highly variable positive and negative valence systems (Gururajan et al., 2019; Slattery & Cryan, 2014), as few studies focus on middle-age. OVX rats at middle-age (10-11 months) were given a daily injection of either a sesame oil vehicle (VEH) or 3μg of E2, that began prior to the rats completing an extensive behavioral battery to assess depressive-like and anxiety behaviors. Depressive profile assessments included SP, FST, social exploration, and sucrose splash tasks. Anxiety profile assessments included defensive marble burying and novelty suppressed feeding (NSF). This low dose of E2 results in circulating levels of estradiol in the diestrous range in OVX female rats (Barha & Galea, 2010; Viau & Meaney, 1991) and this dose and treatment regimen improved cognition (Koebele &

Bimonte-Nelson, 2015; Prakapenka et al., 2018). This experimental approach enabled us to systematically evaluate the hypothesis that E2 treatment during middle-age may improve negative valence outcomes behavior in OVX female rats.

Methods

Subjects

Arizona State University Institutional Animal Care and Use Committee approved the procedures, which align with the Guide for the Care and Use of Laboratory Animals. Thirty-four Middle-aged (9-10 months) female Fischer-344 rats (National Institute on Aging, Hollister, CA, USA) were pair housed in standard laboratory cages (21-22 °C). Except where noted below, animals were allowed food and water *ad libitum*. Animals were housed on a reverse 12:12 light cycle; lights off at 07:00. All procedures occurred during the dark phase of the light cycle.

Ovariectomy (OVX) Surgery

All female rats were OVX after habituating to the animal colony room. Rats were injected with Meloxicam (1 mg/kg) and Buprenorphine (0.03 mg/kg) for pain and anesthetized with isoflurane. The rats were laid prone, and the dorsal surgical sites were shaved and prepared with antiseptic surgical scrub alternating with 70% ethanol three times prior to incision. For each side, a single snip was made and then gently opened to 1.0 to 1.5 cm long dorsolateral incision was made in the skin and peritoneum caudal to the last rib. The ovary and uterine horn tip were ligated with dissolvable Vicryl suture

(Stoelting Co.) and removed with scissors. The muscle was then closed with dissolvable suture, Marcaine was applied, and then the skin closed with wound clips (George Teimann & Co.). Post-surgical care included treating the surgical site with antibiotic ointment, and injections of Meloxicam (1 mg/kg) and Buprenorphine (0.03 mg/kg) daily for two days. Remaining staples were removed 10-14 days after surgery. One rat expired due to surgical complications leaving 31 rats for experimental procedures**.**

Estradiol (E2) Treatment

E2 treatment began 21 days after OVX and continued daily throughout behavioral testing as the goal was to assess the impacts of E2 in a model without ovarian-derived hormones (Prakapenka et al., 2018). Rats were randomly assigned to receive daily subcutaneous injections of either a sesame oil vehicle or a hormone injection of 3μg/ml E2 (Sigma-Aldrich) in 0.1 ml of sesame oil (Sigma-Aldrich). Both VEH and E2 injections occurred between 7:00-8:00 am and behavioral tasks were conducted at least an hour after the final treatment injection.

Behavioral Timeline

After 2-weeks of E2 injections, the rats began a battery of behavioral tasks to assess depressive-like behavior and anxiety. The task order was as follows: Sucrose preference, sucrose splash test, defensive marble burying, novelty suppressed feeding, elevated plus maze, forced swim test, elevated platform with ultrasonic vocalization recording, and social exploration (Fig 5.1). To reduce potential behavioral effects that could result from rats being exposed to various experimenters, unwashed t-shirts were

placed in the testing room, concealed from the rat's view during testing (Sorge et al., 2014). To provide white noise and ensure the dispersion of odors, fans were placed in testing rooms.

Assessments Not Included

Two of the tests were not analyzed further. With the elevated platform, several rats fell within 15 seconds of being placed on the apparatus, making it difficult to reach statistical significance with the remainder. For the ultrasonic vocalization assessments, this was a relatively new technique being performed by a colleague, but apparently, too much noise was present in the data to make it useful to analyze.

Sucrose Preference (SP)

SP was utilized to measure hedonic profile by exposing the rats to a free choice between a highly palatable sucrose solution or their standard drinking water. A reduced preference for the sucrose solution served as an indicator of anhedonia (M. Y. Liu et al., 2018; Willner, 2005; Willner et al., 1987). This study utilized an extended SP assessment without the need for food or water deprivation procedures (Najjar et al., 2018; Taliaz et al., 2010). Rats were single housed and habituated to two drinking bottles, one 2% sucrose in their typical drinking water and one their typical drinking water and food was provided *ad libitum* for 3 days. After 3 days, the amount of sucrose solution and typical water consumed was recorded. Thereafter the rats were habituated to 1% sucrose and typical water were used to assess sucrose preference for 7 days with the test days as the final 3 days.

An SP index was calculated (amount of sucrose drink consumed compared to total amount of sucrose and water consumed) over the final three 1% sucrose test days and three 2% sucrose habituation days and reported as a percentage. Rodent models of depression show reduced SPI, an effect that is validated with antidepressant treatment (Willner, 2005). The bottle locations were counter-balanced and swapped daily to avoid location preference.

Defensive Marble Burying

The marble burying task was employed to evaluate active (burying) or passive (freezing or immobility) coping responses associated with anxiety-like behavior (De Boer & Koolhaas, 2003; de Brouwer et al., 2019). Rats were brought to the testing room by cart and tested simultaneously in squads of 7-8 in isolated by sound-attenuating cabinets (Coulbourn, E10-23, 78.7cm W x 53.3 cm D x 50.8 cm H) or custom (Melamine: 63.5 cm W x 61.0 cm D x 71.1 cm H). Rats were positioned in a standard home cage with a deep 5cm later of bedding and allowed to freely explore for 10 minutes. The subject was then placed in their home cage as 12 marbles were placed in a 3 x 4 pattern along one end of the cage and on the surface of the bedding. The subject was then placed in the test cage and allowed to explore for 15 minutes while being recorded. After 15 minutes, the subject was removed from the test cage and the number of marbles buried by at least 2/3 were counted.

Marble burying behavior was recorded using an overhead GoPro camera for later quantification. Behaviors quantified include, marble investigation, marble burying,

grooming and immobility. Marble investigation was defined as the rat facing and/or interacting with a marble within 2 cm, but not actively burying the object, increased marble investigation was considered a lower anxiety-like profile. Marble burying was considered an active anxiety-like behavior and was defined as actively moving the bedding in the direction of the marbles with either forepaws or hindlimbs. Grooming was considered a passive anxiety-like behavior and was defined as licking body or actively moving forepaws on face/body. Immobility was also considered a passive anxiety-like behavior and was defined as the absence of active movement, grooming, or interaction with the environment. The first 10 minutes of the test were reported as the behavior in the last 5 minutes was indistinct between groups as the rats had acclimated to the environment.

Novelty Suppressed Feeding (NSF)

NSF was utilized to assess anxiety profile by measuring rats' willingness to consume food in a novel environment (Blasco-Serra et al., 2017; Bodnoff et al., 1988; David et al., 2009; Gould et al., 2015; Snyder et al., 2011). NSF occurred in an open field arena located in a novel, brightly lit (170-180 lux) testing room without obvious spatial cues. The arena consisted of a black square field (96.5cm X 96.5cm) with high walls (38.1cm) to prevent escape.

Before each rat was tested, any previous chow was discarded, the arena was cleaned with Lime/Sea Salt scented, Method All-Purpose cleaner, and new chow was placed in the arena. Twenty-four hours prior to NSF, rats were food deprived, but with

unlimited access to drinking water. On the day of testing, rats were carted, two pairs at a time in their home cages, to a holding room prior to testing. Then, an investigator retrieved the cage and brought it to the NSF testing arena. In the center of the arena there was a small pile of standard rodent chow (4-5 pieces). The rat was placed in one corner of the field, and the amount of time it took the rat to approach and begin eating the food was recorded. If the rat did not approach the food after 8 min, the testing was terminated, and the animal was given a score of 480 sec. Latency to approach the food in eating was used as a measure of anxiety, with higher latency indicating an elevated anxiety profile. When completed, the animals were returned to the animal colony and placed individually in their cages for 10-minutes. In each cage was a pre-weighed piece of rat chow with water still available. The amount of food consumed during the home-cage feeding was measured to assess the eating behavior in a familiar environment as a control for the novel environment measures. Littermates were re-united after the end of the home-cage eating assessment and food was provided *ad libitum*.

Forced Swim Test (FST)

The FST was implemented to assess depressive-like behavior as it has been shown to reflect behavioral despair (Huynh et al., 2011; Lino-De-Oliveira et al., 2005). The apparatus consisted of a clear, cylindrical Plexiglas tank (45 cm height, 20 cm diameter) filled with water (28◦ C) to about 30 cm, such that when floating, a rat could did not touch the bottom of the tank. 4 tanks were utilized at a time and vision to nearby

tanks and experimenters was obscured with partitions. Behavior was recorded with side and overhead GoPro cameras to be quantified later.

FST consisted of a two-day protocol with one trial occurring each day. Rats were tested simultaneously in squads of 4, which were counterbalanced between groups. On the first day of testing, squads were transported via cart one at a time into an adjacent room to habituate for 30 minutes prior to home cages being carried to a novel testing room. Each rat was placed into individual tanks for 10 min and then immediately removed and placed under a heat lamp for 1-hour prior to being returned to the housing colony. The FST tanks were rinsed between rats and refilled with fresh water for each rat (24 ◦C). On day 2 of testing, each rat was placed back into the tank for 5 min and behavior was recorded with a GoPro for later quantification. After testing, each rat was again placed under a heat lamp prior to being returned to the housing colony.

FST behaviors quantified included time spent swimming, climbing and immobility. Swimming was defined as paw movement underwater as well as diving behavior. Climbing was defined as actively pawing at the walls of the apparatus with paws breaching the water surface. Immobility was defined as floating or the lack of movement. Rats that spend less time swimming and climbing, and more time immobile are considered to have elevated depressive-like profiles (Detke et al., 1995; Huynh et al., 2011; Lino-De-Oliveira et al., 2005; Slattery & Cryan, 2012).

Social Exploration

The social exploration assessment was utilized as a measure of anxiety and depressive-like behavior, as social withdrawal is an element of depression (American Psychiatric Association, 2013; File & Seth, 2003; Goñi-Balentziaga et al., 2018; Hackenberg et al., 2021). Social exploration testing was conducted in a 3-chamber apparatus composed of 3 identical clear-plastic chambers (32 liters), connected by 2 PVC tubes (9cm length, 11cm diameter) to provide access to each of the chambers. Each apparatus chamber had a plastic lid with holes and objects and animals were confined in 64-ounce Glad food storage containers (The Clorox Co.) with lids and holes punctured throughout the top of the container. Testing was recorded with an overhead GoPro camera for later quantification.

The test rat was acclimated to each of the chambers individually, with access to the other chambers restricted. Next, the rat was acclimated to the entire 3-chamber apparatus without restricted access. On the first test trial, one end chamber contained a stranger female conspecific (i.e., rats have never seen each other before) in a confined box. The rats were able to see, hear, and smell each other, but without physical interactions. The other end chamber contained an inanimate plastic object, confined similarly as for the stranger rat. The test rat was placed in the center chamber and could explore all three chambers for 10 min. Sociability-index was computed as a percentage based on the time spent in the chamber with the stranger vs. object for the 10 min trial. Immediately after the first test trial social novelty preference was assessed. The arena was

cleaned with French Lavender scented, Method All-Purpose cleaner, and the original stranger was placed in a new container and into one end of the arena. On the opposite side of the original stranger, a novel stranger was placed in an identical container and placed on the opposite side as the original stranger rat. The test rat was placed in the center chamber and could explore all three chambers for 10 min. Social Novelty-index was computed as a percentage based on the time spent in the chamber with the novel rat vs. familiar rat for 10 min. Time spent in each of the side chambers was later quantified and time spent in the center chamber calculated as the overall time (10-min) with the time in each side chamber subtracted.

Splash Test

The sucrose splash test was utilized as a measure of behavioral despair, a depressive-like behavior (Bouguiyoud et al., 2022; Hu et al., 2017; Isingrini et al., 2010). Testing occurred within chambers in the rat colony room where the rats were housed. First, a 10% sucrose solution (40g sucrose with 400ml RO water) was prepared and placed in 200 ml spray bottles. Testing cages for each rat were prepared as home cages with normal bedding. Two rats were tested simultaneously as two investigators efficiently sprayed the dorsal side and head of the rat twice prior to closing the chamber door and setting a 5-minute timer. Following the 5 minutes, test rats were removed to their home cage and new testing cages were prepared for the next set of rats. Testing order was counterbalanced across groups throughout the day. Behavior was recorded with overhead

GoPro cameras for later quantification. Behaviors assessed included latency to begin grooming, total duration spent grooming, and number of grooming bouts.

Statistical Analysis

The statistical software package, SPSS (Version 28) was used to analyze the data. When two groups were compared, student's t-tests were utilized with Cohen's d serving as a measure of effect size (J. Cohen, 2013). For analyses with more than one dependent measure, a mixed factors ANOVA was performed. Levene's test for homogeneity of variance was used to ensure that the statistical analysis did not violate the ANOVA assumption of homogeneity between groups. One rat died following surgery and so the final group sizes were as follows: Veh (n=15) and E2 (n=16). Data were represented as means (*M)* $\pm$ S.E.M. Statistical significance was defined when *p*-values were equal to or less than 0.05.

Results

Sucrose Preference

E2 reduced 1% SP during the three days of final testing without altering the total amount of liquid consumed, which contrasts to the initial exposure to 2% SP, whereby E2 reduced 2% SP while also lowering total fluid consumption. During the final testing phase over 3 days of assessment, E2 treatment reduced 1% SP as a student's unpaired t-test revealed an effect of treatment on 1% SP index ($t(19.13) = 1.829$, $p = 0.042$, $d = .641$, Fig. 5.2B). E2 treated rats had a significantly lower 1% SP index (44.4 $\pm$ 9.0) compared

to VEH rats (62.1 ± 3.4). E2 treated rats drank similar amounts of sucrose (14.8 ± 3.0 ml/day) compared to water (11.4 ± 1.3 ml/day, $t(15) = .830$, $p = 0.419$, $d = .208$), while VEH rats consumed significantly more sucrose (20.7 ± 1.1 ml/day) compared to water (5.1 ± 0.3 ml/day, $t(14) = 12.307$, $p < 0.001$, $d = 3.178$). Importantly, the overall fluid consumption during the final test phase was similar between groups (VEH, 77.5 ± 3.1 ml: E2, 78.6 ± 6.4 ml, Fig 5.2C) to support that E2 treatment reduced 1% SP. For the initial acclimation period when the rats were single housed and introduced to the two bottles with one containing 2% sucrose, E2 treatment reduced both 2% SP and total fluid consumption (student's unpaired t-tests, 2% SP index ($t(29) = 6.586$, $p < 0.001$, $d = 2.295$, Fig 5.2D); total volume consumed, ($t(29) = 6.775$, $p < 0.001$, $d = 2.435$, Fig. 5.2E). The results show that E2 treated rats (52.8 ± 5.2) had a significantly lower index during 2% SP acclimation compared to VEH treated (87.7 ± 1.0) and that E2 treated rats consumed less fluid overall (25.7 ± 1.5 ml) compared to VEH treated rats (44.7 ± 2.4 ml).

Marble Bury

During the marble burying task, E2 and VEH treated rats performed similarly in terms of marble investigation, grooming and immobility (Fig. 5.3). Student's t-tests revealed no significance difference between groups in marble investigation ($t(29) = -0.327$, $p = 0.616$, $d = -.182$, VEH, 196.1 ± 36.3 s: E2, 221.0 ± 33.1 s), grooming ($t(29) = 1.179$, $p = 0.248$, $d = .122$, VEH, 165.9 ± 30.8 s: E2, 122.3 ± 21.2 s), and immobility ($t(29) = 0.432$, $p = 0.669$, $d = .155$, VEH, 94.0 ± 37.3 s: E2, 75.0 ± 24.3 s).

Novelty Suppressed Feeding

E2 treatment showed no effect on latency to approach food during NSF and groups consumed similar quantities of food during home cage feeding (Fig. 5.4). A student's t-test revealed no significant difference between groups in latency to approach food ($t(29) = 0.214$, $p = 0.832$, $d = .077$, VEH, 179.5 $\pm$ 52.2 s: E2, 164.8 $\pm$ 45.2 s). Home cage feeding totals were corrected by body weight (mg consumed/ g body weight) as groups weighed significantly different amounts at this point in the experiment. Groups showed similar home cage feeding behavior as they consumed similar amounts of food per body weight during this duration ($t(29) = 0.638$, $p = 0.529$, $d = .229$, VEH, 6.4 mg $\pm$ 0.42 g: E2, 6.0 mg $\pm$ 0.27 g).

Forced Swim Test

E2 treatment significantly affected three key behaviors assessed during FST: immobility, climbing, and swimming. E2 treated rats spent significantly less time immobile (72.4 $\pm$ 5.4 s) compared to VEH rats ($t(29) = 5.685$, $p < 0.001$, $d = 2.043$, 117.8 $\pm$ 5.9 s, Fig. 5.5B). E2 treated rats also spent more time climbing (127.0 $\pm$ 4.7 s) compared to VEH rats ($t(29) = -3.891$, $p < 0.001$, $d = -1.399$, 99.5 $\pm$ 5.3 s, Fig. 5.5C). Lastly, E2 rats spent more time actively swimming (17.6 $\pm$ 1.9 s) compared to VEH rats ($t(29) = -2.783$, $p = 0.009$, $d = -1.00$, 10.4 $\pm$ 1.7 s, Fig. 5.5D).

Social Exploration

VEH-treated (but not E2-treated) rats demonstrated sociability preference by spending more time with a novel conspecific compared to an inanimate object, and

neither treatment group showed a preference for another novel female rat over a previously explored stranger rat. Specifically, no group differences were found in the sociability index when test rats could choose to spend time with the area containing a novel conspecific or an inanimate object ($t(29) = 0.327$, $p = 0.746$, $d = .118$, VEH, 68.3 $\pm$ 11.3 s: E2, 63.2 $\pm$ 10.6 s, Fig. 5.6B,). Separate t-tests were performed within each group to determine whether each treatment spent more time with the conspecific or object and showed that VEH treated rats spent significantly more time with the stranger conspecific compared to the inanimate object ($t(14) = 2.730$, $p = 0.016$, $d = .705$, 320.1 $\pm$ 60.8 s for rat, 81.7 $\pm$ 39.7 s for inanimate object), whereas the E2 treated rats performed at chance ($t(15) = 1.557$, $p = 0.140$, $d = .389$, 247.4 $\pm$ 49.7 s with the novel rat, 116.9 $\pm$ 47.1 s with inanimate object, Fig. 5.6C). Another assessment was performed to determine whether E2 would influence time spent with a new unfamiliar conspecific rat compared to the previously exposed, familiar rat, termed social novelty. No group differences in social novelty was found ($t(29) = -0.868$, $p = 0.392$, $d = -.312$, VEH, 31.4 $\pm$ 11.7 s: E2, 45.9 $\pm$ 11.9 s, Fig. 5.6D). For this assessment, both treatments performed at chance to suggest no preference: VEH ($t(14) = -1.768$, $p = 0.099$, $d = -.456$, 311.2 $\pm$ 71.7 s for familiar rat, 112.2 $\pm$ 54.0 s for novel rat) and E2 ($t(15) = -.025$, $p = 0.009$, $d = -.006$, 235.1 $\pm$ 65.9 s for familiar rat, 238.1 $\pm$ 65.6 s for novel rat, Fig. 5.6E). Finally, the groups showed similar overall explorative activity, as they spent similar amounts of total time in the side compartments during the sociability trial ($t(29) = 0.517$, $p = 0.609$, $d = .186$, VEH, 401.8

± 53.9 s: E2, 364.4 ± 48.5 s), as well as the social novelty trial ($t(29) = -689$, $p = 0.496$, $d = -.248$, VEH, 423.5 ± 58.5 s: E2, 473.2 ± 43.2 s).

Sucrose Splash Test

E2 and VEH treated rats showed similar grooming behavior during the splash test in several measures. There were no group differences in latency to groom after receiving the sucrose spray ($t(29) = 0.084$, $p = 0.934$, $d = .030$, VEH, 179.5 ± 52.2 s: E2, 164.8 ± 45.2 s, Fig. 5.7B), total grooming duration ($t(29) = 0.339$, $p = 0.737$, $d = .122$, VEH, 68.7 ± 10.5 s: E2, 63.8 ± 9.7 s, Fig. 5.7C), and total grooming bouts ($t(29) = 1.262$, $p = 0.217$, $d = .454$, VEH, 6.7 ± 1.0: E2, 5.3 ± 0.5, Fig. 5.7D).

Body Weight

E2 treatment reduced weight gain throughout the experiment. A repeated measures ANOVA for Ovarian Hormone treatment (VEH, E2) across weeks (1-10) for weight revealed a significant effect of week ($F(9,252) = 25.244$, $p < 0.001$, $\eta_p^2 = 0.474$, Fig. 5.8A), and a significant interaction between week and Ovarian Hormone ($F(9,252) = 31.914$, $p < 0.001$, $\eta_p^2 = 0.533$). Weight differences began at week 2 ($p < 0.027$, $\eta_p^2 = 0.162$) and continued throughout the 10-week experiment, in which E2 treated rats showed a lack of weight gain (1.2 ± 1.9 g) compared to VEH rats ($t(28) = 12.063$, $p < 0.001$, $d = 4.405$, 38.4 ± 2.4 g).

Discussion

The current study investigated whether E2 had anti-depressant qualities and could improve anxiety-like behavior during middle-age when females were particularly susceptible to mood disorders (Schmidt & Rubinow, 2009; Soares, 2017). In this study, a behavioral battery was implemented using OVX middle-aged rats given E2 or VEH to explore characteristics of depressive-like behavior across a variety of well-described tasks: anhedonia using SP, immobility and struggling behaviors with FST, social withdrawal using a social exploration assessment, and self-grooming in the sucrose splash test. Also included were anxiety profile assessments with marble burying and NSF due to clinically depressed females showing high comorbidity with anxiety (Jalnapurkar et al., 2018; Kalin, 2020). The results showed mixed outcomes depending upon the depressive assessment. For the FST, E2 treatment had anti-depressive-like qualities by reducing immobility and increasing climbing, and swimming. In contrast, other depressive tests showed that E2 decreased 1% SP during final testing and reduced social behavior when assessing preference for a novel conspecific compared to an inanimate object. For anxiety assessments, E2 failed to alter behavior during the marble bury and NSF assessments. In summary, the results showed that E2 produced anti-depressant qualities in OVX middle-aged rats during FST, depressant-like qualities in SP and social exploration, and had no effect on anxiety assessments.

The overarching goal for this study was to interrogate the potential beneficial effects of E2 on a variety of behavioral assays commonly used to assess mood and

anxiety profile. We found that E2 decreased depressive-like behavior on the FST only, with E2 reducing immobility and increasing climbing/swimming. The literature supports that E2 and other estrogens such as ethynyl-estradiol have antidepressant effects in in young adult female rodents during FST (Estrada-Camarena et al., 2003; Koss et al., 2012; Rachman et al., 1998; Vega-Rivera et al., 2013). Interpretation of these results may be difficult, as behaviors on the FST may be influenced by increased locomotive performance (Trunnell & Carvalho, 2021) and one of the reasons why other depressive-like tests were included in this study. To counter the locomotive comment, we found no indication of locomotor differences during tasks such as marble bury and social exploration to suggest locomotion was less likely to influence performance on the FST in this study. Moreover, the FST was the most aversive and stressful assessment in this study, as the day before the test trial, rats completed a 10-minute trial in the water-filled apparatus. In rodents, exposure to relatively cold water is stress inducing and increases corticosterone response (Agrawal et al., 2011; Bali & Jaggi, 2015; Sántha et al., 2013). Thus, the antidepressant effects of E2 during FST may reflect the affective state of the rats following the water exposure. Future studies are needed to determine the possible antidepressant role of E2 in models of depression such as chronically stressed rodents. This research is novel as it demonstrated antidepressant-like effects of E2 on multiple behaviors (Immobility, Climbing, and Swimming) during FST in middle-aged rodents.

Unexpectedly, E2 failed to alter performance on many of the other assessments including the splash test, marble bury and NSF, and then had opposite effects on the 1%

SP and one of the social exploration tests. Literature shows that E2 can improve mood and anxiety in a variety of tasks such as SP, social exploration, and open field (Bowman et al., 2002; L. A. M. Galea et al., 2001; Garcia et al., 2017; Gogos et al., 2018; Romano-Torres & Fernández-Guasti, 2010; Walf & Frye, 2005). Differences between these studies and ours is that most utilize young adult rodents, and some include rodents that were in a compromised state via a stress manipulation, whereas the current study used uncompromised (no stressor), OVX middle-aged rats. The age of the subject may be critical as anxiolytic effects of E2 in middle-age rats may be test specific, as some research demonstrates anxiolytic effects of E2 on tasks such as open field, while failing to show similar effects during EPM testing (Renczés et al., 2020) Moreover, we found in young males that performance on the open field and EPM failed to correlate even though these tasks are meant to assess anxiety profile (Bellani et al., 2006). In addition, treatments such as chronic stress (Bondi et al., 2008; D'Aquila et al., 1994; Duman et al., 2016; Gregus et al., 2005; Park et al., 2019; Seewoo et al., 2020; Willner et al., 1992) or chronic exposure to the stress steroid, corticosterone via pellet, injection or drinking water (Chapter 4) are used to elicit a depressive-like state in rats (Ardayfio & Kim, 2006; Brymer et al., 2020; Ding et al., 2018; Gregus et al., 2005; Ngoupaye et al., 2018; Nickle et al., 2020). Perhaps E2 may show benefits toward mood and anxiety when the test subjects are exposed to chronic stress or corticosterone (Bérubé et al., 2006; Filova et al., 2015; L. A. M. Galea et al., 2001; Khaleghi et al., 2021). Consequently, many of the behavioral assays used in this study could potentially demonstrate improved mood and

anxiety in rodents that are of a particular age or were compromised by a chronic stress or chronic corticosterone manipulation.

For the social exploration test, the findings were unexpected for another reason in that social exploration and social novelty exploration are commonly observed in rodents. Previous research demonstrates that when given a choice, rodents typically explore a conspecific more than an inanimate object, and that they will explore a novel conspecific more than a familiar conspecific (Beery & Kaufer, 2015; Beery & Shambaugh, 2021; File & Seth, 2003; Hackenberg et al., 2021; Moy et al., 2004; Nadler et al., 2004; Schweinfurth et al., 2017; Worley et al., 2019). The only metric that aligned with the literature was that the VEH-treated females spent more time with a novel conspecific than an inanimate object (sociability). On this metric, E2-treated rats performed at chance, and moreover, both the VEH and E2 treated rats spent a similar amount of time with novel and familiar conspecifics (novel sociability). The failure to explore a novel conspecific over the familiar stranger (novel sociability) by both E2 and VEH treated rats in this study may be explained by several variables. One variable is age, which may lead to aged rodents avoiding or failing to recognize novel stimuli (Arias-Cavieres et al., 2017; Canatelli-Mallat et al., 2022; Koebele, Nishimura, et al., 2020a; Vetter-O'Hagen & Spear, 2012). Additionally, hormonal manipulations may be a variable as all of the rats in this study were OVX, which may lead to social instability and lack of social exploration in rodents (Hlinak, 1993; Karlsson et al., 2016; Spiteri & Ågmo, 2009). Another possible explanation for the lack of time spent with the novel stranger may be the rats displaying

social withdrawal (Fan et al., 2023; Laviola et al., 2004; Si et al., 2023). However, social withdrawal is unlikely to explain the results as the groups spent a similar amount of time in the side chambers with each of the stranger rats. Another explanation could be that long-term E2 treatment in rodents may also disrupt social behavior and recognition of a social conspecific in OVX rodents (Ferguson et al., 2002; Gabor et al., 2012; Tang et al., 2005). Finally, the behavior may also be due to a disruption in social memory, as aged rodents may display a lack of social exploration and social recognition memory (Boyer et al., 2019; Markham & Juraska, 2007). These results underscore the importance of considering age and hormonal status when assessing behavioral outcomes in rodents.

For another measure of anxiety using marble bury, the rats in this study failed to exhibit significant defensive burying behavior and instead, would often play with or hoard the marbles. These behaviors were atypical when interacting with aversive stimuli (Fucich & Morilak, 2018; Poling et al., 1981) and we failed to find this behavior documented in the literature, other than a mention of pilot work in a previous study (Ku et al., 2016). Aversive perception can be a critical aspect to differentiate defensive burying tasks from novel object interaction tasks (Bolles, 1970). Some labs increase the aversive nature of the marbles by coating them in a noxious substance such as hot sauce (de Brouwer et al., 2019; Ho et al., 2002) and would be an approach we would consider in the future when studying middle-aged females. More importantly, most studies using this defensive marble burying task utilized young adult rodents (de Brouwer et al., 2019; Nicolas et al., 2006; Thomas et al., 2009). Given the few studies investigating middle-

aged OVX females, this finding adds to the several documented and unique behaviors observed in middle-aged females when compared to young adult rodents.

Utilization of multiple behavioral assessments delivers comprehensive results and clarifies patterns in different behavioral dimensions, especially in systems that are relatively understudied (Feyissa et al., 2017). We found that E2 improved performance on the FST, but impaired 1% SP and sociability to report that two of three negative valence systems show E2 to impair mood and to have no effect on anxiety. A factor to consider in this study is testing order, as experience on one test may influence behavioral outcomes in subsequent tasks (Blokland et al., 2012; McIlwain et al., 2001). For example, when assessing cognition following chronic stress, testing order influenced behavior, especially when aversive tasks were implemented, such as those involving water exposure (Blokland et al., 2012; McIlwain et al., 2001; Peay et al., 2020). In terms of test order, the SP test was first and had no preceding test. However, the FST and social exploration occurred later and could have been influenced by tasks preceding it. The FST likely was sufficiently aversive to produce E2 effects regardless of the testing order, but the robust and aversive FST may have influenced following tasks (social exploration. Consequently, one interpretation is that testing order masked the E2 effects, apart from the SP and FST assessments.

An important takeaway from the current study is the implementation of a battery of behavioral assessments in middle-aged OVX females, which are an understudied demographic. Although the final 1% SP test and sociability investigations showed signs

that E2 may have induced a depressive-like state, the FST showed that E2 produced anti-depressant effects across both passive and active behaviors, which may perhaps weigh FST assessments more than SP and social investigations by having several behavioral measures. Nonetheless, combining the outcomes from the FST behaviors with the 1% SP test and sociability assessments suggest that E2 effects on negative-valence systems may cancel each other to imply that E2 has null effects on OVX middle-aged females. In other words, E2 by itself is not a germane trigger for changes in mood and anxiety when applied in middle-aged OVX female rats. Consequently, we propose that E2 likely has beneficial effects on mood and anxiety when provided in the context of a compromised state. For example, stressors can trigger depression in humans (K. M. Albert & Newhouse, 2019; Eberhart et al., 2011; Hammen, 2005; Łosiak et al., 2019; Park et al., 2019; Tafet & Nemeroff, 2016). In the current study, the one task that showed beneficial effects of E2 was a two-trial, highly aversive FST. As such, the rats may be in a highly aversive/anxious state that can benefit from E2 treatment. In rodents, chronic stressors provide a foundation to study depressive-like behavior (Bondi et al., 2008; Park et al., 2019; Seewoo et al., 2020). Consequently, we interpret E2 to improve the negative-valence systems, based upon the FST, and perhaps the beneficial effects of E2 would have been observed in more assessments following chronic stress. Future studies will test this hypothesis to further explore the neuroprotective benefits of E2 in the face of a chronic stress challenge.

CHAPTER 6

CHRONIC ESTRADIOL AND CORTICOSTERONE TREATMENT ON DEPRESSIVE-LIKE PROFILE IN GONADECTOMIZED MIDDLE-AGED FEMALE AND MALE RATS

Introduction

Major Depressive Disorder (MDD) is a prevalent mental health disorder, affecting more than 264 million people worldwide. MDD is the global leading cause of disability and is estimated to affect about 10% of the population at any given time (*Major Depression*, 2020; SAMHSA, 2020). Distinct sex differences in the prevalence, expression, and therapeutic outcome of MDD are observed. Specifically, women are at a significantly higher lifetime risk for MDD and may face greater anxiety and mood dysregulation than do men (K. M. Albert & Newhouse, 2019; P. R. Albert, 2015; Kessler et al., 2005; Seedat et al., 2009). MDD diagnosis requires the persistent presence of one of two core symptoms, depressed mood or loss of interest or pleasure, also known as anhedonia (American Psychiatric Association, 2013). In addition to the core symptoms, diagnosis requires a combination of various symptoms including but not limited to, significant weight loss or weight gain, fatigue or loss of energy, reduction of movement, diminished ability to concentrate, feelings of worthlessness, and suicidal ideation. Another complication with treatment is that MDD is associated with high rates of comorbid anxiety, which the DSM-V specifies as anxious distress, this further impacts the diagnosis, treatment options, and ultimate prognosis for individuals with MDD (American Psychiatric Association, 2013; Thase et al., 2017). MDD is also closely associated with various cognitive impairments, including deficits in attention, memory, executive function, and information processing (LeMoult & Gotlib, 2019). The combination of cognitive impairments with affective MDD symptomology significantly

impacts an individual's daily functioning and it is therefore essential to understand the link between MDD and cognition in order to develop effective treatments for the disorder and its range of symptoms.

Aging plays a critical role in the development of MDD and there are several timepoints in the lives of women where MDD risk increases, including middle age. Women transition through natural menopause at an average age of 51 (McKinlay et al., 1992). Biological menopause is defined to have occurred following 12 consecutive months of amenorrhea with no obvious pathological cause, and perimenopause is defined as the time leading to menopause through the year following menopause (Mishra, 2011; Santoro et al., 1996; World Health Organization, 1996). During the perimenopausal period, estrogen levels fluctuate and eventually result in a low estrogen state (H. Burger et al., 2007). Research highlights the fact that the prevalence of MDD in women is significantly higher than in men following puberty and sex differences remain through the perimenopausal period, with 45-68% of perimenopausal women reporting clinically significant elevations in MDD symptomology (Maki et al., 2019). Increased MDD symptomology in women is associated with variability in estradiol (E2) levels (Freeman et al., 2006). Moreover, studies reveal that greater mood sensitivity is closely associated with acute changes in E2 during the perimenopausal transition and greater E2 variability may result in increased overall MDD symptomology (J. Gordon et al., 2019; J. L. Gordon, Eisenlohr-Moul, et al., 2016; J. L. Gordon, Rubinow, et al., 2016b). The specific mechanisms which E2 influences MDD symptomology during the perimenopausal period

are unclear. However, studies underscore that this period is linked to increased sensitivity to psychosocial stress and that this increased sensitivity may contribute to the risk for MDD (J. L. Gordon et al., 2015; Pariante & Lightman, 2008). Few studies directly compare aging men and women, therefore sex differences in aging and MDD risk warrant further investigation.

Though the exact pathology of MDD is unclear, clinical studies demonstrate a strong relationship between the stress response and the development of MDD (Brown et al., 1999; Gold et al., 2015; Heim & Nemeroff, 2001; Park et al., 2019). Research shows that MDD patients exhibit hypothalamic-pituitary-adrenal (HPA) axis dysfunction (Colla et al., 2007b; Pariante & Lightman, 2008). The HPA is activated by stress, which leads to the release of glucocorticoids, cortisol in humans and corticosterone (CORT) in rodents, from the adrenal cortex. The HPA axis (and glucocorticoid release) is typically regulated by a negative feedback mechanism, though in patients with MDD present elevated cortisol compared to healthy controls (Herbert, 2013; Joseph & Golden, 2017; J. Keller et al., 2017; Staufenbiel et al., 2013). Moreover, chronic cortisol elevation in MDD patients is associated with significant memory disruption and disrupted neuronal activity (Egeland et al., 2005; Hinkelmann et al., 2009; J. Keller et al., 2017; Lebedeva et al., 2018). Accordingly, stress and depression the link between stress and MDD necessitates further investigation.

Stress-based rodent models for MDD can be indispensable to investigate depressive etiology. Gonadally intact, adult male rodents, chronic stress results in

depressive-like behavior, including anhedonia, behavioral despair, heightened anxiety, and impaired spatial memory (Bondi et al., 2008; Chiba et al., 2012; Conrad, 2010; Kleen et al., 2006; V. Luine et al., 1994b; Willner et al., 1987). However, gonadally intact, adult female rodents often fail to exhibit many of these impacts following chronic stress (Bangasser & Valentino, 2014; Bowman et al., 2001; Conrad et al., 2003; Gaspar et al., 2021; Huzian et al., 2021; V. Luine et al., 2017; Peay et al., 2020). Chronic stress research in gonadally-intact adult rodents highlights the need for rodent MDD models that allow for investigation in both sexes. Variables that deserve attention include gonadal hormone presence and age of subjects. For instance, compared to gonadally intact counterparts, ovariectomized (OVX) female rodents show increased susceptibility to depressive-like outcomes following chronic stress (Ge et al., 2020; Lagunas et al., 2010). Moreover, compared to young adults, middle aged female rodents show more susceptibility to impaired cognition negative valence outcomes following chronic stress (Bale & Epperson, 2015; Bowman et al., 2006; Hodes & Epperson, 2019). A well-documented characteristic of E2 treatment in middle-aged rodents is that E2 benefits spatial working memory (Bimonte & Denenberg, 1999; Daniel et al., 2006; Koebele, Nishimura, et al., 2020a; Taxier et al., 2020b), a dimension of mood disorders in women (Del Río et al., 2018; Kataja et al., 2017). Consequently, stress based rodent MDD models utilizing age and gonadal hormone modifications offer provide an opportunity to study MDD pathology in both males and females. Compared to traditional chronic stress paradigms, exogenous CORT administration provides a unique opportunity to directly

elevate systemic CORT and mimic HPA axis hyperactivity. Chronic CORT administration, by drinking water, pellet implant or injection, results in elevated depressive-like behavior, including impaired spatial memory in adult male rodents (Demuyser et al., 2016; Ding et al., 2018; Lui et al., 2017; V. N. Luine et al., 1993; Marks et al., 2009; McLay et al., 1998; Xie et al., 2018). Rodent models for MDD involving HPA axis manipulation deserve further investigation to uncover similarities and differences in male and female depressive etiology.

There is substantial evidence highlighting the significance of androgens, such as testosterone, on learning and memory as well as affective behaviors in male rodents (Celec et al., 2015). For instance, male gonadectomy (GDX) impairs behavior on tasks involving, avoidance behavior, social memory (Havens & Rose, 1992; Vetter-O'Hagen & Spear, 2012), and various spatial learning and memory tasks (Gibbs & Johnson, 2008; McConnell et al., 2012). Additionally, testosterone replacement reverses many of the learning and memory deficits in male rodents (Gibbs & Johnson, 2008; McConnell et al., 2012). While substantial evidence shows testosterone to have anxiolytic effects in tasks such as burying, elevated plus maze (EPM) and open field (Aikey et al., 2002; Fernández-Guasti & Martínez-Mota, 2005; Hodosy et al., 2012), many studies show no effects of testosterone on cognitive and affective behaviors (Domonkos et al., 2017; C. Frye et al., 2010). Consequently, further studies are needed to assess the complex effects of gonadal hormones in male rats.

Gonadal hormones can influence cognition and affect, but their mechanisms are far from clear. Testosterone may exert its impact on cognitive processes through various mechanisms that can include androgen receptors, via testosterone or an active metabolite such as 5*a*-dihydrotestosterone (DHT). Alternatively, testosterone can be converted to E2 by the aromatase enzyme (Andriole et al., 2004; Celec et al., 2015; Jones et al., 2006) and act via estrogen receptors (Celec et al., 2015; Edinger & Frye, 2007). Although less is known about how E2 affects cognition in males, the studies that exist show conflicting results (Frick et al., 2018). Some reports find that E2 is beneficial in GDX male rats by attenuating spatial learning and memory deficits in the Barnes maze, Morris water maze, radial arm maze and object exploration tasks (Locklear & Kritzer, 2014; V. Luine & Rodriguez, 1994; Packard et al., 1996). Additionally, E2 is anxiolytic in GDX male rodents during tasks such as the EPM and open field (Domonkos et al., 2018; Filova et al., 2015). Conversely, there is research showing that testosterone, but not E2, attenuates GDX-induced memory deficits in male rats during object recognition (Aubele et al., 2008). Moreover, DHT, a metabolite of testosterone, enhances hippocampal neurogenesis in male rodents (Leranth et al., 2003; Spritzer & Galea, 2007) and male rats given an estradiol inhibitor exhibited enhanced spatial memory (Alejandre-Gomez et al., 2007). Given the contrasting evidence, more studies are needed to elucidate which GDX-induced effects may or may not be modified by E2 in males when studying cognition and affect.

This study investigated interactions between chronic CORT and E2 treatments on a variety of depressive-like behaviors, including cognitive and anxiety-like behaviors, in middle-aged, gonadectomized female and male rats. The sex differences in MDD prevalence makes it critical to model MDD in both females and males to gain insight to mechanisms which lead to susceptibility. Additionally, this study utilized middle-aged and GDX rats as this is a vulnerable timepoint for depressive-like effects and the GDX model provides the opportunity to assess the impact of E2 without other gonadally-derived hormones. This study also included multiple assessments to investigate various depressive domains including, working memory, anhedonia, social exploration, and heightened anxiety. Finally, this study implemented Spearman correlation analyses in order to gain insight to how treatments affected relationships between the depressive domains (cognitive, depressive-like, anxiety-like).

Methods

Subjects

Arizona State University Institutional Animal Care and Use Committee approved the procedures, which align with the Guide for the Care and Use of Laboratory Animals. Two cohorts of Middle-aged (9-10 months) male (n=52) and female (n=52) Fischer-344 rats (National Institute on Aging, Hollister, CA, USA) were utilized in this experiment. Upon arrival to the Arizona State University animal facility, rats were pair housed in standard laboratory cages (21-22 °C). Male and female rats were housed in same-sex

housing units and were tested on separate days or in different rooms. Except where noted below, animals were allowed food and water *ad libitum*. Animals were housed on a reverse 12:12 light cycle; lights off at 07:00. All procedures occurred during the dark phase of the light cycle. All animals in this study were gonadectomized (GDX) and two rats of each sex from each cohort were reserved as conspecifics for social exploration testing. Surgical complications occurred during male GDX and resulted in a loss of male rats (n= 11), this resulted in the addition of male as replacements (n= 14). Although all rats began treatment and behavior at the same time, the original rats began treatment 3 weeks following GDX and the replacement rats began treatment 3 days following GDX. Due to surgical complications with the first cohort, the Arizona State University veterinary team assisted with surgeries for the second cohort. After final attrition, male (n=50) and female (n=41) rats remained for the experimental analyses.

Ovariectomy (OVX) Surgery

All female rats were OVX after habituating to the animal colony room. Rats were injected with Meloxicam (1 mg/kg) and Buprenorphine (0.03 mg/kg) for prophylactic treatment of pain and anesthetized with isoflurane. The rats were laid prone, and the dorsal surgical sites were shaved and prepared with antiseptic surgical scrub alternating with 70% ethanol three times prior to incision. For each side, a single snip was made and then gently opened to 1.0 to 1.5 cm long dorsolateral incision was made in the skin and peritoneum caudal to the last rib. The ovary and uterine horn tip were ligated with dissolvable Vicryl suture (Stoelting Co.) and removed with scissors. The muscle was then

closed with dissolvable suture, Marcaine was applied, and then the skin closed with wound clips (George Teimann & Co.). Post-surgical care included treating the surgical site with antibiotic ointment, and injections of Meloxicam (1 mg/kg) and Buprenorphine (0.03 mg/kg) daily for two days. Remaining staples were removed 10-14 days after surgery.

Male Gonadectomy/Orchiectomy Surgery (Testes Removal)

All male rats were gonadectomized (GDX) after habituating to the animal colony room. Rats were injected with Meloxicam (1 mg/kg) and anesthetized with isoflurane. The rats were laid supine, and the scrotal surgical sites were shaved and prepared with antiseptic surgical scrub alternating with 70% ethanol three times prior to incision. The scrotum skin was tented, and a single snip was made in the skin and muscle which was then gently opened, and the first testis was identified. For each testis, the fascia was slowly torn away from the tunica. The tunica was then nicked anterior to the testis to reveal the testis and epididymis. The blood vessels and fat were then sutured independently to ensure the blood supply was secure. The teste was then cut above the suture, removed from the body and the suture was returned to the tunica. The incision was then closed with wound clips (George Teimann & Co.), triple antibiotic ointment was applied, and Buprenorphine (0.03 mg/kg) was given for pain management once the rat showed coordination. Post-surgical care included treating the surgical site with antibiotic ointment, and injections of Meloxicam (1 mg/kg) and Buprenorphine (0.03 mg/kg) daily for two days. Remaining staples were removed 10-14 days after surgery.

Corticosterone (CORT) Treatment

CORT treatment began 21 days after OVX and continued throughout the day of tissue collection, as the goal was to assess the impacts of CORT in a model without gonadally-derived hormones. Rats were randomly assigned to receive daily subcutaneous injections of either a 10% ethanol vehicle solution or a stress hormone injection of 40mg/ml CORT in 10% ethanol. The CORT treatment suspension was first made by preparing a 400mg/ml CORT stock solution in 100% ethanol, which was then diluted with sesame oil to a final concentration of 40mg/ml CORT in 10% ethanol. Injections were prepared daily to ensure consistent treatment delivery as crystalline CORT (Spectrum) was utilized resulting in a suspension that may settle over time.

Estradiol (E2) Treatment

E2 treatment began 21 days after OVX and continued throughout the day of tissue collection as the goal was to assess the impacts of E2 in a model without ovarian-derived hormones (Prakapenka et al., 2018). Rats were randomly assigned to receive daily subcutaneous injections of either a sesame oil vehicle or a hormone injection of 3μg/ml E2 (Sigma-Aldrich) in 0.1 ml of sesame oil (Sigma-Aldrich). This low-dose, daily E2 injection results in circulating E2 concentrations in the diestrous scope in OVX females (Barha & Galea, 2010; Viau & Meaney, 1991). Both CORT and E2 injections occurred between 7:00-8:00 am and behavioral tasks were conducted at least an hour after the final treatment injection.

Treatment Groups

Groups were treated with, vehicle for both CORT the stress hormone and E2 the ovarian hormone (V-V), CORT and ovarian hormone vehicle (C-V), stress hormone vehicle and E2 (V-E), or both CORT and E2 (C-E). Group sizes were as follows: Female: F-V-V n=10, F-C-V n=10, F-V-E n=10, F-C-E n=11, Male: M-V-V n=11, M-C-V n=12, M-V-E n=13, M-C-E n=14. Note: the first letter denotes the sex, the second denotes the CORT-treatment and the third denotes the E2 treatment.

Behavior Assessment

After 2-weeks of CORT and E2 injections, the rats began a battery of behavioral tasks. The task order was as follows: Radial Arm Water Maze, visible platform, sucrose preference, social exploration, defensive marble burying, elevated plus maze (Fig. 6.1). To reduce potential behavioral effects that may result from rats being exposed to different experimenters, worn and unlaundered t-shirts were placed in the testing room, concealed from the rat's view during testing (Sorge et al., 2014). To disperse odors and provide white noise, fans were placed in testing rooms.

Radial Arm Water Maze (RAWM)

The RAWM apparatus was made with black Plexiglas and consisted of a circular arena (48 cm diameter) with eight identically sized and spaced arms (27.9 cm long X 12.7 cm wide) radiating symmetrically from the center. The apparatus was filled with water (18-20 °C) rendered opaque with non-toxic black paint and was in a room with salient extra-maze cues on the surrounding walls. Four rubber platforms (10 cm diameter) served as an escape from the maze and were placed 2.5-3 cm under the water at the ends

in four of the eight arms. The assigned platform locations remained consistent throughout testing for a given rat but were counterbalanced within and across treatment groups. In addition, platform location criteria required that platforms not be placed in the starting arm, nor immediately across from a starting arm, and that a maximum of two platforms could be placed in adjacent arms.

Testing occurred over 12 consecutive days and consisted of 4 trials per day. Rats were brought to the testing room in individual testing cages via cart in squads of 9 or 10 with testing order counterbalanced across groups. In each trial, the rat was given 3 minutes to locate a platform and the experimenter recorded each arm entry throughout the trial. The rat was given 15 seconds to remain on the platform before being removed from the maze and returned to its individual heated testing cage for a 30 second interval. During the inter-trial-interval, the located platform was removed, any floating debris discarded, and the water stirred to distribute any potential olfactory cues. The removal of the platform after it was located on each trial required the rat to locate one of the remaining platforms and avoid entering an arm once the platform was found within a given day. This procedure was repeated until the end of trial 4 when all platforms were located, and testing concluded for that rat for the day. Throughout the process, the experimenter was visible to the rats during testing and stood in the same position while the rats were tested.

Arm entries were quantified when the rat's nose crossed 11 cm into the arm, which was marked outside of the arm, visible to the experimenter and concealed to the

test rat. Entries into arms without platforms were categorized as one of three error types. Working memory correct errors (WMC), were entries into arms that previously contained a platform within a day, but no longer contained a platform as it was previously located (trials 2-4 only). Reference memory errors (RM) were first time entries into arms that never contained a platform. Working memory incorrect errors (WMI) were repeat entries into arms that never contained a platform. Total errors were the sum of all three error types (WMC + RM + WMI) on each trial.

For analyses, data were parted into three phases based on the learning curve, based upon past work showing changes in the overall errors being made (Koebele, Nishimura, et al., 2020a; Prakapenka et al., 2018; Talboom et al., 2008). In this study, changes in errors were observed between days 5 and 6 and again between days 9 and 10 to produce the following phases: days 2-5 were grouped together and considered Early Acquisition, days 6-9 were grouped together and considered Late Acquisition, and days 10-12 were grouped together and considered the Final Testing phase. Note, day 1 was considered habituation and excluded from analyses as this was the first exposure to RAWM testing. WMC and WMI errors were combined as a measure of overall working memory.

Visible Platform (VP)

The VP was implemented to assess each rat's motivational, visual, and/or motor capability. The apparatus was a 100 x 60 cm rectangular tub filled with clear water. A visible rubber platform (10 cm in diameter) was placed in one of three marked locations

in the tub and sat approximately 5 cm above the surface of the water. Opaque curtains surrounded the maze in a circular fashion to remove extra-maze spatial cues. Lighting in the room was ambient during testing (120-130 lux). Males and females were tested on separate consecutive days.

Rats were tested in squads of 9-10 and completed 6 trials within the testing day. After transporting the squads from the colony room via cart to the testing room, cages were placed under a heat lamp. The experimenter brought each rat into the curtained area and dropped the rat off at a constant start location in the tub. The platform was placed in one of three possible marked locations along the opposite wall of the start location (left, center and right), exposing the rat to each platform location twice across the 6 trials. Each subject began in the same start location and had 90 seconds to locate the platform, once located the rat remained on the platform for 15 seconds before being removed from the curtained area and placed back in its heated testing cage for a 10-minute inter-trial-interval and their squad mates completed their trial. The squad testing was completed when the final rat concluded its 6^{th} trial. The experimenter recorded the latency (seconds) to locate the platform on each trial.

Sucrose Preference (SP) Testing

SP was utilized to measure hedonic profile by exposing the rats to a free choice between a highly palatable sucrose solution or their standard drinking water. A reduced preference for the sucrose solution served as an indicator of anhedonia (M. Y. Liu et al., 2018; Willner, 2005; Willner et al., 1987). This study utilized an extended SP assessment

without the need for food or water deprivation procedures (Najjar et al., 2018; Taliaz et al., 2010). Rats were single housed and habituated to two drinking bottles, one 2% sucrose in their typical drinking water and one their typical drinking water and food was provided *ad libitum* for 3 days. After 3 days, the amount of sucrose solution and typical water consumed was recorded. Thereafter the rats were habituated to 1% sucrose and typical water were used to assess sucrose preference for 7 days with the test days as the final 3 days.

An SP index was calculated (amount of sucrose drink consumed compared to total amount of sucrose and water consumed) over the final three 1% sucrose test days and three 2% sucrose habituation days and reported as a percentage. Rodent models of depression show reduced SPI, an effect that is validated with antidepressant treatment (Willner, 2005). The bottle locations were counter-balanced and swapped daily to avoid location preference.

Social Exploration

The social exploration assessment was utilized as a measure of anxiety and depressive-like behavior, as social withdrawal is an element of depression (American Psychiatric Association, 2013; File & Seth, 2003; Goñi-Balentziaga et al., 2018; Hackenberg et al., 2021). Social exploration testing was conducted in a 3-chamber apparatus composed of 3 identical clear-plastic chambers (32 liters), connected by 2 PVC tubes (9cm length, 11cm diameter) to provide access to each of the chambers. Each apparatus chamber had a plastic lid with holes and objects and animals were confined in

64-ounce Glad food storage containers (The Clorox Co.) with lids and holes punctured throughout the top of the container. Testing was recorded with an overhead GoPro camera for later quantification. Due to the number of rats, testing occurred over six consecutive days. Males and females were tested on separate days.

The test rat was acclimated to each of the chambers individually, with access to the other chambers restricted. Next, the rat was acclimated to the entire 3-chamber apparatus without restricted access. On the first test trial, one end chamber contained a stranger female conspecific (i.e., rats have never seen each other before) in a confined box. The rats were able to see, hear, and smell each other, but without physical interactions. The other end chamber contained an inanimate plastic object, confined similarly as for the stranger rat. The test rat was placed in the center chamber and could explore all three chambers for 10 min. Sociability-index was computed as a percentage based on the time spent in the chamber with the stranger vs. object for the 10 min trial. Immediately after the first test trial social novelty preference was assessed. The arena was cleaned with French Lavender scented, Method All-Purpose cleaner, and the original stranger was placed in a new container and into one end of the arena. On the opposite side of the original stranger, a novel stranger was placed in an identical container and placed on the opposite side as the original stranger rat. The test rat was placed in the center chamber and could explore all three chambers for 10 min. Social Novelty-index was computed as a percentage based on the time spent in the chamber with the novel rat vs. familiar rat for 10 min. Time spent in each of the side chambers was later quantified

and time spent in the center chamber calculated as the overall time (10-min) with the time in each side chamber subtracted.

Defensive Marble Burying

The defensive marble burying task was employed to evaluate active (burying) or passive (freezing or immobility) coping responses associated with anxiety-like behavior (De Boer & Koolhaas, 2003; de Brouwer et al., 2019). Rats were brought to the testing room by cart and tested simultaneously in squads of 7-8 in isolated by sound-attenuating cabinets (Coulbourn, E10-23, 78.7cm W x 53.3 cm D x 50.8 cm H) or custom (Melamine: 63.5 cm W x 61.0 cm D x 71.1 cm H). Rats were positioned in a standard home cage with a deep 5cm later of bedding and allowed to freely explore for 10 minutes. The subject was then placed in their home cage as 4 marbles were placed in a line along one end of the cage and on the surface of the bedding. Furthermore, pilot work demonstrated that middle-aged OVX rats sometimes hoarded the marbles (unpublished), thus the aversive nature of the task was increased (Ho et al., 2002; Ku et al., 2016; O'Connor et al., 2016) by topping each marble with 500ul Tabasco hot sauce (McIlhenny Co.). The subject was then placed in the test cage and allowed to explore for 15 minutes while being recorded. After 15 minutes, the subject was removed from the test cage and the number of marbles buried by at least 2/3 were counted.

Defensive marble burying behavior was recorded using an overhead GoPro camera for later quantification. Behaviors quantified include, marble investigation, marble burying, grooming and immobility. Marble investigation was defined as the rat

facing and/or interacting with a marble within 2 cm, but not actively burying the object, increased marble investigation was considered a lower anxiety-like profile. Marble burying was considered an active anxiety-like behavior and was defined as actively moving the bedding in the direction of the marbles with either forepaws or hindlimbs. Grooming was considered a passive anxiety-like behavior and was defined as licking body or actively moving forepaws on face/body. Immobility was also considered a passive anxiety-like behavior and was defined as the absence of active movement, grooming, or interaction with the environment. The first 10 minutes of the test were reported as the behavior in the last 5 minutes was indistinct between groups as the rats had acclimated to the environment.

Elevated Plus Maze (EPM)

EPM was utilized as a measure of anxiety-like behavior in by differentiating time spent exploring either open and unprotected or closed and protected contexts (Handley & Mithani, 1984; Knight et al., 2021). The EPM apparatus consisted of open arms and opposing arms (50 cm long, 10 cm wide) containing 30 cm high opaque walls, crossed in the middle perpendicularly to each other, and a center area. The open arms of the EPM also contained a small Plexiglas lip (0.5 mm) to reduce the likelihood that the rats would fall. The maze was surrounded by curtains to obscure the surrounding environment and experimenter while the rat was in the maze. Lighting during testing was ambient (130-150 lux).

On the day of EPM testing, rats were transported in their home cage via cart from the colony room to an adjacent room to habituate for a minimum of 30 minutes prior to testing. Rats were brought to the testing room in their home cage in alternating pairs of VEH and CORT rats. Each rat was tested individually, first being placed in an open arm, facing the center area, and given 5 minutes to explore the EPM before being returned to its home cage. Cage mates started in opposing open arms, which were counterbalanced across trials. The EPM was cleaned thoroughly using 70% isopropyl alcohol between each rat.

EPM performance was recorded using an overhead GoPro camera for later quantification. Open and closed arm entries were defined as the front two paws entering the arm, and open arm time began the moment the forelimbs entered the open arm and ended upon exit. Entries and time spent in the closed arms indicated anxiety-like behavior. An anxiety index was calculated using an equation which unifies all EPM factors into one ratio (Huynh et al., 2011); anxiety index values range from 0 to 1, with higher values indicating increased anxiety profiles.

$$Anxiety\ index = 1 - \left[\frac{(open\ arm\ time \div total\ time) + (open\ arm\ entries \div total\ entries)}{2}\right]$$

Euthanasia

The day following behavioral testing, rats were euthanized with CO2 at least 1 hour after treatment administration and trunk blood was collected no more than 5 minutes after home cage disruption to capture baseline CORT levels (Bekhbat et al., 2018). Brains

were removed rapidly and dissected on ice for later analyses. Trunk blood was collected immediately following decapitation and placed on ice. Following collection, blood was centrifuged for 20 minutes at 2000 x g and plasma was collected and stored at -80 °C. Adrenal glands and thymus were excised and weighed a secondary measures of treatment effectiveness (Hara et al., 1981).

CORT ELISA

Serum samples were diluted 1:80 and processed in duplicate. Final values were represented as nanogram per milliliter. CORT levels were determined using an enzyme-linked immunoassay kit (IDS). Intra-assay precision was within 7% and inter-assay precision was within 9%. Antibody cross-reactivity to other steroids did not exceed 0.07%. Optical density values were measured at 450nm using a microplate reader.

Statistical Analysis

The statistical software package, SPSS (Version 28) was used to analyze the data. For analyses with more than one dependent measure, a mixed factors ANOVA was performed with partial eta-squared (η_p^2) serving as a measure of effect size (Keppel, 1991; Lakens, 2013; Richardson, 2011). Levene's test for homogeneity of variance was used to ensure that the statistical analysis did not violate the ANOVA assumption of homogeneity between groups. Bonferroni post-hoc analyses were used when ANOVA reached significance (Armstrong, 2014). Two-tailed Spearman correlations were calculated per group between measures to circumvent influences of kurtosis (Bonett & Wright, 2000; de Winter et al., 2016). Relevant significant correlations were represented

in circular relationship plots (Fig. 6.8-6.11). Data were represented as means (*M)* ± S.E.M. Statistical significance was defined when p-values were equal to or less than 0.05.

Results

Radial Arm Water Maze (RAWM)

The first analyses were performed on total working memory errors committed to confirm the designation of the three phases (early acquisition, late acquisition, final testing) of the learning curve. An omnibus repeated measures ANOVA for Sex (F, M), Stress Hormone (CORT, VEH), and Ovarian Hormone (E2, VEH) across phases (Early Acquisition, Late Acquisition, Asymptotic) for total working memory errors revealed a significant repeated effect of Phase ($F(2,166) = 103.508$, $p < 0.001$, $\eta p2 = 0.555$, Fig. 6.2A). Simple effects post-hoc analyses revealed that each of the three phases significantly differed from each other ($p < 0.001$) and confirmed that these phases were distinct. In addition, significant interactions were found for Phase and Sex ($F(2,166) = 3.798$, $p = 0.024$, $\eta p2 = 0.044$), and Phase, Sex and Ovarian Hormone ($F(2,166) = 3.224$, $p = 0.042$, $\eta p2 = 0.037$) with no other significant effects (and RM errors were also found to be not significant). Consequently, the subsequent analyses were performed on each sex separately with each of the three phases analyses independently (Fig 6.2A, B). Furthermore, we focused on Trials 3 and 4 because these carry the highest working memory load (compared to trials 1 and 2) and prior research, including Chapter 4 of this dissertation, demonstrated that hormone treatment effects manifest when working

memory was highest (Koebele, Mennenga, et al., 2020; Koebele, Nishimura, et al., 2020a; Prakapenka et al., 2018).

Early Acquisition Phase (Days 2-5): During Early Acquisition, E2 altered working memory in female rats with no effects in males (Fig. 6.2C, D). In females, An ANOVA for Stress Hormone and Ovarian Hormone on total working memory errors made during trials 3 and 4 of early acquisition (days 2-5) was performed. Female rats treated with E2 (24.0 $\pm$ 8.0) made fewer working memory errors compared to females that received sex hormone vehicle (33.3 ± 10.0; $F(1,37) = 11.641$, $p = 0.002$, $\eta p2 = 0.235$, Fig. 6.2C). There were no other significant effects in females and no significant effect in males during the early acquisition phase.

Late Acquisition Phase (Days 6-9): During Late Acquisition, E2 altered working memory in female rats with no effects demonstrated in males. An ANOVA for Stress Hormone and Ovarian Hormone on total working memory errors made during trials 3 and 4 of late acquisition (days 6-9) revealed a significant effect of Ovarian Hormone ($F(1,37) = 5.246$, $p = 0.028$, $\eta p2 = 0.124$, Fig 6.2C) with no other significant effects. This showed that female rats treated with E2 made fewer errors (14.8 $\pm$ 1.4) compared to those that received the sex hormone vehicle (19.5 $\pm$ 1.5). There were no significant effects in males during the late acquisition phase.

Asymptotic Phase (Days 10-12): During the asymptotic phase, E2 altered working memory in female rats while males demonstrated no treatment effects. An ANOVA for Stress Hormone and Ovarian Hormone on total working memory errors

made during trials 3 and 4 of final testing (days 10-12) revealed a significant effect of Ovarian Hormone ($F(1,37) = 13.603$, $p < 0.001$, $\eta p2 = 0.269$, Fig 6.2C) with no other significant effects. This showed that female rats treated with E2 made fewer errors (5.8 $\pm$ 1.1) compared to those that received the sex hormone vehicle (11.9 $\pm$ 1.2). There were no significant effects in males during the late acquisition phase

Visible Platform

All groups readily escaped to the VP and performed similarly to support that visual, motor, or motivational aspects were unlikely to influence RAWM behavior. An omnibus repeated measures ANOVA for Sex, Stress Hormone and Ovarian Hormone across trials 1 through 6 revealed a significant trial effect ($F(5,415) = 37.063$, $p < 0.001$, $\eta p2 = 0.309$), and a significant interaction between trial and Sex ($F(5,415) = 25.406$, $p < 0.001$, $\eta p2 = 0.101$), as males found the platform faster than females during early trials as would be expected, and a significant trial effect ($F(5,415) = 37.063$, $p < 0.001$, $\eta p2 = 0.309$). The swim latencies were plotted separately for the sexes to show that regardless of Hormone Treatment, female and male rats took less time to escape as trials progressed (females last trial, 5.8 $\pm$ 0.4 s versus first 19.8 $\pm$ 1.9 s; Males last trial, 4.2 $\pm$ 0.4 s versus first, 9.1 $\pm$ 1.1 s).

Sucrose Preference (SP)

For the 1% SP test phase, an omnibus ANOVA was performed on the SP index using Sex, Stress Hormone, and Ovarian Hormone as the independent variables. A significant main effect of Sex was revealed ($F(1,83) = 4.438$, $p = 0.038$, $\eta_p^2 = 0.051$, Fig.

6.3A), with no other main effects or interactions. Regardless of treatment, females had a significantly greater SP index (86.5 $\pm$ 2.8) compared to males (78.7 $\pm$ 2.5), during the 1% SP test phase, by preferring to drink more of the 1% sucrose than did the males (Fig. 6.3A). This SP index was unlikely caused by total volume of drink consumed as female and males were statistically similar in total liquid consumed (1% sucrose and water) during the same period (Fig. 6.3B), as the omnibus ANOVA for total liquid consumed revealed no significant effects.

For the acclimation phase when rats were single housed and exposed to the two bottles with one containing 2% sucrose, there appeared to be differences among treatments and so this phase was probed further. An omnibus ANOVA was performed on the 2% SP index using Sex (F, M), Stress Hormone (CORT, VEH), and Ovarian Hormone (E2, VEH) as the independent variables. Significant main effects of Sex ($F(1,83) = 7.728$, $p = 0.007$, $\eta_p^2 = 0.085$), and Ovarian Hormones ($F(1,83) = 71.237$, $p < 0.001$, $\eta_p^2 = 0.462$) were found, as well as a significant interaction for Sex and Ovarian Hormone ($F(1,83) = 32.054$, $p < 0.001$, $\eta_p^2 = 0.279$, Fig. 6.3C) and a non-significant three-way interaction for Sex, Stress Hormone, and Ovarian Hormone ($F(1,83) = 3.097$, $p = 0.082$, $\eta_p^2 = 0.036$). Consequently, the data were further investigated separately by Sex.

Both females and males showed a similar effect of E2 decreasing the 2% SP index. A two-way ANOVA for Stress Hormone and Ovarian Hormone was performed on the 2% SP index and revealed a significant main effect for Ovarian Hormone for females ($F(1,37) = 10.228$, $p = 0.003$, $\eta_p^2 = 0.217$) and males ($F(1,46) = 71.996$, $p < 0.001$, $\eta_p^2 =$

0.610) with no other significant effects. E2 significantly lowered the SP index in females (90.2± 0.6 versus sex hormone vehicle 92.8 ± 0.6) and in males (82.6 ± 6.9 versus sex hormone vehicle (95.4 ± 2.5, Fig. 3C). For females, total volume consumed unlikely explained these effects, as follows. In females, a two-way ANOVA for Stress Hormone, and Ovarian Hormone performed on the total volume consumed (during 2% SP acclimation) revealed a significant Stress Hormone and Ovarian Hormone interaction ($F(1,37) = 4.091$, $p = 0.050$, $\eta_p^2 = 0.100$, Fig. 6.3C) with no other significant effects. Bonferroni post-hoc tests showed that CORT alone (F-C-O) or E2 alone (F-V-E) in females increased the volume consumed compared to females treated with vehicle (F-V-O). Interestingly, females treated with E2 and CORT consumed a similar amount of drink compared to all groups. Consequently, volume consumed was unlikely to explain why E2 decreased the 2% SP index during acclimation in females. For males, a two-way ANOVA for Stress Hormone, and Ovarian Hormone was performed on the total volume consumed during 2% SP acclimation and revealed a significant main effect of Ovarian Hormone ($F(1,46) = 41.584$, $p < 0.001$, $\eta_p^2 = 0.475$). During 2% SP acclimation, males treated with E2 consumed significantly less fluid (52.8 ± 20.7 ml) than compared to males that did not receive E2 (84.1 ± 10.3 ml, Fig. 6.3D). Consequently, the volume consumed by the males may have explained the differences in 2% SP Index during acclimation.

Social Exploration

Hormonal treatment altered female sociability, which is the time spent with the novel conspecific compared to an inanimate object and failed to alter male performance. An omnibus ANOVA was performed on the Sociability Index (comparing time with novel conspecific versus inanimate object) using Sex (F, M), Stress Hormone (CORT, VEH), Ovarian Hormone (E2, VEH) as the independent variables to reveal no significant main effects or interactions, to indicate that the sexes and treatments behaved similarly to each other (Fig. 6.4A). Separate analyses were performed on each treatment group to assess time with a novel conspecific versus an inanimate object using individual paired t-tests. Female rats that received no Stress Hormone or Ovarian Hormone treatment (F-V-O) were the only group to show significant preference by spending considerably more time with the novel conspecific (296.1 $\pm$ 62.1 s) compared to the inanimate object (68.7 $\pm$ 22.6 s), ($t(9) = 2.822$, $p = 0.018$, $d = 0.913$, Fig. 6.4B). The remaining groups showed no significant preference and spent statistically similar amounts of time with the novel conspecific and the inanimate object. To determine whether the overall exploratory activity altered performance, the total time spent in the side compartments was analyzed and revealed no significant effects or interactions ($F1,83) = 3.138$, $p = 0.080$, $\eta_p^2 = 0.036$ for the three-way interaction for Sex, Stress Hormone and Ovarian Hormone).

The second social exploration trial assessed social novelty, which determined whether the test subjects preferred a novel stranger compared to the previously exposed novel rat and found no significant effects for either sex. An omnibus ANOVA was

performed on the Social Novelty Index, using Sex (F, M), Stress Hormone (CORT, VEH), Ovarian Hormone (E2, VEH) as the independent variables, and revealed no significant effects. Interestingly, Ovarian Hormone treatment approached significance, ($F(1,71) = 3.454$, $p = 0.067$, $\eta_p^2 = 0.046$), to suggest that E2 treated rats expressed a higher Social Novelty Index (Females and Males treated with E2, 50.0 $\pm$ 38.8) compared to rats that did not receive E2 treatment (Females and Males treated with vehicle, 34.5 $\pm$ 37.0, Fig. 6.4C). Separate analyses were performed on each treatment group to determine whether they explored the novel conspecific over the previously exposed conspecific. Individual paired t-tests for each group showed no significant differences in time spent with a new unfamiliar conspecific compared to the previously exposed conspecific (Fig. 6.4D). To determine whether the overall exploratory activity altered performance, the total time spent in the side compartments (and not in the center arena) during the social novelty trial was assessed with an omnibus ANOVA and revealed a significant three-way interaction between Sex, Stress Hormone and Ovarian Hormone ($F1,83) = 5.306$, $p = 0.024$, $\eta_p^2 = 0.060$) with no other main effects or interactions. The three-way interaction was probed by investigating females and males separately, revealing no significant effects in time spent in the side compartments for the females, but a significant interaction for Stress Hormone and Ovarian hormone in males ($F(1,46) = 6.079$, $p = 0.017$, $\eta_p^2 = 0.117$) for the side compartments. Post-hoc tests revealed that males treated with CORT (M-C-V) spent more time in the side compartments than did males treated with vehicle (M-V-O, $p < 0.05$) and males treated with CORT and E2 (M-C-E, $p < 0.05$).

Defensive Marble Bury

Defensive marble burying behavior was investigated using three different parameters: time spent investigating the marbles, time spent immobile, and time spent grooming. Sex, E2 and CORT all impacted defensive marble burying behavior (Fig. 6.5).

Time Investigating Marbles: CORT and E2 altered behavior during marble bury differently based upon sex, with females being particularly sensitive to both CORT and E2, while males showed marginal changes to E2. An omnibus ANOVA was performed on the time spent investigating marbles using Sex (F, M), Stress Hormone (CORT, VEH), Ovarian Hormone (E2, VEH) as the independent variables. Significant main effects of Stress Hormone ($F(1,83) = 9.597$, $p = 0.003$, $\eta_p^2 = 0.104$), and Ovarian Hormone ($F(1,83) = 8.481$, $p = 0.005$, $\eta_p^2 = 0.093$) were found, with the Stress Hormone and Ovarian Hormone interaction approaching significance ($F(1,83) = 3.790$, $p = 0.055$, $\eta_p^2 = 0.044$) with no other main effects or interactions. The significant effect of Stress Hormone revealed that the rats treated with CORT spent less time investigating marbles compared to those that did not receive CORT Fig. 6.5A and graph insets). The significant effect of Ovarian Hormone showed that rats treated with E2 spent significantly more time investigating marbles compared to rats that did not receive E2 treatment (Fig. 6.5A and graph insets). Given the three-way interaction p-value, the time spent investigating marbles was further probed separately by sex. For females, a two-way ANOVA for Stress Hormone and Ovarian Hormone on time spent investigating marbles revealed significant effects of Stress Hormone ($F(1,37) = 8.976$, $p = 0.005$, $\eta_p^2 = 0.195$), Ovarian Hormone

($F(1,37) = 6.123$, $p = 0.018$, $\eta_p^2 = 0.142$), and interaction between Stress Hormone and Ovarian Hormone ($F(1,37) = 6.114$, $p = 0.018$, $\eta_p^2 = 0.142$). Bonferroni post-hoc tests of the interaction showed that female rats treated with E2 (F-V-E) spent significantly more time investigating marbles (64.2 ± 14.5 s) compared to all other groups ($p < 0.001$). For males, a two-way ANOVA for Stress Hormone and Ovarian Hormone revealed no significant main effects or interactions for time spent investigating marbles.

Time Spent Immobile: An omnibus ANOVA was performed on the time spent immobile during the marble burying task and revealed significant main effects of Sex ($F(1,83) = 5.488$, $p = 0.022$, $\eta_p^2 = 0.062$), Stress Hormone ($F(1,83) = 5.265$, $p = 0.024$, $\eta_p^2 = 0.060$), and Ovarian Hormone ($F(1,83) = 11.058$, $p = 0.001$, $\eta_p^2 = 0.118$) with no interactions. These significant effects demonstrated that males spent more time immobile than females, CORT treatment increased immobility, and E2 treatment reduced immobility (Fig. 6.5B and insets).

Time Spent Grooming: An omnibus ANOVA was performed on the time spent grooming during the marble burying task and revealed a significant main effect of Sex ($F(1,83) = 5.436$, $p = 0.022$, $\eta_p^2 = 0.061$) and a marginal effect of Ovarian Hormone ($F(1,83) = 3.153$, $p = 0.079$, $\eta_p^2 = 0.037$) with no other main effects or interactions.

Regardless of Stress and Ovarian Hormone treatment, female rats spent significantly more time grooming (124.2 $\pm$ 93.9 s) compared to male rats (82.2 $\pm$ 78.9 s, Fig. 6.5C).

Elevated Plus Maze (EPM)

CORT and E2 altered anxiety profile on the EPM based upon sex, with females being particularly sensitive to both CORT and E2, while males showed marginal changes to E2. An omnibus ANOVA was performed on the EPM Anxiety Index using Sex (F, M), Stress Hormone (CORT, VEH), Ovarian Hormone (E2, VEH) as the independent variables. Significant main effects of Sex ($F(1,83) = 11.230$, $p < 0.001$, $\eta_p^2 = 0.119$), and Ovarian Hormone ($F(1,83) = 21.451$, $p < 0.001$, $\eta_p^2 = 0.205$) were found and revealed that the anxiety profiles of females were higher than compared to males and that E2 lowered the anxiety profile compared to vehicle (Fig. 6A). In addition, the omnibus ANOVA revealed near significant interactions for Sex and Ovarian Hormone ($F(1,83) = 3.758$, $p = 0.056$, $\eta_p^2 = 0.043$) and for Sex, Stress Hormone and Ovarian Hormone ($F(1,83) = 3.079$, $p = 0.083$, $\eta_p^2 = 0.036$). Consequently, the data were further investigated separately by Sex.

For females, a two-way ANOVA for Stress Hormone and Ovarian Hormone was performed on EPM anxiety profile and revealed a significant main effect for Ovarian Hormone ($F(1,37) = 35.119$, $p < 0.001$, $\eta_p^2 = 0.395$) and a significant interaction between Stress Hormone and Ovarian Hormone ($F(1,37) = 4.395$, $p = 0.043$, $\eta_p^2 = 0.106$). The interaction was probed further with Bonferroni post-hoc tests and revealed that CORT effects differed depending upon whether females were treated with E2 or sex hormone

vehicle (Fig. 6.6A and inset). Females treated with just CORT (F-C-V) expressed the highest anxiety profiles (0.62 $\pm$ 0.03) compared to females treated with just E2 (F-V-E, 0.52 $\pm$ 0.02, $p < 0.003$) and females treated with both CORT and E2 (F-C-E, 0.46 $\pm$ 0.02. $p < 0.001$). Furthermore, females treated with CORT and E2 had the lowest anxiety profiles and were statistically lower than females given the stress hormone vehicle and sex hormone vehicle treatments (F-V-V, 0.58 $\pm$ 0.02, $p < 0.001$). For males, a two-way ANOVA for Stress Hormone and Ovarian Hormone on EPM anxiety profile revealed no significant main effects or interactions. However, the Ovarian Hormone treatment showed a marginal tendency to decrease anxiety ($F(1,46) = 3.495$, $p = 0.068$, $\eta_p^2 = 0.071$, Fig. 6.6A and inset).

Total arm entries were investigated as a measure of overall maze exploration, a three-way ANOVA revealed a significant interaction for Sex and Ovarian Hormone ($F(1,83) = 4.612$, $p = 0.035$, $\eta_p^2 = 0.053$), thus sexes were analyzed separately. For females, a two-way ANOVA for Stress Hormone and Ovarian Hormone on total arm entries revealed a significant effect of Ovarian Hormone ($F(1,37) = 5.330$, $p = 0.027$, $\eta_p^2 = 0.126$, Fig. 6.6B and inset). Females treated with E2 made significantly more arm entries (5.4 $\pm$ 0.3) compared to vehicle (4.4 $\pm$ 0.3). No significant effects for total entries were found in males.

Physiological Metrics

Body Weights: Body weight measures were investigated separately by Sex due to the inherent differences in body size. For females and males, E2 but not CORT altered

body weight (Fig. 6.7A). A repeated-measures ANOVA on body weight using Stress Hormone and Ovarian Hormone as independent variables by week revealed a significant effect of week ($F(5,185) = 4.763$, $p < 0.001$, $\eta p2 = 0.114$) as well as a Sex Hormone by week interaction ($F(2,185) = 22.580$, $p < 0.001$, $\eta p2 = 0.379$). E2 treated female rats began to weigh significantly less than their VEH counterpart at week 2 of the experiment and the differences continued through the remainder of the experiment. E2 treated females gained less weight, and even lost weight during treatment (-26.9 $\pm$ 4.5 g) compared to females receiving VEH (11.6 $\pm$ 1.7 g). For males, a repeated-measures ANOVA for Stress Hormone and Ovarian Hormone by week revealed a significant effect of week ($F(4,184) = 27.908$, $p < 0.001$, $\eta p2 = 0.378$, Fig. 6.7A) as well as a Sex Hormone by week interaction ($F(4,184) = 4.963$, $p < 0.001$, $\eta p2 = 0.097$). E2 treated male rats began to weigh significantly less than male VEH rats at week 3 of the experiment and the differences continued through the remainder. Although both groups lost weight throughout the experiment, E2 treated males lost more weight during treatment (-26.9 $\pm$ 4.5 g) compared to males receiving VEH (-12.8 $\pm$ 4.7 g). There were no significant body weight effects or interactions with stress hormone treatment.

Adrenal weights confirmed the systemic administration of CORT and E2 in both males and females while thymus weights failed to show treatment effect (Fig. 6.7B-C and insets). Adrenal and thymus weights (per 100 g body weight) were analyzed as an additional measure of CORT effectiveness using Sex, Stress Hormone and Ovarian Hormone as independent variables. Sex was compared because tissue weight was

corrected for body weight. A three-way omnibus ANOVA for adrenal weight (mg/100g body weight) revealed significant effects of Sex ($F(1,80) = 64.003$, $p < 0.001$, $\eta_p^2 = 0.444$), Stress Hormone ($F(1,80) = 42.419$, $p < 0.001$, $\eta_p^2 = 0.347$), and Ovarian Hormone ($F(1,80) = 9.818$, $p = 0.002$, $\eta_p^2 = 0.109$) with no other significant effects. Females showed higher adrenal weights (18.5 $\pm$ 0.7 mg/100g body weight) than did males (11.1 $\pm$ 0.6 mg/100g body weight), CORT reduced adrenal weights (11.8 $\pm$ 0.6 mg/100g body weight) compared to vehicle (17.8 $\pm$ 0.7 mg/100g body weight), and E2 increased adrenal weights (16.2 $\pm$ 0.6 mg/100g body weight) compared to vehicle (13.4 $\pm$ 0.7 mg/100g body weight, Fig. 6.7B and insets). A three-way omnibus ANOVA for thymus weight (mg/100g body weight) revealed no significant effects (Fig. 6.7C).

Serum CORT measurements confirmed systemic administration of the Stress Hormone treatment in both females and males (Fig. 6.7D and inset). A three-way omnibus ANOVA for serum CORT concentration revealed a significant main effect of CORT ($F(1,83) = 56.780$, $p < 0.001$, $\eta_p^2 = 0.406$) and a marginal interaction between Sex and Stress Hormone ($F(1,83) = 3.775$, $p = 0.055$, $\eta_p^2 = 0.044$). CORT treatment elevated baseline serum CORT levels across all conditions (CORT = 412.9 $\pm$ 13.9 ng/ml, VEH = 264.6 $\pm$ 14.3 ng/ml).

Correlational Analysis

Spearman correlations were performed for each group to study the correlations between the measures of cognitive, depressive-like, anxiety-like behaviors and, baseline serum CORT. The cognitive measurements included were, RAWM early acquisition

working memory errors (T3 + T4), RAWM late acquisition and RAWM asymptotic stages. The depressive-like measurements included were, 1% testing SP index, 2% acclimation SP index, sociability index and social novelty index. The anxiety-like measurements included were, defensive marble bury behaviors (investigation, immobility, and grooming) and the EPM anxiety index. Correlation coefficients are displayed as heatmaps (Fig. 6.9), and correlations are plotted in circle diagrams to display relationships between behaviors (Fig. 6.8). Significant correlations ($P \leq 0.05$) are shown for each treatment separately, with both females and males listed together and lines denoting the metrics with correlations. Metrics with positive correlations are shown with solid lines and those with negative correlations are shown with dashed lines. Furthermore, strong correlations ($P \leq 0.01$) are illustrated thicker line compared to weaker correlations (Fig. 6.8). While the sample size requirements were not met for a false discovery rate calculation, Bonferroni corrections were implemented for added control(Benjamini et al., 2001; Colquhoun, 2014).

V-V (Fig. 6.8A): Female rats treated with both stress hormone vehicle, and ovarian hormone vehicle (F-V-V) showed a significant negative correlation for social novelty index with 2% acclimation SP index (-.767, p = .016). The females that had a high 2% acclimation SP Index showed a lower preference for a novel conspecific on the social novelty task. For male rats treated with both stress hormone vehicle and ovarian hormone vehicle (M-V-V), there were significant positive correlations for RAWM asymptotic phase working memory errors with defensive marble bury investigation (.668,

p = .025) as well as negative correlations for social novelty index with 2% acclimation SP index (-.902, p < .001).The males with more marble investigation made more working memory errors and the males with greater SP during acclimation showed greater social novelty exploration.

C-V (Fig. 6.8B): For female rats treated with CORT and ovarian hormone vehicle, there were significant positive correlations for RAWM early acquisition errors with EPM anxiety index (.825, p = .003) and marble bury investigation with social novelty index (.802, p = .017). The females with a greater EPM anxiety index made more working memory errors. There were significant negative correlations for marble bury immobility and social novelty index (-.850, p = .007), sociability index with 1% testing SP index (-.880, p = .021), and sociability index with 2% acclimation SP index (-.826, p = .043). The females that spent more time immobile during defensive marble burying showed less social novelty exploration. Also, the females with greater SP during testing and acclimation showed less social exploration. For male rats treated with CORT and ovarian hormone vehicle, there was a significant positive correlation for RAWM late acquisition working memory errors with 2% acclimation SP index (.701, p = .011). The males with greater SP during acclimation made more working memory errors.

V-E (Fig. 6.8C): For female rats treated with stress hormone vehicle and E2, there was a significant positive correlation for RAWM asymptotic phase working memory errors with social novelty index (.714, p = .031). The females with more social novelty exploration made more working memory errors. There were significant negative

correlations for baseline CORT with EPM anxiety index (-.880, p = .021) and marble bury grooming and EPM anxiety index (-.880, p = .021). The females with greater EPM anxiety index had lower baseline CORT and less grooming during defensive marble burying. For male rats treated with stress hormone vehicle and E2, there were significant positive correlations for RAWM early acquisition working memory errors with marble bury investigation (.653, p = .029) and social novelty index with baseline CORT (.697, p = .012). The males with more defensive marble burying investigation made more working memory errors. There were significant negative correlations for RAWM early acquisition working memory errors with marble bury immobility (-.715, p = .013), RAWM late acquisition working memory errors with EPM anxiety index (-.629, p = .021), and 1% testing SP index with baseline CORT (-.581, p = .037). The males with more defensive marble burying immobility made less working memory errors. The males with a higher EPM anxiety index made fewer working memory errors and the males with a higher baseline CORT showed lower testing SP.

C-E (Fig. 6.8D): For female rats treated with CORT and E2, there was a significant positive correlation for RAWM asymptotic phase working memory errors with marble bury immobility (.823, p = .002). The females with more defensive marble burying immobility made more working memory errors. There was a significant negative correlation for RAWM asymptotic phase working memory errors with marble bury grooming (-.699, p = .017). The females with more defensive marble burying grooming made fewer working memory errors. For male rats treated with CORT and E2, there were

significant positive correlations for RAWM late acquisition working memory errors with marble bury investigation (.546, $p = .043$) and RAWM late acquisition working memory errors with marble bury grooming (.540, $p = .046$). The males that made more working memory errors spent more time investigating marbles and more time grooming during defensive marble burying. There were significant negative correlations for RAWM late acquisition working memory errors with social novelty index (-.761, $p = .017$) and 2% acclimation SP index with baseline CORT (-.889, $p < .001$). The males with greater social novelty exploration made fewer working memory errors and the males with higher baseline CORT had a lower acclimation SP index.

Discussion

The goal of this study was to determine whether CORT and E2 would have interactive effects on depressive-like profiles in middle-aged, GDX female and male rats. Three domains of depressive-like profiles were used to measure cognition (spatial working memory with RAWM), depressive-like behavior (SP, social exploration) and anxiety profile (defensive marble bury, EPM). We found that E2 significantly improved spatial working memory at the highest memory load in females only, with CORT showing no effect in either sex. We also found a significant interaction between CORT and E2 in anxiety profile assessment for marble bury in females without an interaction in males. For anxiety profile (using EPM), spatial working memory and depressive-like behavior, no interactions between CORT and E2 were found for either sex when using

traditional group comparison analyses, but Spearman correlations detected a highly significant relationship in CORT treated females for spatial working memory and anxiety (RAWM and EPM/Marble Burying). For CORT creating a depressive-like profile, we surprisingly failed to find effects on spatial working memory, depressive-like behavior, and anxiety profile with EPM, although CORT elevated anxiety-like behaviors on marble bury as would be expected. Post-mortem serum measurements confirmed CORT treatment was effectively injected to indicate that a lack of effects in other assessments was unlikely attributed to administration failure. Unlike many of CORT actions, E2 affected all three depressive-like domains as measured by spatial working memory using RAWM, depressive-like behavior using SP (but not social exploration) and anxiety profile using marble bury and EPM. Moreover, E2 impacted female performance more often than observed in males and the directionality of effects sometimes contrasted with predictions. Overall, the detailed results provide evidence that E2 provided antidepressant effects in both female and male GDX, middle-aged rats, though the effects in females were more robust. Furthermore, the results highlight that CORT did not exacerbate depressive-like behaviors in GDX middle-aged rats, including cognitive deficits, although the Spearman correlations revealed relationships in females for spatial working memory and anxiety.

This experiment included the spatial working memory version of the RAWM because females often fail to show stress induced deficits on spatial reference memory. For instance females typically express similar or even improved spatial memory

following chronic stress compared to controls, a phenomenon that has been linked to hippocampal synaptic protection (Bowman et al., 2001; Huzian et al., 2021; McCormick et al., 2010; McLaughlin et al., 2005). Similarly, clinical studies highlight working memory deficits in middle-aged females and males with MDD (Altemus et al., 2014; Egeland et al., 2005; Nikolin et al., 2021). To determine proof of concept, it was demonstrated that CORT treatment disrupted working memory in middle-aged, OVX female rats (Chapter 4) and that middle-aged, OVX female rats benefitted from E2-actions on depressive-like pathology (Chapter 5). In the current study, the cognitive component of depressive-like profile was included to investigate E2 and CORT effects in females and males. The current results corroborated the beneficial actions of E2 on memory in middle aged females (Frick et al., 2018; Koebele, Nishimura, et al., 2020a; Taxier et al., 2020b). In addition, the present study provided novel data, exhibiting a failure of E2 to improve spatial working memory in males. While, the literature suggests that E2 enhances spatial memory in both GDX and gonadally-intact male rodents on tasks such as radial arm mazes or spatial object recognition (Frick et al., 2018; Jacome et al., 2016; V. Luine & Rodriguez, 1994; Vázquez-Pereyra et al., 1995), the current results indicated that E2 may not improve spatial working memory in middle-aged male rats. One difference is that the current study focused on spatial working memory, which tapped into overlapping, but different brain regions than these reports (Bimonte & Denenberg, 1999; Gaelle et al., 2019; Galloway et al., 2008; Wirt & Hyman, 2017). Furthermore, visuo-motor functions or motivational drive of middle-aged rodents can be

affected by age (Hamezah et al., 2017; Justice et al., 2014; Topic et al., 2005; Wilson et al., 2004), but the visible platform data demonstrated that the motor capabilities were similar across treatments and that the motivational components of water escape tasks were similar. Interestingly, in both females and males, E2 treatment reduced variability in errors, regardless of stress hormone treatment. The reduced variability suggests that though there may be variability in response to GDX, individuals show similar response to E2 treatment, an observation that warrants further investigation. Previous literature demonstrates that the low dose of E2 utilized in this study produces physiological E2 levels in OVX female rats. In GDX males however, low doses of E2 result in supraphysiological E2 levels and fail to impact testosterone levels (Barha & Galea, 2010; Gibbs, 2005). The failure of E2 treatment to improve spatial memory in male rats in this study suggests that high levels of E2 fail to improve male cognition. As such, present data provide strong indication that E2 provided beneficial actions on cognition in GDX middle-aged female, but not male rats.

In contrast with previous studies, current results demonstrate that stress and CORT may disrupt spatial working memory in tasks such as RAWM, CORT failed to disrupt spatial memory in this study. Studies highlight spatial memory deficits following CORT treatment in males (Bodnoff et al., 1995; Gaelle et al., 2019; McLay et al., 1998), and research also demonstrates memory deficits in CORT treated female rodents (Ngoupaye et al., 2018; Snihur et al., 2008). In the current study we found no effects of CORT on spatial working memory in either sex and few other significant CORT effects,

so we investigated relationships between behaviors within sex to determine or investigate how CORT and E2 influenced relationships between behavioral domains. In doing so, we found a significant positive relationship between spatial working memory errors and EPM anxiety in CORT treated females.

Correlational analysis was implemented to further elucidate the interconnections among the three domains of depressive profile (cognition, depressive-like behavior, and anxiety-like behavior) investigated in the present study. Some benefits to using single assessments compared to a battery of assessments include reproducibility, and avoiding test order effects (Blokland et al., 2012; Feyissa et al., 2017; Saré et al., 2021). However, the goal of the present study was to assess potential relationships amongst a variety of behaviors (Steimer, 2011; van Gaalen & Steckler, 2000). Spearman correlations were utilized as a sophisticated probe to gain further insight to possible relationships between depressive-behavior, anxiety profile and cognition. Our novel and exciting finding is that CORT-treated females (F-C-V and F-C-E) showed a significant positive relationship between spatial working memory and anxiety. Specifically, F-C-V rats that made more errors during RAWM early acquisition demonstrated higher EPM anxiety indexes and F-C-E rats that made more errors during the RAWM asymptotic phase demonstrated more immobility during defensive marble burying. Although the observed correlation does not denote causation, it does highlight a robust relationship between cognitive function and anxiety in response to CORT treatment and that some OVX middle-age female rats may exhibit a vulnerability. As CORT treated rats with more spatial memory errors tended to

show greater anxiety profiles, this suggests that specific rats may be more sensitive or predisposed to alterations in cognitive ability as their anxiety levels fluctuate. Understanding this correlation's implications for individual vulnerability is crucial in identifying potential targets in subsequent studies. In addition to the relationship between cognition and anxiety in CORT-treated females, F-C-V rats showed a significant negative correlation between social novelty preference and immobility during defensive marble burying meaning rats that had greater social novelty preference, exhibited less immobility during marble burying. This relationship was not significant for F-C-E rats highlighting a robust relationship between social novelty exploration and anxiety-like behavior in response to CORT treatment in female rats and that E2 treatment may disrupt this relationship. This work highlights a novel relationship between cognition and anxiety in CORT treated female rats and contributes to the knowledge that CORT and E2 have complex interactions on depressive-like behavioral outcomes.

We also analyzed correlations in male animals that were in CORT (or VEH) and E2 (or VEH) conditions. Particularly, E2-treated male rats (M-V-E and M-C-E) showed a significant negative relationship between baseline CORT and depressive-like profiles in terms of anhedonia. The relationship demonstrated that male rats treated with E2 with higher baseline CORT exhibited a lower SP index. This is expected as research has demonstrated that elevated CORT results in anhedonia and decreased SP in rodents (Dieterich et al., 2019; Ding et al., 2018; Kvarta et al., 2015). Additional relationships were revealed in CORT treated male rats (M-C-V and M-C-E). For instance, M-C-V rats

exhibited a significant positive correlation was between errors made during the RAWM late acquisition phase and 2% acclimation SP, demonstrating rats that made more errors showed greater SP during acclimation. This is unexpected as rats exhibiting anhedonia often exhibit cognitive deficits (Bechtholt-Gompf et al., 2010; Hamer et al., 2019) and M-C-V rats exhibited the opposite relationship. This correlation was insignificant in male rats that received E2 and may represent unique aspects in which males are affected by E2. Therefore, the current results demonstrate that CORT and E2 have differential effects in females and males and may alter the relationship between various behaviors in a complex manner, beyond the assumed directional effects.

In this study, depressive-like behavior was assessed using SP and social exploration as animal models of depression often display anhedonia and withdraw from investigating and spending time with conspecifics. For example, chronically stressed rodents exhibit decreased SP and show reduced social exploration compared to non-stressed controls (Du Preez et al., 2020; Fan et al., 2023; Gaspar et al., 2021; Hackenberg et al., 2021; M. Y. Liu et al., 2018; Willner et al., 1992). In the current study, CORT failed to reduce SP in either sex, which contrasts with previous research (Ding et al., 2018; Kvarta et al., 2015; Nickle et al., 2020). Moreover, E2 decreased SP, findings that were consistent with previous chapters and research demonstrating that E2 reduces food intake (Butera, 2010). Additionally, CORT and E2 failed to influence social exploration and social novelty exploration, adding to previous literature demonstrating social behavioral changes with aging and gonadal hormone manipulation (Boyer et al., 2019;

Vetter-O'Hagen & Spear, 2012). Moreover, the lack of social novelty exploration in this study adds to previous literature suggesting that recognition memory and novelty preference may shift with aging (Arias-Cavieres et al., 2017; Canatelli-Mallat et al., 2022; Koebele, Nishimura, et al., 2020a; Markham & Juraska, 2007; Vetter-O'Hagen & Spear, 2012). Interestingly, males and females exhibited similar social preference in this study, an unexpected finding as previous literature demonstrates that young adult male rats display more social novelty exploration compared to young adult females (Wu et al., 2022). Consequently, the current study demonstrates unique social behavior in GDX, middle-aged females and males.

This study investigated whether CORT treatment would elevate anxiety-like behavior and whether E2 would alleviate CORT effects using defensive marble burying and EPM assessments due to the high comorbidity with MDD and anxiety (Altemus et al., 2014; Anxiety and Depression Association of America, 2017). Additionally, animal models of depression often show increased anxiety like behavior during these tasks (Cryan & Sweeney, 2011; de Brouwer et al., 2019; Huynh et al., 2011; Nicolas et al., 2006; Rodgers & Dalvi, 1997; van Gaalen & Steckler, 2000). Notably, E2 treatment decreased male and female anxiety-like behavior in both assessments. Specifically, in both males and females, E2 increased the time spent actively investigating marbles and decreased the time spent immobile during defensive marble burying. E2 treated female rats also exhibited lower anxiety profiles compared to VEH rats during EPM testing as E2 treated females had a lower anxiety index, as well as more overall maze exploration

compared to females treated with the sex hormone vehicle. Conversely, in both males and females, CORT decreased the time spent actively investigating marbles and increased the time spent immobile during defensive marble buying. Nevertheless, CORT failed to affect time spent exploring and entries made during the EPM in either males or females. Accordingly, E2 and CORT provided contrasting effects on anxiety-like behavior in this study, with CORT increasing and E2 decreasing anxiety-like behavior in both females and males.

As CORT was confirmed to be elevated, the lack of traditional effects was unlikely due to failure of treatment manipulation. Evidence suggests that chronic CORT itself may be ineffective, however previous work with middle aged OVX females in Chapter 4 shows that females displayed working memory deficits following CORT. One difference is the route of administration of CORT treatment, as CORT was administered via drinking water in Chapter 4 and no rats received injections during treatment. In the current study, CORT was administered via injection and all rats received two injections daily as E2 treatment was also included. The method of CORT treatment is a critical difference as it is well documented in literature that injections induce a stress/CORT response and elevate depressive and anxiety-like behaviors (Deutsch-Feldman et al., 2015; Du Preez et al., 2020; Stuart & Robinson, 2015). Several studies investigating spatial working memory in aged and OVX females with the RAWM, implement a delay between trials 2 and 3 as an additional challenge (Bernaud et al., 2022; Braden et al., 2017; Hiroi et al., 2016; Koebele et al., 2019). Moreover, in Chapter 4, CORT effects

were not detectable until a delay was implemented during retention testing. Consequently, a lack of CORT to impair working memory on the WRAM may be a result of the task failing to be sufficiently challenging at a high working memory load.

In summary, E2 improved cognition in females only (not males), specifically during trials 3 and 4 when spatial working memory demand was high. E2 resulted in more robust effects in females than in males during EPM testing, although E2 reduced anxiety-like behavior in both sexes. However, E2 increased depressive-like behavior in both sexes during 1% SP testing. CORT on the other hand failed to impact cognition in both males and females during RAWM testing and CORT failed to impact depressive or anxiety-like behavior in both sexes during SP, Social Exploration and EPM testing. Nevertheless, CORT increased anxiety-like behavior in both sexes during marble Bury testing. Although E2 effects were less frequent in males, and CORT failed to show effects in many assessments, the present study identified robust relationships between behaviors that clearly differed by treatment. For instance, E2 treated male rats had significant correlations between working memory and anxiety-like behaviors in defensive marble bury and EPM testing, that were not reflected in ovarian hormone vehicle treated males. The present results introduce a novel method to assess relationships between depressive profile domains and are supported by differential effects of stress and sex hormones in males and females on an array of behavioral measures.

CHAPTER 7

GENERAL DISCUSSION

The overarching goal of the present studies was to investigate sex differences in chronic stress-induced depressive-like behavior, including cognitive outcomes, to gain a better understanding of female susceptibility to depressive-like outcomes. One conundrum when modeling depressive profiles in rodents, is that female rodents often fail to show chronic stress effects (Bowman et al., 2001; Bowman & Kelly, 2012; Hoffman et al., 2010; Huzian et al., 2021). As females are more likely to be diagnosed with depression in the clinic (P. R. Albert, 2015; University of Texas Health Science Center at Houston & Jalnapurkar, 2018), modeling depressive-like pathology in both male and female rodents provides great insight to vulnerabilities that may be unique to females.

Chapter 2 of this dissertation aimed to determine whether female rats display cognitive and anxiety-like effects following a novel robust stress paradigm. Chapter 2 demonstrated that chronic stress negatively impairs spatial memory in young adult male rats, while leaving spatial memory intact in young adult females (Peay et al., 2020). This supports the notion that young adult female rats exhibit resilience to chronic stress-induced spatial memory deficits (Beery & Kaufer, 2015; Bowman et al., 2001; Bowman & Kelly, 2012; Huzian et al., 2021). Chapter 2 also highlighted the importance of testing order when utilizing a behavioral battery and the transience of chronic stress effects, as male spatial memory deficits waned days after chronic stress ceased. Chapter 3 focused on the hippocampal and amygdalar neuronal morphology of the male and female rats

from Chapter 2, in order to investigate whether male and female rat brains displayed long term effects in these regions following chronic stress. Chapter 3 demonstrated that chronic stress results in enduring enhancement of basolateral amygdala (BLA) dendritic arborization in both male and female young adult rats. Moreover, results revealed sex differences in regional BLA hypertrophy, suggesting specific stress-activated pathology that may lead to susceptible phenotypes for cognitive and emotional dysfunction (Peay et al., 2023). Particularly, UIR stress led to BLA dendritic hypertrophy in both male and females, but with regional differences. BLA dendritic hypertrophy in stellate neurons occurred closest to the soma in males and distally from the soma in females. Stellate neurons within the BLA are primarily a local circuit of excitatory glutamatergic neurons (Beitchman et al., 2020; Hartmann et al., 2017). These glutamatergic BLA neurons can exhibit inhibitory control by targeting GABAergic interneurons, which in turn project to the central amygdala (CeA), a key output region (Pare & Duvarci, 2012; X. Zhang et al., 2018). The CeA targets brain circuits involved in physiological and behavioral responses to stress, including anxiety and fear expression (Duvarci & Pare, 2014; X. Zhang et al., 2018). Brain regions controlling the BLA include the dorsal hippocampus and medial prefrontal cortex, mPFC (Adamec et al., 2012; Maren & Fanselow, 1995). (Almada et al., 2009; Guthman et al., 2020; Lau et al., 2017; McGarry & Carter, 2016; Quirk et al., 2003). The mPFC and dorsal hippocampus can suppress BLA activity, while chronic stress can decrease the mPFC and dorsal hippocampus control of the BLA (Adamec et al., 2012; Colyn et al., 2019; Lau et al., 2017; Quirk et al., 2003). Consequently, changes

in dendritic architecture may reflect the specific sources of afferents and effects on synaptic integration within the BLA and the subsequent BLA output (Andreasen & Lambert, 1998; Blume et al., 2019; Larkum et al., 2004). Further research is needed to elucidate the specific topography of inhibitory and excitatory input to dendrites in the BLA as well as the impacts that they may have within the BLA circuits.

Together, Chapters 2 and 3 showed that chronic stress impairs spatial memory in male rats and fails to alter spatial memory in young adult female rats, although both rats show long term hypertrophy in the amygdala. Thus, young adult female rats are not entirely resistant to chronic stress effects and may be more susceptible to stress with age as suggested by literature (Gobinath et al., 2015; Hodes & Epperson, 2019; Lowy et al., 1995; Sapolsky et al., 1983; Xie et al., 2018). The results from chapters 2 and 3 gave credence the next series of studies in Chapters 4, 5, and 6, which sought to test the impacts of CORT in middle aged OVX females, as this is a model that may be more vulnerable to chronic stress and E2 may show beneficial effects.

Chapter 4 investigated whether CORT would increase depressive-like profile in OVX middle-aged female rats. CORT treatment intensified depressive profile in multiple domains. Specifically, CORT compromised spatial working memory during RAWM testing, increased anhedonia during SP testing, amplified behavioral despair in FST, and increased anxiety profile during marble bury and EPM testing. Overall, Chapter 4 revealed a model for depressive-like profile in female rats, across several domains including cognition, anhedonia, behavioral despair, and anxiety.

Chapter 5 investigated whether E2 offered benefits to depressive-like profile in OVX middle-aged female rats. This study investigated the beneficial properties of E2 on mood and anxiety aspects of depressive profile as the benefits of E2 on cognition in OVX middle-aged female rats are well-documented (Daniel et al., 2006; Kiss et al., 2012; Koebele, Nishimura, et al., 2020a; Koebele & Bimonte-Nelson, 2015; Taxier et al., 2020a). Outcomes from this study demonstrated that E2 significantly may improve depressive-like profile in OVX, middle-aged female rats, though the outcomes may be task-specific.

The previous series of studies necessitated a deeper exploration of the effects of E2 and CORT on depressive-like profile in gonadectomized (GDX) female and male rodents. Chapter 6 investigated possible interactions between CORT and E2 on several domains of depressive profile in middle age, GDX female and male rats. This investigation helped to assess possible beneficial effects of E2 in rats that were in a compromised state due to CORT treatment. In Chapter 6, E2 significantly improved spatial working memory during RAWM in females. E2 also decreased anxiety-like behavior in both males and females during marble bury and in females during EPM. Fundamental outcomes from Chapter 6 demonstrated that E2 improves spatial working memory and reduces anxiety-like behavior in GDX middle-aged female and male rats, with more robust effects in females. The complex and highly variable domains of depressive profile necessitate complementary analyses to gain further understanding of the interrelationships among behavioral measures (Acikgoz et al., 2022; Ho et al., 2002;

Hu et al., 2017; Steimer, 2011). Chapter 6 implemented Spearman correlations to provide unique insight into interrelated behavioral variables, and behaviors that showed different CORT and E2 effects in females and male. Notably, CORT-treated females showed a significant positive relationship between spatial working memory and anxiety, demonstrating that CORT-treated females with the highest anxiety profile made more working memory errors on the RAWM. Moreover, E2 treated males showed a significant negative relationship between baseline CORT and depressive-like profile (anhedonia). These relationships represent specific aspects which CORT and E2 affected female and male behavior respectively. Overall, Chapters 4, 5, and 6 demonstrated that middle aged OVX females are susceptible to CORT-induced cognitive, depressive, and anxiety like outcomes that these negative valence outcomes may be improved by E2.

This work contributed to the understanding of female resilience and susceptibility to chronic stress-induced depressive profile, including spatial working memory, depressive and anxiety-like behavior, as well as long-term amygdala neuron morphological changes. These results indicate that although young adult females exhibit resilience to stress induced spatial memory deficits, ovariectomized middle-aged females exhibit spatial memory deficits following corticosterone treatment, highlighting the importance of age and ovarian hormones. Accordingly, this research demonstrates that female rats respond to stress in distinct ways compared to their male counterparts, indicating the importance of considering sex as a significant variable in stress-related studies. The research demonstrated that female rats exhibit unique benefits from ovarian

hormones such as estradiol that may protect them from some stress provoked impairments. This sex-specific vulnerability to aging underscores the necessity of considering gender-based differences when investigating stress-related depression models. Moreover, age and ovariectomy were identified as crucial factors influencing female rats' susceptibility to stress-induced depressive-like behavior. Chapter 4 demonstrated that the combination of ovariectomy and corticosterone treatment in middle-aged female rats leads to many depressive-like outcomes including impaired spatial memory. The age-related changes and the removal of ovaries may magnify the consequences of chronic stress, highlighting the importance of accounting for age and sex hormones in future studies on depression and stress-related disorders in female subjects. Notably, the research highlights the therapeutic potential of E2 in mitigating depressive-like behaviors in female rats. This suggests that hormonal interventions could offer promising avenues for future treatments aimed at alleviating depression and related mood disorders in women.

The implications of this work extend beyond the realm of animal models, as understanding the nuances of female stress responses may have significant implications for human mental health research. Recognizing the importance of sex-specific factors in stress-related conditions could enhance the development of more personalized and effective interventions for depression in women. Nevertheless, while this dissertation makes notable contributions to the field, there remain areas that warrant further exploration. Future studies should delve deeper into the molecular mechanisms

underlying the interplay between sex hormones and stress pathways, in order to refine our understanding of the complex neurobiological basis of depression in females.

REFERENCES

Acikgoz, B., Dalkiran, B., & Dayi, A. (2022). An overview of the currency and usefulness of behavioral tests used from past to present to assess anxiety, social behavior and depression in rats and mice. *Behavioural Processes, 200,* 104670. https://doi.org/10.1016/j.beproc.2022.104670

Adamec, R., Hebert, M., Blundell, J., & Mervis, R. F. (2012). Dendritic morphology of amygdala and hippocampal neurons in more and less predator stress responsive rats and more and less spontaneously anxious handled controls. *Behavioural Brain Research*, *226*(1), 133–146. https://doi.org/10.1016/j.bbr.2011.09.009

Agrawal, A., Jaggi, A. S., & Singh, N. (2011). Pharmacological investigations on adaptation in rats subjected to cold water immersion stress. *Physiology & Behavior*, *103*(3), 321–329. https://doi.org/10.1016/j.physbeh.2011.02.014

Aikey, J. L., Nyby, J. G., Anmuth, D. M., & James, P. J. (2002). Testosterone rapidly reduces anxiety in male house mice (Mus musculus). *Hormones and Behavior*, *42*(4), 448–460. https://doi.org/10.1006/hbeh.2002.1838

Akama, K. T., & McEwen, B. S. (2003). Estrogen stimulates postsynaptic density-95 rapid protein synthesis via the Akt/protein kinase B pathway. *Journal of Neuroscience, 23*(6). https://doi.org/10.1523/jneurosci.23-06-02333.2003

Albert, K., Ledet, T., Taylor, W., & Newhouse, P. (2020). Estradiol administration differentially affects the response to experimental psychosocial stress in post-menopausal women with or without a history of major depression. *Journal of Affective Disorders*, *261*. https://doi.org/10.1016/j.jad.2019.09.074

Albert, K. M., & Newhouse, P. A. (2019). Estrogen, Stress, and Depression: Cognitive and Biological Interactions. *Annual Review of Clinical Psychology*, *15*. https://doi.org/10.1146/annurev-clinpsy-050718-095557

Albert, P. R. (2015). Why is depression more prevalent in women? *Journal of Psychiatry and Neuroscience*, *40*(4), 219–221. https://doi.org/10.1503/jpn.150205

Alejandre-Gomez, M., Garcia-Segura, L. M., & Gonzalez-Burgos, I. (2007). Administration of an inhibitor of estrogen biosynthesis facilitates working memory acquisition in male rats. *Neuroscience Research*, *58*(3), 272–277. https://doi.org/10.1016/j.neures.2007.03.011

Almada, R. C., Borelli, K. G., Albrechet-Souza, L., & Brandão, M. L. (2009). Serotonergic mechanisms of the median raphe nucleus-dorsal hippocampus in conditioned fear: Output circuit involves the prefrontal cortex and amygdala. *Behavioural Brain Research*, *203*(2), 279–287. https://doi.org/10.1016/j.bbr.2009.05.017

Altemus, M., Sarvaiya, N., & Neill Epperson, C. (2014). Sex differences in anxiety and depression clinical perspectives. *Frontiers in Neuroendocrinology*, *35*(3), 320–330. https://doi.org/10.1016/j.yfrne.2014.05.004

Altshuler, L. L., Cohen, L. S., Moline, M. L., Kahn, D. A., Carpenter, D., Docherty, J. P., & W., R. R. (2001). Treatment of Depression in Women: A Summary of the Expert Consensus Guidelines. *Journal of Psychiatric Practice*, *7*(3). https://doi.org/10.1097/00131746-200105000-00006

Alyea, R. A., & Watson, C. S. (2009). Nongenomic mechanisms of physiological estrogen-mediated dopamine efflux. *BMC Neuroscience*, *10*. https://doi.org/10.1186/1471-2202-10-59

American Psychiatric Association. (2013). DSM-V. In *American Journal of Psychiatry* (Issue 1). https://doi.org/10.1176/appi.books.9780890425596.744053

Anderson, R. M., Johnson, S. B., Lingg, R. T., Hinz, D. C., Romig-Martin, S. A., & Radley, J. J. (2020). Evidence for Similar Prefrontal Structural and Functional Alterations in Male and Female Rats following Chronic Stress or Glucocorticoid Exposure. *Cerebral Cortex*, *30*(1). https://doi.org/10.1093/cercor/bhz092

Andreasen, M., & Lambert, J. D. (1998). Factors determining the efficacy of distal excitatory synapses in rat hippocampal CA1 pyramidal neurones. *The Journal of Physiology*, *507 (Pt 2)*(Pt 2), 441–462. https://doi.org/10.1111/j.1469-7793.1998.441bt.x

Andrewes, D. G., & Jenkins, L. M. (2019). The Role of the Amygdala and the Ventromedial Prefrontal Cortex in Emotional Regulation: Implications for Post-traumatic Stress Disorder. *Neuropsychology Review*, *29*(2), 220–243. https://doi.org/10.1007/s11065-019-09398-4

Andriole, G., Bruchovsky, N., Chung, L. W. K., Matsumoto, A. M., Rittmaster, R., Roehrborn, C., Russell, D., & Tindall, D. (2004). Dihydrotestosterone and the

prostate: The scientific rationale for 5alpha-reductase inhibitors in the treatment of benign prostatic hyperplasia. *The Journal of Urology*, *172*(4 Pt 1), 1399–1403. https://doi.org/10.1097/01.ju.0000139539.94828.29

Anxiety and Depression Association of America. (2017). *Facts & Statistics | Anxiety and Depression Association of America, ADAA*. Anxiety and Depression Association of America.

Ardayfio, P., & Kim, K. S. (2006). Anxiogenic-like effect of chronic corticosterone in the light-dark emergence task in mice. *Behavioral Neuroscience*, *120*(2). https://doi.org/10.1037/0735-7044.120.2.249

Arias-Cavieres, A., Adasme, T., Sánchez, G., Muñoz, P., & Hidalgo, C. (2017). Aging Impairs Hippocampal- Dependent Recognition Memory and LTP and Prevents the Associated RyR Up-regulation. *Frontiers in Aging Neuroscience*, *9*. https://www.frontiersin.org/articles/10.3389/fnagi.2017.00111

Armstrong, R. A. (2014). When to use the Bonferroni correction. *Ophthalmic and Physiological Optics*, *34*(5), 502–508. https://doi.org/10.1111/opo.12131

Arnsten, A. F. T. (2009). Stress signalling pathways that impair prefrontal cortex structure and function. *Nature Reviews Neuroscience*, *10*(6), 410–422. https://doi.org/10.1038/nrn2648

Arnsten, A. F. T. (2015). Stress weakens prefrontal networks: Molecular insults to higher cognition. *Nature Neuroscience*, *18*(10), Article 10. https://doi.org/10.1038/nn.4087

Aschenbrenner, A. J., Gordon, B. A., Benzinger, T. L. S., Morris, J. C., & Hassenstab, J. J. (2018). Influence of tau PET, amyloid PET, and hippocampal volume on cognition in Alzheimer disease. *Neurology*, *91*(9), e859–e866. https://doi.org/10.1212/WNL.0000000000006075

Aubele, T., Kaufman, R., Montalment, F., & Kritzer, M. F. (2008). Effects of gonadectomy and hormone replacement on a spontaneous novel object recognition task in adult male rats. *Hormones and Behavior*, *54*(2), 244–252. https://doi.org/10.1016/j.yhbeh.2008.04.001

Austin, M. P., Mitchell, P., & Goodwin, G. M. (2001). Cognitive deficits in depression: Possible implications for functional neuropathology. *British Journal of Psychiatry*, *178*(MARCH.). https://doi.org/10.1192/bjp.178.3.200

Babb, J. A., Masini, C. V., Day, H. E. W., & Campeau, S. (2014). Habituation of hypothalamic-pituitary-adrenocortical axis hormones to repeated homotypic stress and subsequent heterotypic stressor exposure in male and female rats. *Stress*. https://doi.org/10.3109/10253890.2014.905534

Baka, J., Csakvari, E., Huzian, O., Dobos, N., Siklos, L., Leranth, C., MacLusky, N. J., Duman, R. S., & Hajszan, T. (2017). Stress induces equivalent remodeling of hippocampal spine synapses in a simulated postpartum environment and in a female rat model of major depression. *Neuroscience*, *343*, 384–397. https://doi.org/10.1016/j.neuroscience.2016.12.021

Balderas, I., Rodriguez-Ortiz, C. J., Salgado-Tonda, P., Chavez-Hurtado, J., McGaugh, J. L., & Bermudez-Rattoni, F. (2008). The consolidation of object and context recognition memory involve different regions of the temporal lobe. *Learning & Memory*, *15*(9), 618–624. https://doi.org/10.1101/lm.1028008

Bale, T. L., & Epperson, C. N. (2015). Sex differences and stress across the lifespan. *Nature Neuroscience*, *18*(10), Article 10. https://doi.org/10.1038/nn.4112

Bali, A., & Jaggi, A. S. (2015). Preclinical experimental stress studies: Protocols, assessment and comparison. *European Journal of Pharmacology*, *746*, 282–292. https://doi.org/10.1016/j.ejphar.2014.10.017

Bangasser, D. A., & Valentino, R. J. (2014). Sex differences in stress-related psychiatric disorders: Neurobiological perspectives. *Frontiers in Neuroendocrinology*. https://doi.org/10.1016/j.yfrne.2014.03.008

Bangasser, D. A., Wiersielis, K. R., & Khantsis, S. (2016). Sex differences in the locus coeruleus-norepinephrine system and its regulation by stress. *Brain Research*. https://doi.org/10.1016/j.brainres.2015.11.021

Barfield, E., Moser, V., Hand, A., & Grisel, J. (2013). β-endorphin modulates the effect of stress on novelty-suppressed feeding. *Frontiers in Behavioral Neuroscience*, *7*. https://www.frontiersin.org/articles/10.3389/fnbeh.2013.00019

Barha, C. K., & Galea, L. A. M. (2010). Influence of different estrogens on neuroplasticity and cognition in the hippocampus. *Biochimica Et Biophysica Acta*, *1800*(10), 1056–1067. https://doi.org/10.1016/j.bbagen.2010.01.006

Barker, G. R. I., & Warburton, E. C. (2011). When Is the Hippocampus Involved in Recognition Memory? *Journal of Neuroscience*, *31*(29), 10721–10731. https://doi.org/10.1523/JNEUROSCI.6413-10.2011

Bartels, T., Berk, J., Cramer, K., Kanitz, E., & Otten, W. (2021). Research Note: A sip of stress. Effects of corticosterone supplementation in drinking water on feather corticosterone concentrations in layer pullets. *Poultry Science*, *100*(9), 101361. https://doi.org/10.1016/j.psj.2021.101361

Bechtholt-Gompf, A. J., Walther, H. V., Adams, M. A., Carlezon, W. A., Öngür, D., & Cohen, B. M. (2010). Blockade of Astrocytic Glutamate Uptake in Rats Induces Signs of Anhedonia and Impaired Spatial Memory. *Neuropsychopharmacology*, *35*(10), Article 10. https://doi.org/10.1038/npp.2010.74

Beck, K. D., & Luine, V. N. (2002). Sex differences in behavioral and neurochemical profiles after chronic stress: Role of housing conditions. *Physiology and Behavior*. https://doi.org/10.1016/S0031-9384(02)00670-4

Beery, A. K., & Kaufer, D. (2015). Stress, social behavior, and resilience: Insights from rodents. *Neurobiology of Stress*, *1*(1). https://doi.org/10.1016/j.ynstr.2014.10.004

Beery, A. K., & Shambaugh, K. L. (2021). Comparative Assessment of Familiarity/Novelty Preferences in Rodents. *Frontiers in Behavioral Neuroscience*, *15*. https://www.frontiersin.org/articles/10.3389/fnbeh.2021.648830

Beitchman, J. A., Griffiths, D. R., Hur, Y., Ogle, S. B., Bromberg, C. E., Morrison, H. W., Lifshitz, J., Adelson, P. D., & Thomas, T. C. (2020). Experimental Traumatic Brain Injury Induces Chronic Glutamatergic Dysfunction in Amygdala Circuitry Known to Regulate Anxiety-Like Behavior. *Frontiers in Neuroscience*, *13*. https://www.frontiersin.org/articles/10.3389/fnins.2019.01434

Bekhbat, M., Glasper, E. R., Rowson, S. A., Kelly, S. D., & Neigh, G. N. (2018). Measuring corticosterone concentrations over a physiological dynamic range in female rats. *Physiology & Behavior*, *194*, 73–76. https://doi.org/10.1016/j.physbeh.2018.04.033

Bellani, R., Luecken, L. J., & Conrad, C. D. (2006). Peripubertal anxiety profile can predict predisposition to spatial memory impairments following chronic stress. *Behavioural Brain Research*, *166*(2), 263–270. https://doi.org/10.1016/j.bbr.2005.08.006

Belleau, E. L., Treadway, M. T., & Pizzagalli, D. A. (2019). The Impact of Stress and Major Depressive Disorder on Hippocampal and Medial Prefrontal Cortex Morphology. *Biological Psychiatry*, *85*(6), 443–453. https://doi.org/10.1016/j.biopsych.2018.09.031

Benjamini, Y., Drai, D., Elmer, G., Kafkafi, N., & Golani, I. (2001). Controlling the false discovery rate in behavior genetics research. *Behavioural Brain Research*, *125*(1), 279–284. https://doi.org/10.1016/S0166-4328(01)00297-2

Benmansour, S., Arroyo, L. D., & Frazer, A. (2016). Comparison of the Antidepressant-Like Effects of Estradiol and That of Selective Serotonin Reuptake Inhibitors in Middle-Aged Ovariectomized Rats. *Frontiers in Aging Neuroscience*, *8*. https://www.frontiersin.org/articles/10.3389/fnagi.2016.00311

Berger, T., Lee, H., Young, A. H., Aarsland, D., & Thuret, S. (2020). Adult Hippocampal Neurogenesis in Major Depressive Disorder and Alzheimer's Disease. *Trends in Molecular Medicine*, *26*(9), 803–818. https://doi.org/10.1016/j.molmed.2020.03.010

Bernaud, V. E., Bulen, H. L., Peña, V. L., Koebele, S. V., Northup-Smith, S. N., Manzo, A. A., Valenzuela Sanchez, M., Opachich, Z., Ruhland, A. M., & Bimonte-Nelson, H. A. (2022). Task-dependent learning and memory deficits in the TgF344-AD rat model of Alzheimer's disease: Three key timepoints through middle-age in females. *Scientific Reports*, *12*(1), Article 1. https://doi.org/10.1038/s41598-022-18415-1

Bérubé, G., Rabouin, D., Perron, V., N'Zemba, B., Gaudreault, R. C., Parent, S., & Asselin, É. (2006). Synthesis of unique 17β-estradiol homo-dimers, estrogen receptors binding affinity evaluation and cytocidal activity on breast, intestinal and skin cancer cell lines. *Steroids*, *71*(10). https://doi.org/10.1016/j.steroids.2006.06.007

Beshai, S., Dobson, K. S., Bockting, C. L. H., & Quigley, L. (2011). Relapse and recurrence prevention in depression: Current research and future prospects. *Clinical Psychology Review*, *31*(8). https://doi.org/10.1016/j.cpr.2011.09.003

Bhatnagar, S., & Dallman, M. (1998). Neuroanatomical basis for facilitation of hypothalamic-pituitary- adrenal responses to a novel stressor after chronic stress. *Neuroscience*, *84*(4), 1025–1039. https://doi.org/10.1016/S0306-4522(97)00577-0

Biesheuvel-Leliefeld, K. E. M., Kok, G. D., Bockting, C. L. H., Cuijpers, P., Hollon, S. D., Van Marwijk, H. W. J., & Smit, F. (2015). Effectiveness of psychological interventions in preventing recurrence of depressive disorder: Meta-analysis and meta-regression. *Journal of Affective Disorders*, *174*. https://doi.org/10.1016/j.jad.2014.12.016

Bimonte, H. A., & Denenberg, V. H. (1999). Estradiol facilitates performance as working memory load increases. *Psychoneuroendocrinology*, *24*(2). https://doi.org/10.1016/S0306-4530(98)00068-7

Birrell, J. M., & Brown, V. J. (2000). Medial frontal cortex mediates perceptual attentional set shifting in the rat. *The Journal of Neuroscience : The Official Journal of the Society for Neuroscience*.

Bisagno, V., Ferguson, D., & Luine, V. N. (2003). Chronic D-amphetamine induces sexually dimorphic effects on locomotion, recognition memory, and brain monoamines. *Pharmacology Biochemistry and Behavior*. https://doi.org/10.1016/S0091-3057(03)00017-0

Bisagno, V., Grillo, C. A., Piroli, G. G., Giraldo, P., McEwen, B., & Luine, V. N. (2004). Chronic stress alters amphetamine effects on behavior and synaptophysin levels in female rats. *Pharmacology Biochemistry and Behavior*. https://doi.org/10.1016/j.pbb.2004.04.023

Blackburn, T. P. (2019). Depressive disorders: Treatment failures and poor prognosis over the last 50 years. *Pharmacology Research and Perspectives*, *7*(3). https://doi.org/10.1002/prp2.472

Blasco-Serra, A., González-Soler, E. M., Cervera-Ferri, A., Teruel-Martí, V., & Valverde-Navarro, A. A. (2017). A standardization of the Novelty-Suppressed Feeding Test protocol in rats. *Neuroscience Letters*, *658*. https://doi.org/10.1016/j.neulet.2017.08.019

Blokland, A., ten Oever, S., van Gorp, D., van Draanen, M., Schmidt, T., Nguyen, E., Krugliak, A., Napoletano, A., Keuter, S., & Klinkenberg, I. (2012). The use of a test battery assessing affective behavior in rats: Order effects. *Behavioural Brain Research*, *228*(1), 16–21. https://doi.org/10.1016/j.bbr.2011.11.042

Bloss, E. B., Janssen, W. G., McEwen, B. S., & Morrison, J. H. (2010). Interactive effects of stress and aging on structural plasticity in the prefrontal cortex. *Journal of Neuroscience*, *30*(19). https://doi.org/10.1523/JNEUROSCI.0759-10.2010

Blume, S. R., Padival, M., Urban, J. H., & Rosenkranz, J. A. (2019). Disruptive effects of repeated stress on basolateral amygdala neurons and fear behavior across the estrous cycle in rats. *Scientific Reports*, *9*(1), Article 1. https://doi.org/10.1038/s41598-019-48683-3

Bodnoff, S. R., Humphreys, A. G., Lehman, J. C., Diamond, D. M., Rose, G. M., & Meaney, M. J. (1995). Enduring effects of chronic corticosterone treatment on spatial learning, synaptic plasticity, and hippocampal neuropathology in young and mid-aged rats. *The Journal of Neuroscience : The Official Journal of the Society for Neuroscience*.

Bodnoff, S. R., Suranyi-Cadotte, B., Aitken, D. H., Quirion, R., & Meaney, M. J. (1988). The effects of chronic antidepressant treatment in an animal model of anxiety. *Psychopharmacology*, *95*(3). https://doi.org/10.1007/BF00181937

Bolles, R. C. (1970). Species-specific defense reactions and avoidance learning. *Psychological Review*, *77*(1), 32–48. https://doi.org/10.1037/h0028589

Bollinger, J. L., Bergeon Burns, C. M., & Wellman, C. L. (2016). Differential effects of stress on microglial cell activation in male and female medial prefrontal cortex. *Brain, Behavior, and Immunity*. https://doi.org/10.1016/j.bbi.2015.10.003

Bondi, C. O., Rodriguez, G., Gould, G. G., Frazer, A., & Morilak, D. A. (2008). Chronic unpredictable stress induces a cognitive deficit and anxiety-like behavior in rats that is prevented by chronic antidepressant drug treatment. *Neuropsychopharmacology*. https://doi.org/10.1038/sj.npp.1301410

Bonett, D. G., & Wright, T. A. (2000). Sample size requirements for estimating pearson, kendall and spearman correlations. *Psychometrika*, *65*(1), 23–28. https://doi.org/10.1007/BF02294183

Bouguiyoud, N., Roullet, F., Bronchti, G., Frasnelli, J., & Al Aïn, S. (2022). Anxiety and Depression Assessments in a Mouse Model of Congenital Blindness. *Frontiers in Neuroscience, 15*. https://www.frontiersin.org/articles/10.3389/fnins.2021.807434

Bowman, R. E., Beck, K. D., & Luine, V. N. (2003). Chronic stress effects on memory: Sex differences in performance and monoaminergic activity. *Hormones and Behavior*. https://doi.org/10.1016/S0018-506X(02)00022-3

Bowman, R. E., Ferguson, D., & Luine, V. N. (2002). Effects of chronic restraint stress and estradiol on open field activity, spatial memory, and monoaminergic neurotransmitters in ovariectomized rats. *Neuroscience*. https://doi.org/10.1016/S0306-4522(02)00156-2

Bowman, R. E., & Kelly, R. (2012). Chronically stressed female rats show increased anxiety but no behavioral alterations in object recognition or placement memory: A preliminary examination. *Stress*. https://doi.org/10.3109/10253890.2011.645926

Bowman, R. E., Maclusky, N. J., Diaz, S. E., Zrull, M. C., & Luine, V. N. (2006). Aged rats: Sex differences and responses to chronic stress. *Brain Research*, *1126*(1), 156–166. https://doi.org/10.1016/j.brainres.2006.07.047

Bowman, R. E., Zrull, M. C., & Luine, V. N. (2001). Chronic restraint stress enhances radial arm maze performance in female rats. *Brain Research*, *904*(2), 279–289. https://doi.org/10.1016/S0006-8993(01)02474-X

Boyer, F., Jaouen, F., Ibrahim, E. C., & Gascon, E. (2019). Deficits in Social Behavior Precede Cognitive Decline in Middle-Aged Mice. *Frontiers in Behavioral Neuroscience*, *13*. https://www.frontiersin.org/articles/10.3389/fnbeh.2019.00055

Braden, B. B., Andrews, M. G., Acosta, J. I., Mennenga, S. E., Lavery, C., & Bimonte-Nelson, H. A. (2017). A comparison of progestins within three classes: Differential effects on learning and memory in the aging surgically menopausal rat. *Behavioural Brain Research*, *322*(Pt B), 258–268. https://doi.org/10.1016/j.bbr.2016.06.053

Bremner, J. D., Elzinga, B., Schmahl, C., & Vermetten, E. (2007). Structural and functional plasticity of the human brain in posttraumatic stress disorder. *Progress in Brain Research, 167*. https://doi.org/10.1016/S0079-6123(07)67012-5

Bremner, J. D., Narayan, M., Anderson, E. R., Staib, L. H., Miller, H. L., & Charney, D. S. (2000a). Hippocampal volume reduction in major depression. *American Journal of Psychiatry*, *157*(1). https://doi.org/10.1176/ajp.157.1.115

Bremner, J. D., Narayan, M., Anderson, E. R., Staib, L. H., Miller, H. L., & Charney, D. S. (2000b). Hippocampal volume reduction in major depression. *The American Journal of Psychiatry*, *157*(1), 115–118. https://doi.org/10.1176/ajp.157.1.115

Brenes, J. C., Padilla, M., & Fornaguera, J. (2009). A detailed analysis of open-field habituation and behavioral and neurochemical antidepressant-like effects in postweaning enriched rats. *Behavioural Brain Research*, *197*(1), 125–137. https://doi.org/10.1016/j.bbr.2008.08.014

Brown, J., Cohen, P., Johnson, J. G., & Smailes, E. M. (1999). Childhood abuse and neglect: Specificity of effects on adolescent and young adult depression and suicidality. *Journal of the American Academy of Child and Adolescent Psychiatry*, *38*(12). https://doi.org/10.1097/00004583-199912000-00009

Brummelte, S., & Galea, L. A. M. (2010). Chronic high corticosterone reduces neurogenesis in the dentate gyrus of adult male and female rats. *Neuroscience*, *168*(3), 680–690. https://doi.org/10.1016/j.neuroscience.2010.04.023

Brymer, K. J., Johnston, J., Botterill, J. J., Romay-Tallon, R., Mitchell, M. A., Allen, J., Pinna, G., Caruncho, H. J., & Kalynchuk, L. E. (2020). Fast-acting antidepressant-like effects of Reelin evaluated in the repeated-corticosterone chronic stress paradigm. *Neuropsychopharmacology*, *45*(10), Article 10. https://doi.org/10.1038/s41386-020-0609-z

Burger, H. G., Dudley, E. C., Hopper, J. L., Shelley, J. M., Green, A., Smith, A., Dennerstein, L., & Morse, C. (1995). The endocrinology of the menopausal transition: A cross-sectional study of a population-based sample. *Journal of Clinical Endocrinology and Metabolism*, *80*(12). https://doi.org/10.1210/jcem.80.12.8530596

Burger, H., Woods, N. F., Dennerstein, L., Alexander, J. L., Kotz, K., & Richardson, G. (2007). Nomenclature and endocrinology of menopause and perimenopause. *Expert Review of Neurotherapeutics*, *7*(sup1), S35–S43. https://doi.org/10.1586/14737175.7.11s.S35

Burn, C. C. (2008). What is it like to be a rat? Rat sensory perception and its implications for experimental design and rat welfare. *Applied Animal Behaviour Science*, *112*(1), 1–32. https://doi.org/10.1016/j.applanim.2008.02.007

Butera, P. C. (2010). ESTRADIOL AND THE CONTROL OF FOOD INTAKE. *Physiology & Behavior*, *99*(2), 175. https://doi.org/10.1016/j.physbeh.2009.06.010

Cameron, H. A., & Schoenfeld, T. J. (2018). Behavioral and structural adaptations to stress. *Frontiers in Neuroendocrinology*. https://doi.org/10.1016/j.yfrne.2018.02.002

Canatelli-Mallat, M., Chiavellini, P., Lehmann, M., Goya, R. G., & Morel, G. R. (2022). Age-related loss of recognition memory and its correlation with hippocampal and perirhinal cortex changes in female Sprague Dawley rats. *Behavioural Brain Research*, *435*, 114026. https://doi.org/10.1016/j.bbr.2022.114026

Caspi, A., Sugden, K., Moffitt, T. E., Taylor, A., Craig, I. W., Harrington, H. L., McClay, J., Mill, J., Martin, J., Braithwaite, A., & Poulton, R. (2003). Influence of life stress on depression: Moderation by a polymorphism in the 5-HTT gene. *Science*, *301*(5631). https://doi.org/10.1126/science.1083968

Cavus, I., & Duman, R. S. (2003). Influence of estradiol, stress, and 5-HT2A agonist treatment on brain-derived neurotrophic factor expression in female rats. *Biological Psychiatry*, *54*(1). https://doi.org/10.1016/S0006-3223(03)00236-1

Celec, P., Ostatníková, D., & Hodosy, J. (2015). On the effects of testosterone on brain behavioral functions. *Frontiers in Neuroscience*, *9*. https://www.frontiersin.org/articles/10.3389/fnins.2015.00012

Chiba, S., Numakawa, T., Ninomiya, M., Richards, M. C., Wakabayashi, C., & Kunugi, H. (2012). Chronic restraint stress causes anxiety- and depression-like behaviors, downregulates glucocorticoid receptor expression, and attenuates glutamate release induced by brain-derived neurotrophic factor in the prefrontal cortex. *Progress in Neuro-Psychopharmacology and Biological Psychiatry*, *39*(1). https://doi.org/10.1016/j.pnpbp.2012.05.018

Cohen, J. (2013). *Statistical Power Analysis for the Behavioral Sciences*. Academic Press.

Cohen, S., Janicki-Deverts, D., & Miller, G. E. (2007). Psychological stress and disease. *Journal of the American Medical Association*, *298*(14). https://doi.org/10.1001/jama.298.14.1685

Colla, M., Kronenberg, G., Deuschle, M., Meichel, K., Hagen, T., Bohrer, M., & Heuser, I. (2007a). Hippocampal volume reduction and HPA-system activity in major depression. *Journal of Psychiatric Research*, *41*(7). https://doi.org/10.1016/j.jpsychires.2006.06.011

Colla, M., Kronenberg, G., Deuschle, M., Meichel, K., Hagen, T., Bohrer, M., & Heuser, I. (2007b). Hippocampal volume reduction and HPA-system activity in major depression. *Journal of Psychiatric Research*, *41*(7), 553–560. https://doi.org/10.1016/j.jpsychires.2006.06.011

Colquhoun, D. (2014). An investigation of the false discovery rate and the misinterpretation of p-values. *Royal Society Open Science*, *1*(3), 140216. https://doi.org/10.1098/rsos.140216

Colyn, L., Venzala, E., Marco, S., Perez-Otaño, I., & Tordera, R. M. (2019). Chronic social defeat stress induces sustained synaptic structural changes in the prefrontal cortex and amygdala. *Behavioural Brain Research*, *373*, 112079. https://doi.org/10.1016/j.bbr.2019.112079

Conrad, C. D. (2006). What is the functional significance of chronic stress-induced CA3 dendritic retraction within the hippocampus? *Behavioral and Cognitive Neuroscience Reviews*. https://doi.org/10.1177/1534582306289043

Conrad, C. D. (2010). A critical review of chronic stress effects on spatial learning and memory. *Progress in Neuro-Psychopharmacology and Biological Psychiatry*, *34*(5), 742–755. https://doi.org/10.1016/j.pnpbp.2009.11.003

Conrad, C. D., Galea, L. A., Kuroda, Y., & McEwen, B. S. (1996a). Chronic stress impairs rat spatial memory on the Y maze, and this effect is blocked by tianeptine pretreatment. *Behavioral Neuroscience*, *110*(6), 1321–1334.

Conrad, C. D., Galea, L. A. M., Kuroda, Y., & McEwen, B. S. (1996b). Chronic stress impairs rat spatial memory on the Y maze, and this effect is blocked by tianeptine pretreatment. *Behavioral Neuroscience*, *110*(6), 1321–1334. https://doi.org/10.1037/0735-7044.110.6.1321

Conrad, C. D., Grote, K. A., Hobbs, R. J., & Ferayorni, A. (2003). Sex differences in spatial and non-spatial Y-maze performance after chronic stress. *Neurobiology of Learning and Memory*, *79*(1), 32–40. https://doi.org/10.1016/S1074-7427(02)00018-7

Conrad, C. D., LeDoux, J. E., Magariños, A. M., & McEwen, B. S. (1999). Repeated restraint stress facilitates fear conditioning independently of causing hippocampal CA3 dendritic atrophy. *Behavioral Neuroscience*, *113*(5), 902–913. https://doi.org/10.1037//0735-7044.113.5.902

Conrad, C. D., Mauldin-Jourdain, M. L., & Hobbs, R. J. (2001). Metyrapone reveals that previous chronic stress differentially impairs hippocampal-dependent memory. *Stress*. https://doi.org/10.3109/10253890109014754

Conrad, C. D., McLaughlin, K. J., Huynh, T. N., El-Ashmawy, M., & Sparks, M. (2012). Chronic stress and a cyclic regimen of estradiol administration separately facilitate spatial memory: Relationship with hippocampal CA1 spine density and dendritic complexity. *Behavioral Neuroscience*. https://doi.org/10.1037/a0025770

Conrad, C. D., Ortiz, J. B., & Judd, J. M. (2017). Chronic stress and hippocampal dendritic complexity: Methodological and functional considerations. *Physiology and Behavior*, *178*. https://doi.org/10.1016/j.physbeh.2016.11.017

Coryell, W., & Young, E. A. (2005). Clinical predictors of suicide in primary major depressive disorder. *Journal of Clinical Psychiatry*, *66*(4). https://doi.org/10.4088/JCP.v66n0401

Cryan, J. F., Markou, A., & Lucki, I. (2002). Assessing antidepressant activity in rodents: Recent developments and future needs. *Trends in Pharmacological Sciences*, *23*(5). https://doi.org/10.1016/S0165-6147(02)02017-5

Cryan, J. F., & Sweeney, F. F. (2011). The age of anxiety: Role of animal models of anxiolytic action in drug discovery. *British Journal of Pharmacology*, *164*(4), 1129–1161. https://doi.org/10.1111/j.1476-5381.2011.01362.x

Cui, M., Yang, Y., Yang, J., Zhang, J., Han, H., Ma, W., Li, H., Mao, R., Xu, L., Hao, W., & Cao, J. (2006). Enriched environment experience overcomes the memory deficits and depressive-like behavior induced by early life stress. *Neuroscience Letters*. https://doi.org/10.1016/j.neulet.2006.05.048

Daniel, J. M., Fader, A. J., Spencer, A. L., & Dohanich, G. P. (1997). Estrogen enhances performance of female rats during acquisition of a radial arm maze. *Hormones and Behavior*, *32*(3). https://doi.org/10.1006/hbeh.1997.1433

Daniel, J. M., Hulst, J. L., & Berbling, J. L. (2006). Estradiol replacement enhances working memory in middle-aged rats when initiated immediately after ovariectomy but not after a long-term period of ovarian hormone deprivation. *Endocrinology*, *147*(1). https://doi.org/10.1210/en.2005-0998

D'Aquila, P. S., Brain, P., & Willner, P. (1994). Effects of chronic mild stress on performance in behavioural tests relevant to anxiety and depression. *Physiology and Behavior*, *56*(5). https://doi.org/10.1016/0031-9384(94)90316-6

Darcet, F., Mendez-David, I., Tritschler, L., Gardier, A. M., Guilloux, J. P., & David, D. J. (2014). Learning and memory impairments in a neuroendocrine mouse model of anxiety/depression. *Frontiers in Behavioral Neuroscience*, *8*(MAY). https://doi.org/10.3389/fnbeh.2014.00136

David, D. J., Samuels, B. A., Rainer, Q., Wang, J. W., Marsteller, D., Mendez, I., Drew, M., Craig, D. A., Guiard, B. P., Guilloux, J. P., Artymyshyn, R. P., Gardier, A. M., Gerald, C., Antonijevic, I. A., Leonardo, E. D., & Hen, R. (2009). Neurogenesis-Dependent and -Independent Effects of Fluoxetine in an Animal Model of Anxiety/Depression. *Neuron*, *62*(4). https://doi.org/10.1016/j.neuron.2009.04.017

De Boer, S. F., & Koolhaas, J. M. (2003). Defensive burying in rodents: Ethology, neurobiology and psychopharmacology. *European Journal of Pharmacology*, *463*(1–3), 145–161. https://doi.org/10.1016/s0014-2999(03)01278-0

de Brouwer, G., Fick, A., Harvey, B. H., & Wolmarans, D. W. (2019). A critical inquiry into marble-burying as a preclinical screening paradigm of relevance for anxiety and obsessive–compulsive disorder: Mapping the way forward. *Cognitive, Affective, & Behavioral Neuroscience*, *19*(1), 1–39. https://doi.org/10.3758/s13415-018-00653-4

de Bruin, N. M. W. J., Prickaerts, J., van Loevezijn, A., Venhorst, J., de Groote, L., Houba, P., Reneerkens, O., Akkerman, S., & Kruse, C. G. (2011). Two novel 5-HT6 receptor antagonists ameliorate scopolamine-induced memory deficits in the object recognition and object location tasks in Wistar rats. *Neurobiology of Learning and Memory*, *96*(2), 392–402. https://doi.org/10.1016/j.nlm.2011.06.015

De Kloet, E. R. (2004). Hormones and the stressed brain. *Annals of the New York Academy of Sciences*, *1018*. https://doi.org/10.1196/annals.1296.001

De Kloet, E. R., Joëls, M., & Holsboer, F. (2005). Stress and the brain: From adaptation to disease. *Nature Reviews Neuroscience*. https://doi.org/10.1038/nrn1683

De Novaes Soares, C., Almeida, O. P., Joffe, H., & Cohen, L. S. (2001). Efficacy of estradiol for the treatment of depressive disorders in perimenopausal women: A double-blind, randomized, placebo-controlled trial. *Archives of General Psychiatry*, *58*(6). https://doi.org/10.1001/archpsyc.58.6.529

de Winter, J. C. F., Gosling, S. D., & Potter, J. (2016). Comparing the Pearson and Spearman correlation coefficients across distributions and sample sizes: A tutorial using simulations and empirical data. *Psychological Methods*, *21*(3), 273–290. https://doi.org/10.1037/met0000079

Del Río, J. P., Alliende, M. I., Molina, N., Serrano, F. G., Molina, S., & Vigil, P. (2018). Steroid Hormones and Their Action in Women's Brains: The Importance of Hormonal Balance. *Frontiers in Public Health*, *6*. https://www.frontiersin.org/articles/10.3389/fpubh.2018.00141

Dellu, F., Mayo, W., Cherkaoui, J., Le Moal, M., & Simon, H. (1992). A two-trial memory task with automated recording: Study in young and aged rats. *Brain Research*, *588*(1), 132–139. https://doi.org/10.1016/0006-8993(92)91352-F

Demuyser, T., Bentea, E., Deneyer, L., Albertini, G., Massie, A., & Smolders, I. (2016). Disruption of the HPA-axis through corticosterone-release pellets induces robust depressive-like behavior and reduced BDNF levels in mice. *Neuroscience Letters*, *626*. https://doi.org/10.1016/j.neulet.2016.05.026

Denny, B. T., Inhoff, M. C., Zerubavel, N., Davachi, L., & Ochsner, K. N. (2015). Getting over it: Long-lasting effects of emotion regulation on amygdala response. *Psychological Science*, *26*(9), 1377–1388. https://doi.org/10.1177/0956797615578863

Detke, M. J., Rickels, M., & Lucki, I. (1995). Active behaviors in the rat forced swimming test differentially produced by serotonergic and noradrenergic antidepressants. *Psychopharmacology*, *121*(1). https://doi.org/10.1007/BF02245592

Deutsch-Feldman, M., Picetti, R., Seip-Cammack, K., Zhou, Y., & Kreek, M. J. (2015). Effects of Handling and Vehicle Injections on Adrenocorticotropic and Corticosterone Concentrations in Sprague–Dawley Compared with Lewis Rats. *Journal of the American Association for Laboratory Animal Science : JAALAS*, *54*(1), 35–39.

Diamond, D. M., Park, C. R., Heman, K. L., & Rose, G. M. (1999). Exposing rats to a predator impairs spatial working memory in the radial arm water maze. *Hippocampus*, *9*(5), 542–552. https://doi.org/10.1002/(SICI)1098-1063(1999)9:5<542::AID-HIPO8>3.0.CO;2-N

Dias-Ferreira, E., Sousa, J. C., Melo, I., Morgado, P., Mesquita, A. R., Cerqueira, J. J., Costa, R. M., & Sousa, N. (2009). Chronic stress causes frontostriatal reorganization and affects decision-making. *Science*. https://doi.org/10.1126/science.1171203

Dieterich, A., Srivastava, P., Sharif, A., Stech, K., Floeder, J., Yohn, S. E., & Samuels, B. A. (2019). Chronic corticosterone administration induces negative valence and impairs positive valence behaviors in mice. *Translational Psychiatry*, *9*(1), Article 1. https://doi.org/10.1038/s41398-019-0674-4

Ding, H., Cui, X.-Y., Cui, S.-Y., Ye, H., Hu, X., Zhao, H.-L., Liu, Y.-T., & Zhang, Y.-H. (2018). Depression-like behaviors induced by chronic corticosterone exposure via drinking water: Time-course analysis. *Neuroscience Letters*, *687*, 202–206. https://doi.org/10.1016/j.neulet.2018.09.059

Domonkos, E., Borbélyová, V., Csongová, M., Bosý, M., Kačmárová, M., Ostatníková, D., Hodosy, J., & Celec, P. (2017). Sex differences and sex hormones in anxiety-like behavior of aging rats. *Hormones and Behavior*, *93*, 159–165. https://doi.org/10.1016/j.yhbeh.2017.05.019

Domonkos, E., Hodosy, J., Ostatníková, D., & Celec, P. (2018). On the Role of Testosterone in Anxiety-Like Behavior Across Life in Experimental Rodents. *Frontiers in Endocrinology*, *9*. https://www.frontiersin.org/articles/10.3389/fendo.2018.00441

Drevets, W. C. (2000). Functional anatomical abnormalities in limbic and prefrontal cortical structures in major depression. *Progress in Brain Research*, *126*. https://doi.org/10.1016/S0079-6123(00)26027-5

Du Preez, A., Law, T., Onorato, D., Lim, Y. M., Eiben, P., Musaelyan, K., Egeland, M., Hye, A., Zunszain, P. A., Thuret, S., Pariante, C. M., & Fernandes, C. (2020). The type of stress matters: Repeated injection and permanent social isolation stress in male mice have a differential effect on anxiety- and depressive-like behaviours, and associated biological alterations. *Translational Psychiatry*, *10*, 325. https://doi.org/10.1038/s41398-020-01000-3

Du, Y., Wei, J., Yang, X., Dou, Y., Zhao, L., Qi, X., Yu, X., Guo, W., Wang, Q., Deng, W., Li, M., Lin, D., Li, T., & Ma, X. (2021). Plasma metabolites were associated with spatial working memory in major depressive disorder. *Medicine*, *100*(8), e24581. https://doi.org/10.1097/MD.0000000000024581

Duarte-Guterman, P., Yagi, S., Chow, C., & Galea, L. A. M. (2015). Hippocampal learning, memory, and neurogenesis: Effects of sex and estrogens across the lifespan in adults. *Hormones and Behavior*. https://doi.org/10.1016/j.yhbeh.2015.05.024

Duman, R. S. (2017). Sex-specific disease-Associated modules for depression. *Nature Medicine, 23*(9). https://doi.org/10.1038/nm.4391

Duman, R. S., Aghajanian, G. K., Sanacora, G., & Krystal, J. H. (2016). Synaptic plasticity and depression: New insights from stress and rapid-acting antidepressants. *Nature Medicine, 22*(3). https://doi.org/10.1038/nm.4050

Dunham, J. S., Deakin, J. F. W., Miyajima, F., Payton, A., & Toro, C. T. (2009). Expression of hippocampal brain-derived neurotrophic factor and its receptors in Stanley consortium brains. *Journal of Psychiatric Research*, *43*(14). https://doi.org/10.1016/j.jpsychires.2009.03.008

Duvarci, S., & Pare, D. (2014). Amygdala microcircuits controlling learned fear. *Neuron, 82*(5), 966–980. https://doi.org/10.1016/j.neuron.2014.04.042

Dwivedi, Y., Rizavi, H. S., Conley, R. R., Roberts, R. C., Tamminga, C. A., & Pandey, G. N. (2003). Altered gene expression of brain-derived neurotrophic factor and receptor tyrosine kinase B in postmortem brain of suicide subjects. *Archives of General Psychiatry*, *60*(8). https://doi.org/10.1001/archpsyc.60.8.804

Eberhart, N. K., Auerbach, R. P., Bigda-Peyton, J., & Abela, J. R. Z. (2011). Maladaptive schemas and depression: Tests of stress generation and diathesis-stress models.

Journal of Social and Clinical Psychology, *30*(1). https://doi.org/10.1521/jscp.2011.30.1.75

Edinger, K. L., & Frye, C. A. (2007). Androgens' effects to enhance learning may be mediated in part through actions at estrogen receptor-β in the hippocampus. *Neurobiology of Learning and Memory*, *87*(1), 78–85. https://doi.org/10.1016/j.nlm.2006.07.001

Egeland, J., Lund, A., Landrø, N. I., Rund, B. R., Sundet, K., Asbjørnsen, A., Mjellem, N., Roness, A., & Stordal, K. I. (2005). Cortisol level predicts executive and memory function in depression, symptom level predicts psychomotor speed. *Acta Psychiatrica Scandinavica*, *112*(6). https://doi.org/10.1111/j.1600-0447.2005.00599.x

Ennaceur, A., Neave, N., & Aggleton, J. P. (1997). Spontaneous object recognition and object location memory in rats: The effects of lesions in the cingulate cortices, the medial prefrontal cortex, the cingulum bundle and the fornix. *Experimental Brain Research*, *113*(3), 509–519.

Estrada-Camarena, E., Fernández-Guasti, A., & López-Rubalcava, C. (2003). Antidepressant-Like Effect of Different Estrogenic Compounds in the Forced Swimming Test. *Neuropsychopharmacology*, *28*(5), Article 5. https://doi.org/10.1038/sj.npp.1300097

Fan, Z., Chang, J., Liang, Y., Zhu, H., Zhang, C., Zheng, D., Wang, J., Xu, Y., Li, Q.-J., & Hu, H. (2023). Neural mechanism underlying depressive-like state associated with social status loss. *Cell*, *186*(3), 560-576.e17. https://doi.org/10.1016/j.cell.2022.12.033

Ferguson, J. N., Young, L. J., & Insel, T. R. (2002). The neuroendocrine basis of social recognition. *Frontiers in Neuroendocrinology*, *23*(2). https://doi.org/10.1006/frne.2002.0229

Fernández-Guasti, A., & Martínez-Mota, L. (2005). Anxiolytic-like actions of testosterone in the burying behavior test: Role of androgen and GABA-benzodiazepine receptors. *Psychoneuroendocrinology*, *30*(8), 762–770. https://doi.org/10.1016/j.psyneuen.2005.03.006

Feyissa, D. D., Aher, Y. D., Engidawork, E., Höger, H., Lubec, G., & Korz, V. (2017). Individual Differences in Male Rats in a Behavioral Test Battery: A Multivariate

Statistical Approach. *Frontiers in Behavioral Neuroscience*, *11*. https://www.frontiersin.org/articles/10.3389/fnbeh.2017.00026

Figueiredo, H. F., Dolgas, C. M., & Herman, J. P. (2002). Stress activation of cortex and hippocampus is modulated by sex and stage of estrus. *Endocrinology*, *143*(7). https://doi.org/10.1210/endo.143.7.8888

File, S. E., & Seth, P. (2003). A review of 25 years of the social interaction test. *European Journal of Pharmacology*, *463*(1–3). https://doi.org/10.1016/S0014-2999(03)01273-1

Filova, B., Malinova, M., Babickova, J., Tothova, L., Ostatnikova, D., Celec, P., & Hodosy, J. (2015). Effects of testosterone and estradiol on anxiety and depressive-like behavior via a non-genomic pathway. *Neuroscience Bulletin*, *31*(3), 288–296. https://doi.org/10.1007/s12264-014-1510-8

Fogaça, M. V., & Duman, R. S. (2019). Cortical GABAergic dysfunction in stress and depression: New insights for therapeutic interventions. *Frontiers in Cellular Neuroscience*, *13*. https://doi.org/10.3389/fncel.2019.00087

Freeman, E. W., Sammel, M. D., Lin, H., & Nelson, D. B. (2006). Associations of Hormones and Menopausal Status With Depressed Mood in Women With No History of Depression. *Archives of General Psychiatry*, *63*(4), 375–382. https://doi.org/10.1001/archpsyc.63.4.375

Frick, K. M. (2009). Estrogens and age-related memory decline in rodents: What have we learned and where do we go from here? *Hormones and Behavior*, *55*(1). https://doi.org/10.1016/j.yhbeh.2008.08.015

Frick, K. M., & Gresack, J. E. (2003). Sex Differences in the Behavioral Response to Spatial and Object Novelty in Adult C57BL/6 Mice. *Behavioral Neuroscience*. https://doi.org/10.1037/0735-7044.117.6.1283

Frick, K. M., & Kim, J. (2018). Mechanisms underlying the rapid effects of estradiol and progesterone on hippocampal memory consolidation in female rodents. *Hormones and Behavior*, *104*. https://doi.org/10.1016/j.yhbeh.2018.04.013

Frick, K. M., Kim, J., & Koss, W. A. (2018). Estradiol and hippocampal memory in female and male rodents. *Current Opinion in Behavioral Sciences*, *23*, 65–74. https://doi.org/10.1016/j.cobeha.2018.03.011

Frodl, T. S., Koutsoulcris, N., Bottlender, R., Born, C., Jäger, M., Scupin, I., Reiser, M., Möller, H. J., & Meiscnzahl, E. M. (2008). Depression-related variation in brain morphology over 3 years: Effects of stress? *Archives of General Psychiatry*, *65*(10). https://doi.org/10.1001/archpsyc.65.10.1156

Frye, C. A., & Walf, A. A. (2004). Estrogen and/or Progesterone Administered Systemically or to the Amygdala Can Have Anxiety-, Fear-, and Pain-Reducing Effects in Ovariectomized Rats. *Behavioral Neuroscience*, *118*(2). https://doi.org/10.1037/0735-7044.118.2.306

Frye, C., Edinger, K., Lephart, E., & Walf, A. (2010). 3α-androstanediol, but not testosterone, attenuates age-related decrements in cognitive, anxiety, and depressive behavior of male rats. *Frontiers in Aging Neuroscience*, *2*. https://www.frontiersin.org/articles/10.3389/fnagi.2010.00015

Fucich, E., & Morilak, D. (2018). Shock-probe Defensive Burying Test to Measure Active versus Passive Coping Style in Response to an Aversive Stimulus in Rats. *BIO-PROTOCOL*, *8*(17). https://doi.org/10.21769/BioProtoc.2998

Fukumoto, K., Toki, H., Iijima, M., Hashihayata, T., Yamaguchi, J. I., Hashimoto, K., & Chaki, S. (2017). Antidepressant potential of (R)-ketamine in rodent models: Comparison with (S)-ketamine. *Journal of Pharmacology and Experimental Therapeutics*, *361*(1). https://doi.org/10.1124/jpet.116.239228

Gabor, C. S., Phan, A., Clipperton-Allen, A. E., Kavaliers, M., & Choleris, E. (2012). Interplay of oxytocin, vasopressin, and sex hormones in the regulation of social recognition. *Behavioral Neuroscience*, *126*(1). https://doi.org/10.1037/a0026464

Gaelle, D., Nadia, H., Thomas, P., Vincent, D., Jean-Louis, G., Catherine, B., Nicole, M., & Daniel, B. (2019). Sustained corticosterone rise in the prefrontal cortex is a key factor for chronic stress-induced working memory deficits in mice. *Neurobiology of Stress*, *10*. https://doi.org/10.1016/j.ynstr.2019.100161

Gala, R. R., & Westphal, U. (1965). Corticosteroid-Binding Globulin in the Rat: Studies on the Sex Difference. *Endocrinology*, *77*(5), 841–851. https://doi.org/10.1210/endo-77-5-841

Gale, S. K., & Sclafani, A. (1977). Ovariectomy-induced changes in food motivation in the rat. *Hormones and Behavior*, *9*(2), 120–129. https://doi.org/10.1016/0018-506X(77)90079-4

Galea, L. A. M., Wide, J. K., & Barr, A. M. (2001). Estradiol alleviates depressive-like symptoms in a novel animal model of post-partum depression. *Behavioural Brain Research*, *122*(1). https://doi.org/10.1016/S0166-4328(01)00170-X

Galea, L. A., McEwen, B. S., Tanapat, P., Deak, T., Spencer, R. L., & Dhabhar, F. S. (1997a). Sex differences in dendritic atrophy of CA3 pyramidal neurons in response to chronic restraint stress. *Neuroscience*, *81*(3), 689–697. https://doi.org/S0306-4522(97)00233-9

Galea, L. A., McEwen, B. S., Tanapat, P., Deak, T., Spencer, R. L., & Dhabhar, F. S. (1997b). Sex differences in dendritic atrophy of CA3 pyramidal neurons in response to chronic restraint stress. *Neuroscience*. https://doi.org/10.1016/S0306-4522(97)00233-9

Galkin, S. A., Peshkovskaya, A. G., Simutkin, G. G., Vasil'eva, S. N., Roshchina, O. V., Ivanova, S. A., & Bokhan, N. A. (2020). Impairments to the Functions of Spatial Working Memory in Mild Depression and their Neurophysiological Correlates. *Neuroscience and Behavioral Physiology*, *50*(7), 825–829. https://doi.org/10.1007/s11055-020-00973-4

Galloway, E. M., Woo, N. H., & Lu, B. (2008). Chapter 15 Persistent neural activity in the prefrontal cortex: A mechanism by which BDNF regulates working memory? *Progress in Brain Research*, *169*. https://doi.org/10.1016/S0079-6123(07)00015-5

Garcia, A. N., Bezner, K., Depena, C., Yin, W., & Gore, A. C. (2017). The effects of long-term estradiol treatment on social behavior and gene expression in adult female rats. *Hormones and Behavior*, *87*, 145–154. https://doi.org/10.1016/j.yhbeh.2016.11.011

Garrett, J. E., & Wellman, C. L. (2009). Chronic stress effects on dendritic morphology in medial prefrontal cortex: Sex differences and estrogen dependence. *Neuroscience*, *162*(1). https://doi.org/10.1016/j.neuroscience.2009.04.057

Gaspar, R., Soares-Cunha, C., Domingues, A. V., Coimbra, B., Baptista, F. I., Pinto, L., Ambrósio, A. F., Rodrigues, A. J., & Gomes, C. A. (2021). Resilience to stress and sex-specific remodeling of microglia and neuronal morphology in a rat model

of anxiety and anhedonia. *Neurobiology of Stress*, *14*, 100302. https://doi.org/10.1016/j.ynstr.2021.100302

Gasparini, S. J., Weber, M.-C., Henneicke, H., Kim, S., Zhou, H., & Seibel, M. J. (2016). Continuous corticosterone delivery via the drinking water or pellet implantation: A comparative study in mice. *Steroids*, *116*, 76–82. https://doi.org/10.1016/j.steroids.2016.10.008

Ge, F., Yang, H., Lu, W., Shi, H., Chen, Q., Luo, Y., Liu, L., & Yan, J. (2020). Ovariectomy Induces Microglial Cell Activation and Inflammatory Response in Rat Prefrontal Cortices to Accelerate the Chronic Unpredictable Stress-Mediated Anxiety and Depression. *BioMed Research International*, *2020*, e3609758. https://doi.org/10.1155/2020/3609758

Geary, C. G., Wilk, V. C., Barton, K. L., Jefferson, P. O., Binder, T., Bhutani, V., Baker, C. L., Fernando-Peiris, A. J., Mousley, A. L., Rozental, S. F. A., Thompson, H. M., Touchon, J. C., Esteban, D. J., & Bergstrom, H. C. (2021). Sex differences in gut microbiota modulation of aversive conditioning, open field activity, and basolateral amygdala dendritic spine density. *Journal of Neuroscience Research*, *99*(7), 1780–1801. https://doi.org/10.1002/jnr.24848

Gerrits, M., Bakker, P. L., Koch, T., & Ter Horst, G. J. (2006). Stress-induced sensitization of the limbic system in ovariectomized rats is partly restored by cyclic 17β-estradiol administration. *European Journal of Neuroscience*, *23*(7). https://doi.org/10.1111/j.1460-9568.2006.04701.x

Gibbs, R. B. (1998). Levels of trkA and BDNF mRNA, but not NGF mRNA, fluctuate across the estrous cycle and increase in response to acute hormone replacement. *Brain Research*, *787*(2). https://doi.org/10.1016/S0006-8993(97)01511-4

Gibbs, R. B. (2005). Testosterone and estradiol produce different effects on cognitive performance in male rats. *Hormones and Behavior*, *48*(3), 268–277. https://doi.org/10.1016/j.yhbeh.2005.03.005

Gibbs, R. B., & Johnson, D. A. (2008). Sex-specific effects of gonadectomy and hormone treatment on acquisition of a 12-arm radial maze task by Sprague Dawley rats. *Endocrinology*, *149*(6), 3176–3183. https://doi.org/10.1210/en.2007-1645

Girard, J. M., Cohn, J. F., Mahoor, M. H., Mavadati, S. M., Hammal, Z., & Rosenwald, D. P. (2014). Nonverbal social withdrawal in depression: Evidence from manual

and automatic analyses. *Image and Vision Computing*, *32*(10), 641–647. https://doi.org/10.1016/j.imavis.2013.12.007

Gobinath, A. R., Mahmoud, R., & Galea, L. A. M. (2015). Influence of sex and stress exposure across the lifespan on endophenotypes of depression: Focus on behavior, glucocorticoids, and hippocampus. *Frontiers in Neuroscience*. https://doi.org/10.3389/fnins.2014.00420

Gogos, A., McCarthy, M., Walker, A. J., Udawela, M., Gibbons, A., Dean, B., & Kusljic, S. (2018). Differential effects of chronic 17β-oestradiol treatment on rat behaviours relevant to depression. *Journal of Neuroendocrinology*, *30*(11), e12652. https://doi.org/10.1111/jne.12652

Gold, P. W., Machado-Vieira, R., & Pavlatou, M. G. (2015). Clinical and Biochemical Manifestations of Depression: Relation to the Neurobiology of Stress. *Neural Plasticity*, *2015*, e581976. https://doi.org/10.1155/2015/581976

Goldwater, D. S., Pavlides, C., Hunter, R. G., Bloss, E. B., Hof, P. R., McEwen, B. S., & Morrison, J. H. (2009). Structural and functional alterations to rat medial prefrontal cortex following chronic restraint stress and recovery. *Neuroscience*, *164*(2). https://doi.org/10.1016/j.neuroscience.2009.08.053

Goñi-Balentziaga, O., Perez-Tejada, J., Renteria-Dominguez, A., Lebeña, A., & Labaka, A. (2018). Social instability in female rodents as a model of stress related disorders: A systematic review. *Physiology and Behavior*. https://doi.org/10.1016/j.physbeh.2018.09.001

Gordon, J., Eisenlohr-Moul, T., Sauer, T., & Sykes-Tottenham, L. (2019). The role of sensitivity to estrogen change in the development of perimenopausal depressive symptoms. *Psychoneuroendocrinology*, *100*, S42.

Gordon, J. L., Eisenlohr-Moul, T. A., Rubinow, D. R., Schrubbe, L., & Girdler, S. S. (2016). Naturally Occurring Changes in Estradiol Concentrations in the Menopause Transition Predict Morning Cortisol and Negative Mood in Perimenopausal Depression. *Clinical Psychological Science*, *4*(5), 919–935. https://doi.org/10.1177/2167702616647924

Gordon, J. L., Girdler, S. S., Meltzer-Brody, S. E., Stika, C. S., Thurston, R. C., Clark, C. T., Prairie, B. A., Moses-Kolko, E., Joffe, H., & Wisner, K. L. (2015). Ovarian Hormone Fluctuation, Neurosteroids, and HPA Axis Dysregulation in

Perimenopausal Depression: A Novel Heuristic Model. *American Journal of Psychiatry*, *172*(3), 227–236. https://doi.org/10.1176/appi.ajp.2014.14070918

Gordon, J. L., Peltier, A., Grummisch, J. A., & Sykes Tottenham, L. (2019). Estradiol Fluctuation, Sensitivity to Stress, and Depressive Symptoms in the Menopause Transition: A Pilot Study. *Frontiers in Psychology*, *10*. https://www.frontiersin.org/articles/10.3389/fpsyg.2019.01319

Gordon, J. L., Rubinow, D. R., Eisenlohr-Moul, T. A., Leserman, J., & Girdler, S. S. (2016a). Estradiol Variability, Stressful Life Events and the Emergence of Depressive Symptomatology during the Menopause Transition. *Menopause (New York, N.Y.)*, *23*(3), 257–266. https://doi.org/10.1097/GME.0000000000000528

Gordon, J. L., Rubinow, D. R., Eisenlohr-Moul, T. A., Leserman, J., & Girdler, S. S. (2016b). Estradiol Variability, Stressful Life Events and the Emergence of Depressive Symptomatology during the Menopause Transition. *Menopause (New York, N.Y.)*, *23*(3), 257–266. https://doi.org/10.1097/GME.0000000000000528

Gotlib, I. H., & Joormann, J. (2010). Cognition and depression: Current status and future directions. *Annual Review of Clinical Psychology*, *6*. https://doi.org/10.1146/annurev.clinpsy.121208.131305

Gould, E., Karatsoreos, I. N., Kane, G. A., McEwen, B. S., Kirschen, G. W., LaMarca, E. A., Fasolino, M., & Bocarsly, M. E. (2015). Obesity diminishes synaptic markers, alters microglial morphology, and impairs cognitive function. *Proceedings of the National Academy of Sciences*. https://doi.org/10.1073/pnas.1511593112

Gourley, S. L., Kiraly, D. D., Howell, J. L., Olausson, P., & Taylor, J. R. (2008). Acute Hippocampal Brain-Derived Neurotrophic Factor Restores Motivational and Forced Swim Performance After Corticosterone. *Biological Psychiatry*, *64*(10). https://doi.org/10.1016/j.biopsych.2008.06.016

Gourley, S. L., & Taylor, J. R. (2009). Recapitulation and reversal of a persistent depression-like syndrome in rodents. *Current Protocols in Neuroscience*, *SUPPL.49*. https://doi.org/10.1002/0471142301.ns0932s49

Grafe, L. A., Cornfeld, A., Luz, S., Valentino, R., & Bhatnagar, S. (2017). Orexins Mediate Sex Differences in the Stress Response and in Cognitive Flexibility. *Biological Psychiatry*. https://doi.org/10.1016/j.biopsych.2016.10.013

Gregus, A., Wintink, A. J., Davis, A. C., & Kalynchuk, L. E. (2005). Effect of repeated corticosterone injections and restraint stress on anxiety and depression-like behavior in male rats. *Behavioural Brain Research*. https://doi.org/10.1016/j.bbr.2004.05.013

Gresack, J. E., & Frick, K. M. (2006). Post-training estrogen enhances spatial and object memory consolidation in female mice. *Pharmacology Biochemistry and Behavior*, *84*(1). https://doi.org/10.1016/j.pbb.2006.04.013

Grigoryan, G. A. (2022). Ovariectomy as a Model of Anxiety-Depressive Disorders. *Neurochemical Journal*, *16*(1), 1–13. https://doi.org/10.1134/S1819712422010068

Grissom, N., & Bhatnagar, S. (2009). Habituation to repeated stress: Get used to it. *Neurobiology of Learning and Memory*, *92*(2), 215–224. https://doi.org/10.1016/j.nlm.2008.07.001

Grissom, N., Iyer, V., Vining, C., & Bhatnagar, S. (2007). The physical context of previous stress exposure modifies hypothalamic-pituitary-adrenal responses to a subsequent homotypic stress. *Hormones and Behavior*, *51*(1), 95–103. https://doi.org/10.1016/j.yhbeh.2006.08.011

Gururajan, A., Reif, A., Cryan, J. F., & Slattery, D. A. (2019). The future of rodent models in depression research. *Nature Reviews Neuroscience*, *20*(11), Article 11. https://doi.org/10.1038/s41583-019-0221-6

Guthman, E. M., Garcia, J. D., Ma, M., Chu, P., Baca, S. M., Smith, K. R., Restrepo, D., & Huntsman, M. M. (2020). Cell-type-specific control of basolateral amygdala neuronal circuits via entorhinal cortex-driven feedforward inhibition. *ELife*, *9*, e50601. https://doi.org/10.7554/eLife.50601

Hackenberg, T. D., Vanderhooft, L., Huang, J., Wagar, M., Alexander, J., & Tan, L. (2021). Social preference in rats. *Journal of the Experimental Analysis of Behavior*, *115*(3), 634–649. https://doi.org/10.1002/jeab.686

Hamer, J. A., Testani, D., Mansur, R. B., Lee, Y., Subramaniapillai, M., & McIntyre, R. S. (2019). Brain insulin resistance: A treatment target for cognitive impairment and anhedonia in depression. *Experimental Neurology*, *315*, 1–8. https://doi.org/10.1016/j.expneurol.2019.01.016

Hamezah, H. S., Durani, L. W., Ibrahim, N. F., Yanagisawa, D., Kato, T., Shiino, A., Tanaka, S., Damanhuri, H. A., Ngah, W. Z. W., & Tooyama, I. (2017). Volumetric changes in the aging rat brain and its impact on cognitive and locomotor functions. *Experimental Gerontology*, *99*, 69–79. https://doi.org/10.1016/j.exger.2017.09.008

Hamilton, J. P., Siemer, M., & Gotlib, I. H. (2008). Amygdala volume in Major Depressive Disorder: A meta-analysis of magnetic resonance imaging studies. *Molecular Psychiatry*, *13*(11), 993–1000. https://doi.org/10.1038/mp.2008.57

Hammen, C. (2005). Stress and depression. *Annual Review of Clinical Psychology*, *1*, 293–319. https://doi.org/10.1146/annurev.clinpsy.1.102803.143938

Hammen, C., Kim, E. Y., Eberhart, N. K., & Brennan, P. A. (2009). Chronic and acute stress and the prediction of major depression in women. *Depression and Anxiety*, *26*(8). https://doi.org/10.1002/da.20571

Handley, S. L., & Mithani, S. (1984). Effects of alpha-adrenoceptor agonists and antagonists in a maze-exploration model of 'fear'-motivated behaviour. *Naunyn-Schmiedeberg's Archives of Pharmacology*, *327*(1), 1–5. https://doi.org/10.1007/BF00504983

Hara, C., Manabe, K., & Ogawa, N. (1981). Influence of activity-stress on thymus, spleen and adrenal weights of rats: Possibility for an immunodeficiency model. *Physiology & Behavior*, *27*(2), 243–248. https://doi.org/10.1016/0031-9384(81)90264-X

Hartmann, J., Dedic, N., Pöhlmann, M. L., Häusl, A., Karst, H., Engelhardt, C., Westerholz, S., Wagner, K. V., Labermaier, C., Hoeijmakers, L., Kertokarijo, M., Chen, A., Joëls, M., Deussing, J. M., & Schmidt, M. V. (2017). Forebrain glutamatergic, but not GABAergic, neurons mediate anxiogenic effects of the glucocorticoid receptor. *Molecular Psychiatry*, *22*(3), Article 3. https://doi.org/10.1038/mp.2016.87

Havens, M. D., & Rose, J. D. (1992). Investigation of familiar and novel chemosensory stimuli by golden hamsters: Effects of castration and testosterone replacement. *Hormones and Behavior*, *26*(4), 505–511. https://doi.org/10.1016/0018-506x(92)90017-p

Heim, C., & Nemeroff, C. B. (2001). The role of childhood trauma in the neurobiology of mood and anxiety disorders: Preclinical and clinical studies. *Biological Psychiatry*, *49*(12), 1023–1039. https://doi.org/10.1016/S0006-3223(01)01157-X

Heller, W. (1993). Gender differences in depression: Perspectives from neuropsychology. *Journal of Affective Disorders*, *29*(2–3), 129–143.

Henckens, M. J. A. G., van der Marel, K., van der Toorn, A., Pillai, A. G., Fernández, G., Dijkhuizen, R. M., & Joëls, M. (2015). Stress-induced alterations in large-scale functional networks of the rodent brain. *NeuroImage*. https://doi.org/10.1016/j.neuroimage.2014.10.037

Herbert, J. (2013). Cortisol and depression: Three questions for psychiatry. *Psychological Medicine*, *43*(3). https://doi.org/10.1017/S0033291712000955

Herbert, J., Goodyer, I. M., Grossman, A. B., Hastings, M. H., de Kloet, E. R., Lightman, S. L., Lupien, S. J., Roozendaal, B., & Seckl, J. R. (2006). Do corticosteroids damage the brain? *Journal of Neuroendocrinology*, *18*(6), 393–411. https://doi.org/10.1111/j.1365-2826.2006.01429.x

Herbison, A. E., Simonian, S. X., Thanky, N. R., & Bicknell, R. J. (2000). Oestrogen modulation of noradrenaline neurotransmission. *Novartis Foundation Symposium*, *230*. https://doi.org/10.1002/0470870818.ch7

Herson, M., & Kulkarni, J. (2022). Hormonal Agents for the Treatment of Depression Associated with the Menopause. *Drugs & Aging*, *39*(8), 607–618. https://doi.org/10.1007/s40266-022-00962-x

Hinkelmann, K., Moritz, S., Botzenhardt, J., Riedesel, K., Wiedemann, K., Kellner, M., & Otte, C. (2009). Cognitive Impairment in Major Depression: Association with Salivary Cortisol. *Biological Psychiatry*, *66*(9), 879–885. https://doi.org/10.1016/j.biopsych.2009.06.023

Hiroi, R., Weyrich, G., Koebele, S., Mennenga, S., Talboom, J., Hewitt, L., Lavery, C., Mendoza, P., Jordan, A., & Bimonte-Nelson, H. (2016). Benefits of hormone therapy estrogens depend on estrogen type: 17β-estradiol and conjugated equine estrogens have differential effects on cognitive, anxiety-like, and depressive-like behaviors and increase tryptophan hydroxylase-2 mRNA levels in dorsal raphe nucleus subregions. *Frontiers in Neuroscience*, *10*. https://www.frontiersin.org/articles/10.3389/fnins.2016.00517

Hlinak, Z. (1993). Social recognition in ovariectomized and estradiol-treated female rats. *Hormones and Behavior*, *27*(2). https://doi.org/10.1006/hbeh.1993.1012

Ho, Y.-J., Eichendorff, J., & Schwarting, R. K. W. (2002). Individual response profiles of male Wistar rats in animal models for anxiety and depression. *Behavioural Brain Research*, *136*(1), 1–12. https://doi.org/10.1016/s0166-4328(02)00089-x

Hodes, G. E., & Epperson, C. N. (2019). Sex Differences in Vulnerability and Resilience to Stress Across the Life Span. *Biological Psychiatry*, *86*(6), 421–432. https://doi.org/10.1016/j.biopsych.2019.04.028

Hodosy, J., Zelmanová, D., Majzúnová, M., Filová, B., Malinová, M., Ostatníková, D., & Celec, P. (2012). The anxiolytic effect of testosterone in the rat is mediated via the androgen receptor. *Pharmacology, Biochemistry, and Behavior*, *102*(2), 191–195. https://doi.org/10.1016/j.pbb.2012.04.005

Hoffman, A. N., Armstrong, C. E., Hanna, J. J., & Conrad, C. D. (2010). Chronic stress, cyclic 17β-estradiol, and daily handling influences on fear conditioning in the female rat. *Neurobiology of Learning and Memory*, *94*(3), 422–433. https://doi.org/10.1016/j.nlm.2010.08.010

Hoffman, A. N., Krigbaum, A., Ortiz, J. B., Mika, A., Hutchinson, K. M., Bimonte-Nelson, H. A., & Conrad, C. D. (2011). Recovery after chronic stress within spatial reference and working memory domains: Correspondence with hippocampal morphology. *European Journal of Neuroscience*, *34*(6), 1023–1030. https://doi.org/10.1111/j.1460-9568.2011.07820.x

Hoffman, A. N., Lorson, N. G., Sanabria, F., Foster Olive, M., & Conrad, C. D. (2014). Chronic stress disrupts fear extinction and enhances amygdala and hippocampal Fos expression in an animal model of post-traumatic stress disorder. *Neurobiology of Learning and Memory*, *112*, 139–147. https://doi.org/10.1016/j.nlm.2014.01.018

Hoffman, A. N., Paode, P. R., May, H. G., Ortiz, J. B., Kemmou, S., Lifshitz, J., Conrad, C. D., & Currier Thomas, T. (2017). Early and Persistent Dendritic Hypertrophy in the Basolateral Amygdala following Experimental Diffuse Traumatic Brain Injury. *Journal of Neurotrauma*, *34*(1), 213–219. https://doi.org/10.1089/neu.2015.4339

Hu, C., Luo, Y., Wang, H., Kuang, S., Liang, G., Yang, Y., Mai, S., & Yang, J. (2017). Re-evaluation of the interrelationships among the behavioral tests in rats exposed to chronic unpredictable mild stress. *PLoS ONE*, *12*(9), e0185129. https://doi.org/10.1371/journal.pone.0185129

Hutchinson, K. M., McLaughlin, K. J., Wright, R. L., Bryce Ortiz, J., Anouti, D. P., Mika, A., Diamond, D. M., & Conrad, C. D. (2012). Environmental enrichment protects against the effects of chronic stress on cognitive and morphological measures of hippocampal integrity. *Neurobiology of Learning and Memory*, *97*(2), 250–260. https://doi.org/10.1016/j.nlm.2012.01.003

Huynh, T. N., Krigbaum, A. M., Hanna, J. J., & Conrad, C. D. (2011). Sex differences and phase of light cycle modify chronic stress effects on anxiety and depressive-like behavior. *Behavioural Brain Research*. https://doi.org/10.1016/j.bbr.2011.03.038

Huzian, O., Baka, J., Csakvari, E., Dobos, N., Leranth, C., Siklos, L., Duman, R. S., Farkas, T., & Hajszan, T. (2021). Stress Resilience is Associated with Hippocampal Synaptoprotection in the Female Rat Learned Helplessness Paradigm. *Neuroscience*, *459*, 85–103. https://doi.org/10.1016/j.neuroscience.2021.01.029

Ilin, Y., & Richter-Levin, G. (2009). Enriched environment experience overcomes learning deficits and depressive-like behavior induced by Juvenile stress. *PLoS ONE*. https://doi.org/10.1371/journal.pone.0004329

Iob, E., Kirschbaum, C., & Steptoe, A. (2020). Persistent depressive symptoms, HPA-axis hyperactivity, and inflammation: The role of cognitive-affective and somatic symptoms. *Molecular Psychiatry*, *25*(5). https://doi.org/10.1038/s41380-019-0501-6

Isingrini, E., Camus, V., Le Guisquet, A.-M., Pingaud, M., Devers, S., & Belzung, C. (2010). Association between repeated unpredictable chronic mild stress (UCMS) procedures with a high fat diet: A model of fluoxetine resistance in mice. *PloS One*, *5*(4), e10404. https://doi.org/10.1371/journal.pone.0010404

Jacome, L. F., Barateli, K., Buitrago, D., Lema, F., Frankfurt, M., & Luine, V. N. (2016). Gonadal Hormones Rapidly Enhance Spatial Memory and Increase Hippocampal Spine Density in Male Rats. *Endocrinology*, *157*(4), 1357–1362. https://doi.org/10.1210/en.2015-1959

Jalnapurkar, I., Allen, M., & Pigott, T. (2018). *Sex Differences in Anxiety Disorders: A Review*. https://doi.org/10.24966/PDA-0150/100012

Jaric, I., Rocks, D., Cham, H., Herchek, A., & Kundakovic, M. (2019). Sex and estrous cycle effects on anxiety- and depression-related phenotypes in a two-hit developmental stress model. *Frontiers in Molecular Neuroscience*. https://doi.org/10.3389/fnmol.2019.00074

Jean Kant, G., Eggleston, T., Landman-Roberts, L., Kenion, C. C., Driver, G. C., & Meyerhoff, J. L. (1985). Habituation to repeated stress is stressor specific. *Pharmacology, Biochemistry and Behavior*, *22*(4), 631–634. https://doi.org/10.1016/0091-3057(85)90286-2

Jin, J., Van Snellenberg, J. X., Perlman, G., DeLorenzo, C., Klein, D. N., Kotov, R., & Mohanty, A. (2020). Intrinsic neural circuitry of depression in adolescent females. *Journal of Child Psychology and Psychiatry and Allied Disciplines*, *61*(4). https://doi.org/10.1111/jcpp.13123

Jones, M. E. E., Chin Boon, W., Proietto, J., & Simpson, E. R. (2006). Of mice and men: The evolving phenotype of aromatase deficiency. *Trends in Endocrinology & Metabolism*, *17*(2), 55–64. https://doi.org/10.1016/j.tem.2006.01.004

Joseph, J. J., & Golden, S. H. (2017). Cortisol dysregulation: The bidirectional link between stress, depression, and type 2 diabetes mellitus. *Annals of the New York Academy of Sciences*, *1391*(1), 20–34. https://doi.org/10.1111/nyas.13217

Justice, J. N., Carter, C. S., Beck, H. J., Gioscia-Ryan, R. A., McQueen, M., Enoka, R. M., & Seals, D. R. (2014). Battery of behavioral tests in mice that models age-associated changes in human motor function. *Age*, *36*(2), 583–595. https://doi.org/10.1007/s11357-013-9589-9

Kalin, N. H. (2020). The Critical Relationship Between Anxiety and Depression. *American Journal of Psychiatry*, *177*(5), 365–367. https://doi.org/10.1176/appi.ajp.2020.20030305

Karisetty, B. C., Joshi, P. C., Kumar, A., & Chakravarty, S. (2017). Sex differences in the effect of chronic mild stress on mouse prefrontal cortical BDNF levels: A role of major ovarian hormones. *Neuroscience*, *356*. https://doi.org/10.1016/j.neuroscience.2017.05.020

Karlsson, S., Henningsson, S., Hovey, D., Zettergren, A., Jonsson, L., Cortes, D. S., Melke, J., Laukka, P., Fischer, H., & Westberg, L. (2016). Social memory associated with estrogen receptor polymorphisms in women. *Social Cognitive and Affective Neuroscience*, *11*(6). https://doi.org/10.1093/scan/nsw010

Karten, Y. J. G., Nair, S. M., van Essen, L., Sibug, R., & Joëls, M. (1999). Long-term exposure to high corticosterone levels attenuates serotonin responses in rat hippocampal CA1 neurons. *Proceedings of the National Academy of Sciences of the United States of America*, *96*(23), 13456–13461.

Kataja, E.-L., Karlsson, L., Huizink, A. C., Tolvanen, M., Parsons, C., Nolvi, S., & Karlsson, H. (2017). Pregnancy-related anxiety and depressive symptoms are associated with visuospatial working memory errors during pregnancy. *Journal of Affective Disorders*, *218*, 66–74. https://doi.org/10.1016/j.jad.2017.04.033

Keller, J., Gomez, R., Williams, G., Lembke, A., Lazzeroni, L., Murphy, G. M., & Schatzberg, A. F. (2017). HPA axis in major depression: Cortisol, clinical symptomatology and genetic variation predict cognition. *Molecular Psychiatry*, *22*(4), 527–536. https://doi.org/10.1038/mp.2016.120

Keller, M. B. (2005). Issues in treatment-resistant depression. *Journal of Clinical Psychiatry*, *66*(SUPPL. 8), 5–12.

Keppel, G. (1991). *Design and analysis: A researcher's handbook* (3rd ed). Prentice Hall. http://catalog.hathitrust.org/api/volumes/oclc/22735012.html

Kessler, R. C., Berglund, P., Demler, O., Jin, R., Merikangas, K. R., & Walters, E. E. (2005). Lifetime Prevalence and Age-of-Onset Distributions of DSM-IV Disorders in the National Comorbidity Survey Replication. *Archives of General Psychiatry*, *62*(6), 593–602. https://doi.org/10.1001/archpsyc.62.6.593

Khaleghi, M., Rajizadeh, M. A., Bashiri, H., Kohlmeier, K. A., Mohammadi, F., Khaksari, M., & Shabani, M. (2021). Estrogen attenuates physical and psychological stress-induced cognitive impairments in ovariectomized rats. *Brain and Behavior*, *11*(5), e02139. https://doi.org/10.1002/brb3.2139

Kircanski, K., Joormann, J., & Gotlib, I. H. (2012). Cognitive aspects of depression. *Wiley Interdisciplinary Reviews: Cognitive Science*, *3*(3). https://doi.org/10.1002/wcs.1177

Kishi, T., Yoshimura, R., Ikuta, T., & Iwata, N. (2018). Brain-derived neurotrophic factor and major depressive disorder: Evidence from meta-analyses. *Frontiers in Psychiatry*, *8*(JAN). https://doi.org/10.3389/fpsyt.2017.00308

Kiss, Á., Delattre, A. M., Pereira, S. I. R., Carolino, R. G., Szawka, R. E., Anselmo-Franci, J. A., Zanata, S. M., & Ferraz, A. C. (2012). 17β-Estradiol replacement in young, adult and middle-aged female ovariectomized rats promotes improvement of spatial reference memory and an antidepressant effect and alters monoamines and BDNF levels in memory- and depression-related brain areas. *Behavioural Brain Research*, *227*(1), 100–108. https://doi.org/10.1016/j.bbr.2011.10.047

Kitraki, E., Kremmyda, O., Youlatos, D., Alexis, M. N., & Kittas, C. (2004). Gender-dependent alterations in corticosteroid receptor status and spatial performance following 21 days of restraint stress. *Neuroscience*, *125*(1), 47–55. https://doi.org/10.1016/j.neuroscience.2003.12.024

Kleen, J. K., Sitomer, M. T., Killeen, P. R., & Conrad, C. D. (2006). Chronic stress impairs spatial memory and motivation for reward without disrupting motor ability and motivation to explore. *Behavioral Neuroscience*, *120*(4), 842–851. https://doi.org/10.1037/0735-7044.120.4.842

Knight, P., Chellian, R., Wilson, R., Behnood-Rod, A., Panunzio, S., & Bruijnzeel, A. W. (2021). Sex differences in the elevated plus-maze test and large open field test in adult Wistar rats. *Pharmacology Biochemistry and Behavior*, *204*, 173168. https://doi.org/10.1016/j.pbb.2021.173168

Koebele, S. V., & Bimonte-Nelson, H. A. (2015). Trajectories and phenotypes with estrogen exposures across the lifespan: What does Goldilocks have to do with it? *Hormones and Behavior*, *74*, 86–104. https://doi.org/10.1016/j.yhbeh.2015.06.009

Koebele, S. V., Mennenga, S. E., Hiroi, R., Quihuis, A. M., Hewitt, L. T., Poisson, M. L., George, C., Mayer, L. P., Dyer, C. A., Aiken, L. S., Demers, L. M., Carson, C., & Bimonte-Nelson, H. A. (2017). Cognitive changes across the menopause transition: A longitudinal evaluation of the impact of age and ovarian status on spatial memory. *Hormones and Behavior*. https://doi.org/10.1016/j.yhbeh.2016.10.010

Koebele, S. V., Mennenga, S. E., Poisson, M. L., Hewitt, L. T., Patel, S., Mayer, L. P., Dyer, C. A., & Bimonte-Nelson, H. A. (2020). Characterizing the effects of tonic

17β-estradiol administration on spatial learning and memory in the follicle-deplete middle-aged female rat. *Hormones and Behavior*, *126*. https://doi.org/10.1016/j.yhbeh.2020.104854

Koebele, S. V., Nishimura, K. J., Bimonte-Nelson, H. A., Kemmou, S., Ortiz, J. B., Judd, J. M., & Conrad, C. D. (2020a). A long-term cyclic plus tonic regimen of 17β-estradiol improves the ability to handle a high spatial working memory load in ovariectomized middle-aged female rats. *Hormones and Behavior*, *118*, 104656. https://doi.org/10.1016/J.YHBEH.2019.104656

Koebele, S. V., Nishimura, K. J., Bimonte-Nelson, H. A., Kemmou, S., Ortiz, J. B., Judd, J. M., & Conrad, C. D. (2020b). A long-term cyclic plus tonic regimen of 17β-estradiol improves the ability to handle a high spatial working memory load in ovariectomized middle-aged female rats. *Hormones and Behavior*. https://doi.org/10.1016/j.yhbeh.2019.104656

Koebele, S. V., Palmer, J. M., Hadder, B., Melikian, R., Fox, C., Strouse, I. M., DeNardo, D. F., George, C., Daunis, E., Nimer, A., Mayer, L. P., Dyer, C. A., & Bimonte-Nelson, H. A. (2019). Hysterectomy Uniquely Impacts Spatial Memory in a Rat Model: A Role for the Nonpregnant Uterus in Cognitive Processes. *Endocrinology*, *160*(1), 1–19. https://doi.org/10.1210/en.2018-00709

Konarski, J. Z., Mcintyre, R. S., Kennedy, S. H., Rafi-tari, S., Soczynska, J. K., & Ketter, T. A. (2008). Volumetric neuroimaging investigations in mood disorders: Bipolar disorder versus major depressive disorder. *Bipolar Disorders*, *10*(1). https://doi.org/10.1111/j.1399-5618.2008.00435.x

Koolhaas, J. M., Bartolomucci, A., Buwalda, B., de Boer, S. F., Flügge, G., Korte, S. M., Meerlo, P., Murison, R., Olivier, B., Palanza, P., Richter-Levin, G., Sgoifo, A., Steimer, T., Stiedl, O., van Dijk, G., Wöhr, M., & Fuchs, E. (2011). Stress revisited: A critical evaluation of the stress concept. *Neuroscience and Biobehavioral Reviews*. https://doi.org/10.1016/j.neubiorev.2011.02.003

Koolhaas, J. M., de Boer, S. F., & Buwalda, B. (2006). Stress and Adaptation. *Current Directions in Psychological Science*. https://doi.org/10.1111/j.0963-7214.2006.00417.x

Koolhaas, J. M., De Boer, S. F., De Ruitter, A. J. H., Meerlo, P., & Sgoifo, A. (1997). Social stress in rats and mice. *Acta Physiologica Scandinavica, Supplement*.

Koss, W. A., Einat, H., Schloesser, R. J., Manji, H. K., & Rubinow, D. R. (2012). Estrogen effects on the forced swim test differ in two outbred rat strains. *Physiology & Behavior*, *106*(2), 81–86. https://doi.org/10.1016/j.physbeh.2012.01.004

Ku, K. M., Weir, R. K., Silverman, J. L., Berman, R. F., & Bauman, M. D. (2016). Behavioral Phenotyping of Juvenile Long-Evans and Sprague-Dawley Rats: Implications for Preclinical Models of Autism Spectrum Disorders. *PLoS ONE*, *11*(6), e0158150. https://doi.org/10.1371/journal.pone.0158150

Kuo, J. R., Kaloupek, D. G., & Woodward, S. H. (2012). Amygdala Volume in Combat-Exposed Veterans With and Without Posttraumatic Stress Disorder: A Cross-sectional Study. *Archives of General Psychiatry*, *69*(10), 1080–1086. https://doi.org/10.1001/archgenpsychiatry.2012.73

Kvarta, M. D., Bradbrook, K. E., Dantrassy, H. M., Bailey, A. M., & Thompson, S. M. (2015). Corticosterone mediates the synaptic and behavioral effects of chronic stress at rat hippocampal temporoammonic synapses. *Journal of Neurophysiology*, *114*(3), 1713–1724. https://doi.org/10.1152/jn.00359.2015

Lagunas, N., Calmarza-Font, I., Diz-Chaves, Y., & Garcia-Segura, L. M. (2010). Long-term ovariectomy enhances anxiety and depressive-like behaviors in mice submitted to chronic unpredictable stress. *Hormones and Behavior*, *58*(5), 786–791. https://doi.org/10.1016/j.yhbeh.2010.07.014

Lakens, D. (2013). Calculating and reporting effect sizes to facilitate cumulative science: A practical primer for t-tests and ANOVAs. *Frontiers in Psychology*, *4*, 863. https://doi.org/10.3389/fpsyg.2013.00863

Lakshminarasimhan, H., & Chattarji, S. (2012). Stress leads to contrasting effects on the levels of brain derived neurotrophic factor in the hippocampus and amygdala. *PLoS ONE*, *7*(1). https://doi.org/10.1371/journal.pone.0030481

Lapiz-Bluhm, M. D. S., Bondi, C. O., Doyen, J., Rodriguez, G. A., Bédard-Arana, T., & Morilak, D. A. (2008). Behavioural assays to model cognitive and affective dimensions of depression and anxiety in rats. *Journal of Neuroendocrinology*, *20*(10), 1115–1137. https://doi.org/10.1111/j.1365-2826.2008.01772.x

Larkum, M. E., Senn, W., & Lüscher, H.-R. (2004). Top-down dendritic input increases the gain of layer 5 pyramidal neurons. *Cerebral Cortex (New York, N.Y.: 1991)*, *14*(10), 1059–1070. https://doi.org/10.1093/cercor/bhh065

Lau, T., Bigio, B., Zelli, D., McEwen, BS., & Nasca, C. (2017). Stress-induced structural plasticity of medial amygdala stellate neurons and rapid prevention by a candidate antidepressant. *Molecular Psychiatry*, *22*(2), 227–234. https://doi.org/10.1038/mp.2016.68

Laviola, G., Adriani, W., Rea, M., Aloe, L., & Alleva, E. (2004). Social withdrawal, neophobia, and stereotyped behavior in developing rats exposed to neonatal asphyxia. *Psychopharmacology*, *175*(2), 196–205. https://doi.org/10.1007/s00213-004-1800-3

Lebedeva, A., Sundström, A., Lindgren, L., Stomby, A., Aarsland, D., Westman, E., Winblad, B., Olsson, T., & Nyberg, L. (2018). Longitudinal relationships among depressive symptoms, cortisol, and brain atrophy in the neocortex and the hippocampus. *Acta Psychiatrica Scandinavica*, *137*(6), 491–502. https://doi.org/10.1111/acps.12860

Lecorps, B., Rödel, H. G., & Féron, C. (2016). Assessment of anxiety in open field and elevated plus maze using infrared thermography. *Physiology & Behavior*, *157*, 209–216. https://doi.org/10.1016/j.physbeh.2016.02.014

Ledford, H. (2014). Medical research: If depression were cancer. *Nature*, *515*(7526), 182–184. https://doi.org/10.1038/515182a

Lee, B. K., Glass, T. A., McAtee, M. J., Wand, G. S., Bandeen-Roche, K., Bolla, K. I., & Schwartz, B. S. (2007). Associations of salivary cortisol with cognitive function in the Baltimore memory study. *Archives of General Psychiatry*, *64*(7). https://doi.org/10.1001/archpsyc.64.7.810

LeMoult, J., & Gotlib, I. H. (2019). Depression: A cognitive perspective. *Clinical Psychology Review*, *69*. https://doi.org/10.1016/j.cpr.2018.06.008

Leranth, C., Petnehazy, O., & MacLusky, N. J. (2003). Gonadal Hormones Affect Spine Synaptic Density in the CA1 Hippocampal Subfield of Male Rats. *Journal of Neuroscience*, *23*(5), 1588–1592. https://doi.org/10.1523/JNEUROSCI.23-05-01588.2003

Leuner, B., & Gould, E. (2010). Structural plasticity and hippocampal function. *Annual Review of Psychology*, *61*, 111–140, C1-3. https://doi.org/10.1146/annurev.psych.093008.100359

Leuner, B., & Shors, T. J. (2013). Stress, anxiety, and dendritic spines: What are the connections? *Neuroscience*, *251*, 108–119. https://doi.org/10.1016/j.neuroscience.2012.04.021

Lin, Y., Ter Horst, G. J., Wichmann, R., Bakker, P., Liu, A., Li, X., & Westenbroek, C. (2009). Sex differences in the effects of acute and chronic stress and recovery after long-term stress on stress-related brain regions of rats. *Cerebral Cortex*, *19*(9). https://doi.org/10.1093/cercor/bhn225

Lino-De-Oliveira, C., De Lima, T. C. M., & Carobrez, A. D. P. (2005). Structure of the rat behaviour in the forced swimming test. *Behavioural Brain Research*, *158*(2). https://doi.org/10.1016/j.bbr.2004.09.004

Liu, B., Liu, J., Wang, M., Zhang, Y., & Li, L. (2017). From serotonin to neuroplasticity: Evolvement of theories for major depressive disorder. *Frontiers in Cellular Neuroscience*, *11*. https://doi.org/10.3389/fncel.2017.00305

Liu, M. Y., Yin, C. Y., Zhu, L. J., Zhu, X. H., Xu, C., Luo, C. X., Chen, H., Zhu, D. Y., & Zhou, Q. G. (2018). Sucrose preference test for measurement of stress-induced anhedonia in mice. *Nature Protocols*, *13*(7). https://doi.org/10.1038/s41596-018-0011-z

Liu, Q., He, H., Yang, J., Feng, X., Zhao, F., & Lyu, J. (2020). Changes in the global burden of depression from 1990 to 2017: Findings from the Global Burden of Disease study. *Journal of Psychiatric Research, 126*. https://doi.org/10.1016/j.jpsychires.2019.08.002

Liu, W. Z., Zhang, W. H., Zheng, Z. H., Zou, J. X., Liu, X. X., Huang, S. H., You, W. J., He, Y., Zhang, J. Y., Wang, X. D., & Pan, B. X. (2020). Identification of a prefrontal cortex-to-amygdala pathway for chronic stress-induced anxiety. *Nature Communications*, *11*(1). https://doi.org/10.1038/s41467-020-15920-7

Locklear, M., & Kritzer, M. (2014). Assessment of the effects of sex and sex hormones on spatial cognition in adult rats using the Barnes maze. *Hormones and Behavior*, *66*(2), 298–308. https://doi.org/10.1016/j.yhbeh.2014.06.006

Łosiak, W., Blaut, A., Kłosowska, J., & Łosiak-Pilch, J. (2019). Stressful Life Events, Cognitive Biases, and Symptoms of Depression in Young Adults. *Frontiers in Psychology, 10*. https://doi.org/10.3389/fpsyg.2019.02165

Lowy, M. T., Wittenberg, L., & Yamamoto, B. K. (1995). Effect of Acute Stress on Hippocampal Glutamate Levels and Spectrin Proteolysis in Young and Aged Rats. *Journal of Neurochemistry*, *65*(1). https://doi.org/10.1046/j.1471-4159.1995.65010268.x

Lui, E., Salim, M., Chahal, M., Puri, N., Marandi, E., Quadrilatero, J., & Satvat, E. (2017). Chronic corticosterone-induced impaired cognitive flexibility is not due to suppressed adult hippocampal neurogenesis. *Behavioural Brain Research*, *332*, 90–98. https://doi.org/10.1016/j.bbr.2017.05.060

Luine, V. (2002). Sex differences in chronic stress effects on memory in rats. *Stress*. https://doi.org/10.1080/1025389021000010549

Luine, V., & Frankfurt, M. (2013). Interactions between estradiol, BDNF and dendritic spines in promoting memory. *Neuroscience*, *239*. https://doi.org/10.1016/j.neuroscience.2012.10.019

Luine, V., & Frankfurt, M. (2020). Estrogenic regulation of memory: The first 50 years. *Hormones and Behavior*, *121*. https://doi.org/10.1016/j.yhbeh.2020.104711

Luine, V., Gomez, J., Beck, K., & Bowman, R. (2017). Sex differences in chronic stress effects on cognition in rodents. *Pharmacology Biochemistry and Behavior*, *152*, 13–19. https://doi.org/10.1016/j.pbb.2016.08.005

Luine, V. N., Richards, S. T., Wu, V. Y., & Beck, K. D. (1998). Estradiol enhances learning and memory in a spatial memory task and effects levels of monoaminergic neurotransmitters. *Hormones and Behavior*, *34*(2). https://doi.org/10.1006/hbeh.1998.1473

Luine, V. N., Spencer, R. L., & McEwen, B. S. (1993). Effects of chronic corticosterone ingestion on spatial memory performance and hippocampal serotonergic function. *Brain Research*, *616*(1–2). https://doi.org/10.1016/0006-8993(93)90193-Q

Luine, V., & Rodriguez, M. (1994). Effects of estradiol on radial arm maze performance of young and aged rats. *Behavioral and Neural Biology*, *62*(3), 230–236. https://doi.org/10.1016/s0163-1047(05)80021-4

Luine, V., Villegas, M., Martinez, C., & McEwen, B. S. (1994a). Repeated stress causes reversible impairments of spatial memory performance. *Brain Research*. https://doi.org/10.1016/0006-8993(94)91778-7

Luine, V., Villegas, M., Martinez, C., & McEwen, B. S. (1994b). Repeated stress causes reversible impairments of spatial memory performance. *Brain Research*, *639*(1), 167–170. https://doi.org/10.1016/0006-8993(94)91778-7

Ma, L., Xu, Y., Wang, G., & Li, R. (2019). What do we know about sex differences in depression: A review of animal models and potential mechanisms. *Progress in Neuro-Psychopharmacology and Biological Psychiatry*, *89*, 48–56. https://doi.org/10.1016/j.pnpbp.2018.08.026

MacQueen, G. M., Campbell, S., McEwen, B. S., Macdonald, K., Amano, S., Joffe, R. T., Nahmias, C., & Young, L. T. (2003). Course of illness, hippocampal function, and hippocampal volume in major depression. *Proceedings of the National Academy of Sciences of the United States of America*, *100*(3), 1387–1392. https://doi.org/10.1073/pnas.0337481100

Maeng, L. Y., & Shors, T. J. (2013). The stressed female brain: Neuronal activity in the prelimbic but not infralimbic region of the medial prefrontal cortex suppresses learning after acute stress. *Frontiers in Neural Circuits*, *7*(DEC). https://doi.org/10.3389/fncir.2013.00198

Maeng, L. Y., Waddell, J., & Shors, T. J. (2010). The prefrontal cortex communicates with the amygdala to impair learning after acute stress in females but not in males. *Journal of Neuroscience*, *30*(48). https://doi.org/10.1523/JNEUROSCI.2265-10.2010

Magariños, A. M., Orchinik, M., & McEwen, B. S. (1998). Morphological changes in the hippocampal CA3 region induced by non- invasive glucocorticoid administration: A paradox. *Brain Research*, *809*(2). https://doi.org/10.1016/S0006-8993(98)00882-8

Major Depression. (2020). National Institute of Mental Health (NIMH). https://www.nimh.nih.gov/health/statistics/major-depression

Maki, P. M., Kornstein, S. G., Joffe, H., Bromberger, J. T., Freeman, E. W., Athappilly, G., Bobo, W. V., Rubin, L. H., Koleva, H. K., Cohen, L. S., & Soares, C. N.

(2019). Guidelines for the Evaluation and Treatment of Perimenopausal Depression: Summary and Recommendations. *Journal of Women's Health*, *28*(2), 117–134. https://doi.org/10.1089/jwh.2018.27099.mensocrec

Maren, S., & Fanselow, M. (1995). Synaptic plasticity in the basolateral amygdala induced by hippocampal formation stimulation in vivo. *The Journal of Neuroscience*, *15*(11), 7548–7564. https://doi.org/10.1523/JNEUROSCI.15-11-07548.1995

Marin, M. F., Lord, C., Andrews, J., Juster, R. P., Sindi, S., Arsenault-Lapierre, G., Fiocco, A. J., & Lupien, S. J. (2011). Chronic stress, cognitive functioning and mental health. *Neurobiology of Learning and Memory*. https://doi.org/10.1016/j.nlm.2011.02.016

Marin, M. T., Cruz, F. C., & Planeta, C. S. (2007). Chronic restraint or variable stresses differently affect the behavior, corticosterone secretion and body weight in rats. *Physiology and Behavior*. https://doi.org/10.1016/j.physbeh.2006.08.021

Markham, J. A., & Juraska, J. M. (2007). Social Recognition Memory: Influence of Age, Sex, and Ovarian Hormonal Status. *Physiology & Behavior*, *92*(5), 881–888. https://doi.org/10.1016/j.physbeh.2007.06.020

Markowska, A. L., & Savonenko, A. V. (2002). Effectiveness of estrogen replacement in restoration of cognitive function after long-term estrogen withdrawal in aging rats. *Journal of Neuroscience*, *22*(24). https://doi.org/10.1523/jneurosci.22-24-10985.2002

Marks, W., Fournier, N. M., & Kalynchuk, L. E. (2009). Repeated exposure to corticosterone increases depression-like behavior in two different versions of the forced swim test without altering nonspecific locomotor activity or muscle strength. *Physiology & Behavior*, *98*(1–2), 67–72. https://doi.org/10.1016/j.physbeh.2009.04.014

Marti & Armario. (1997). Influence of Regularity of Exposure to Chronic Stress on the Pattern of Habituation of Pituitary-Adrenal Hormones, Prolactin and Glucose. *Stress (Amsterdam, Netherlands)*, *1*(3), 179–189.

Martí, O., Martí, J., & Armario, A. (1994). Effects of chronic stress on food intake in rats: Influence of stressor intensity and duration of daily exposure. *Physiology and Behavior*. https://doi.org/10.1016/0031-9384(94)90055-8

Martinez, M., Calvo-Torrent, A., & Pico-Alfonso, M. A. (1998). Social defeat and subordination as models of social stress in laboratory rodents: A review. *Aggressive Behavior*. https://doi.org/10.1002/(sici)1098-2337(1998)24:4<241::aid-ab1>3.3.co;2-z

McConnell, S. E. A., Alla, J., Wheat, E., Romeo, R. D., McEwen, B., & Thornton, J. E. (2012). The role of testicular hormones and luteinizing hormone in spatial memory in adult male rats. *Hormones and Behavior*, *61*(4), 479–486. https://doi.org/10.1016/j.yhbeh.2012.01.003

McCormick, C. M., Nixon, F., Thomas, C., Lowie, B., & Dyck, J. (2010). Hippocampal cell proliferation and spatial memory performance after social instability stress in adolescence in female rats. *Behavioural Brain Research*. https://doi.org/10.1016/j.bbr.2009.11.003

McEwen, B. S. (2017). Neurobiological and Systemic Effects of Chronic Stress. *Chronic Stress*, *1*. https://doi.org/10.1177/2470547017692328

McEwen, B. S., & Akil, H. (2020). Revisiting the Stress Concept: Implications for Affective Disorders. *The Journal of Neuroscience*, *40*(1), 12–21. https://doi.org/10.1523/JNEUROSCI.0733-19.2019

McEwen, B. S., & Morrison, J. H. (2013). The Brain on Stress: Vulnerability and Plasticity of the Prefrontal Cortex over the Life Course. *Neuron*, *79*(1), 16–29. https://doi.org/10.1016/j.neuron.2013.06.028

McEwen, B. S., Nasca, C., & Gray, J. D. (2016). Stress Effects on Neuronal Structure: Hippocampus, Amygdala, and Prefrontal Cortex. *Neuropsychopharmacology*, *41*(1), Article 1. https://doi.org/10.1038/npp.2015.171

McFadden, L. M., Paris, J. J., Mitzelfelt, M. S., McDonough, S., Frye, C. A., & Matuszewich, L. (2011a). Sex-dependent effects of chronic unpredictable stress in the water maze. *Physiology & Behavior*, *102*(3), 266–275. https://doi.org/10.1016/j.physbeh.2010.10.022

McFadden, L. M., Paris, J. J., Mitzelfelt, M. S., McDonough, S., Frye, C. A., & Matuszewich, L. (2011b). Sex-dependent effects of chronic unpredictable stress in the water maze. *Physiology and Behavior*. https://doi.org/10.1016/j.physbeh.2010.10.022

McGarry, L. M., & Carter, A. G. (2016). Inhibitory Gating of Basolateral Amygdala Inputs to the Prefrontal Cortex. *The Journal of Neuroscience*, *36*(36), 9391–9406. https://doi.org/10.1523/JNEUROSCI.0874-16.2016

McIlwain, K. L., Merriweather, M. Y., Yuva-Paylor, L. A., & Paylor, R. (2001). The use of behavioral test batteries: Effects of training history. *Physiology and Behavior*, *73*(5), 705–717. https://doi.org/10.1016/S0031-9384(01)00528-5

McKinlay, S. M., Brambilla, D. J., & Posner, J. G. (1992). The normal menopause transition. *Maturitas*, *14*(2), 103–115. https://doi.org/10.1016/0378-5122(92)90003-M

McKittrick, C. R., Magariños, A. M., Blanchard, D. C., Blanchard, R. J., McEwen, B. S., & Sakai, R. R. (2000). Chronic social stress reduces dendritic arbors in CA3 of hippocampus and decreases binding to serotonin transporter sites. *Synapse*. https://doi.org/10.1002/(SICI)1098-2396(200005)36:2<85::AID-SYN1>3.0.CO;2-Y

McLaughlin, K. J., Baran, S. E., Wright, R. L., & Conrad, C. D. (2005). Chronic stress enhances spatial memory in ovariectomized female rats despite CA3 dendritic retraction: Possible involvement of CA1 neurons. *Neuroscience*. https://doi.org/10.1016/j.neuroscience.2005.06.083

McLaughlin, K. J., Bimonte-Nelson, H., Neisewander, J. L., & Conrad, C. D. (2008). Assessment of estradiol influence on spatial tasks and hippocampal CA1 spines: Evidence that the duration of hormone deprivation after ovariectomy compromises 17β-estradiol effectiveness in altering CA1 spines. *Hormones and Behavior*. https://doi.org/10.1016/j.yhbeh.2008.04.010

McLaughlin, K. J., Gomez, J. L., Baran, S. E., & Conrad, C. D. (2007). The effects of chronic stress on hippocampal morphology and function: An evaluation of chronic restraint paradigms. *Brain Research*, *1161*(Supplement C), 56–64. https://doi.org/10.1016/j.brainres.2007.05.042

McLaughlin, K. J., Wilson, J. O., Harman, J., Wright, R. L., Wieczorek, L., Gomez, J., Korol, D. L., & Conrad, C. D. (2010). Chronic 17β-estradiol or cholesterol prevents stress-induced hippocampal CA3 dendritic retraction in ovariectomized female rats: Possible correspondence between CA1 spine properties and spatial acquisition. *Hippocampus*. https://doi.org/10.1002/hipo.20678

McLay, R. N., Freeman, S. M., & Zadina, J. E. (1998). Chronic corticosterone impairs memory performance in the Barnes maze. *Physiology and Behavior*, *63*(5). https://doi.org/10.1016/S0031-9384(97)00529-5

Mennenga, S. E., & Bimonte-Nelson, H. A. (2013). Translational cognitive endocrinology: Designing rodent experiments with the goal to ultimately enhance cognitive health in women. *Brain Research*, *1514*, 50–62. https://doi.org/10.1016/j.brainres.2013.01.020

Mika, A., Mazur, G. J., Hoffman, A. N., Talboom, J. S., Bimonte-Nelson, H. A., Sanabria, F., & Conrad, C. D. (2012a). Chronic stress impairs prefrontal cortex-dependent response inhibition and spatial working memory. *Behavioral Neuroscience*, *126*(5), 605–619. https://doi.org/10.1037/a0029642

Mika, A., Mazur, G. J., Hoffman, A. N., Talboom, J. S., Bimonte-Nelson, H. A., Sanabria, F., & Conrad, C. D. (2012b). Chronic stress impairs prefrontal cortex-dependent response inhibition and spatial working memory. *Behavioral Neuroscience*, *126*(5), 605–619. https://doi.org/10.1037/a0029642

Miller, B. R., & Hen, R. (2015). The current state of the neurogenic theory of depression and anxiety. *Current Opinion in Neurobiology*, *30*. https://doi.org/10.1016/j.conb.2014.08.012

Mishra, S. K. (2011). Menopausal transition and postmenopausal health problems: A review on its bio-cultural perspectives. *Health*, *03*(04), Article 04. https://doi.org/10.4236/health.2011.34041

Mitra, R., & Sapolsky, R. M. (2008). Acute corticosterone treatment is sufficient to induce anxiety and amygdaloid dendritic hypertrophy. *Proceedings of the National Academy of Sciences of the United States of America*, *105*(14). https://doi.org/10.1073/pnas.0705615105

Mizoguchi, K., Yuzurihara, M., Ishige, A., Sasaki, H., Chui, D. H., & Tabira, T. (2000). Chronic stress induces impairment of spatial working memory because of prefrontal dopaminergic dysfunction. *The Journal of Neuroscience : The Official Journal of the Society for Neuroscience*.

Moench, K. M., Breach, M. R., & Wellman, C. L. (2019). Chronic stress produces enduring sex- and region-specific alterations in novel stress-induced c-Fos expression. *Neurobiology of Stress*. https://doi.org/10.1016/j.ynstr.2019.100147

Moench, K. M., & Wellman, C. L. (2017). Differential dendritic remodeling in prelimbic cortex of male and female rats during recovery from chronic stress. *Neuroscience*. https://doi.org/10.1016/j.neuroscience.2017.05.049

Monsey, M. S., Boyle, L. M., Zhang, M. L., Nguyen, C. P., Kronman, H. G., Ota, K. T., Duman, R. S., Taylor, J. R., & Schafe, G. E. (2014). Chronic corticosterone exposure persistently elevates the expression of memory-related genes in the lateral amygdala and enhances the consolidation of a Pavlovian fear memory. *PLoS ONE*, *9*(3). https://doi.org/10.1371/journal.pone.0091530

Moosavi, M., Naghdi, N., Maghsoudi, N., & Zahedi Asl, S. (2007). Insulin protects against stress-induced impairments in water maze performance. *Behavioural Brain Research*. https://doi.org/10.1016/j.bbr.2006.10.011

Moy, S. S., Nadler, J. J., Perez, A., Barbaro, R. P., Johns, J. M., Magnuson, T. R., Piven, J., & Crawley, J. N. (2004). Sociability and preference for social novelty in five inbred strains: An approach to assess autistic-like behavior in mice. *Genes, Brain and Behavior*, *3*(5), 287–302. https://doi.org/10.1111/j.1601-1848.2004.00076.x

Mumby, D. G. (2002). Hippocampal Damage and Exploratory Preferences in Rats: Memory for Objects, Places, and Contexts. *Learning & Memory*, *9*(2), 49–57. https://doi.org/10.1101/lm.41302

Musazzi, L., Treccani, G., & Popoli, M. (2015). Functional and Structural Remodeling of Glutamate Synapses in Prefrontal and Frontal Cortex Induced by Behavioral Stress. *Frontiers in Psychiatry*, *6*. https://www.frontiersin.org/articles/10.3389/fpsyt.2015.00060

Myers, B., McKlveen, J. M., & Herman, J. P. (2014). Glucocorticoid actions on synapses, circuits, and behavior: Implications for the energetics of stress. *Frontiers in Neuroendocrinology*, *35*(2). https://doi.org/10.1016/j.yfrne.2013.12.003

Nacher, J., Gomez-Climent, M. A., & McEwen, B. (2004). Chronic non-invasive glucocorticoid administration decreases polysialylated neural cell adhesion molecule expression in the adult rat dentate gyrus. *Neuroscience Letters*, *370*(1). https://doi.org/10.1016/j.neulet.2004.07.062

Nadler, J. J., Moy, S. S., Dold, G., and Simmons, N., Perez, A., Young, N. B., Barbaro, R. P., Piven, J., Magnuson, T. R., & Crawley, J. N. (2004). Automated apparatus

for quantitation of social approach behaviors in mice. *Genes, Brain and Behavior*, *3*(5), 303–314. https://doi.org/10.1111/j.1601-183X.2004.00071.x

Najjar, F., Ahmad, M., Lagace, D., & Leenen, F. H. H. (2018). Sex differences in depression-like behavior and neuroinflammation in rats post-MI: role of estrogens. *American Journal of Physiology-Heart and Circulatory Physiology*. https://doi.org/10.1152/ajpheart.00615.2017

Nestler, E. J., Barrot, M., DiLeone, R. J., Eisch, A. J., Gold, S. J., & Monteggia, L. M. (2002). Neurobiology of depression. *Neuron*, *34*(1). https://doi.org/10.1016/S0896-6273(02)00653-0

Nestler, E. J., & Hyman, S. E. (2010). Animal models of neuropsychiatric disorders. *Nature Neuroscience*. https://doi.org/10.1038/nn.2647

Newhouse, P., & Albert, K. (2015). Estrogen, stress, and depression: A neurocognitive model. *JAMA Psychiatry*, *72*(7). https://doi.org/10.1001/jamapsychiatry.2015.0487

Ngoupaye, G. T., Yassi, F. B., Bahane, D. A. N., & Bum, E. N. (2018). Combined corticosterone treatment and chronic restraint stress lead to depression associated with early cognitive deficits in mice. *Metabolic Brain Disease*, *33*(2), 421–431. https://doi.org/10.1007/s11011-017-0148-4

Nickle, T. R., Stanley, E. M., & Middlemas, D. S. (2020). Corticosterone Induces Depressive-Like Behavior in Female Peri-Pubescent Rats, but Not in Pre-Pubescent Rats. *Chronic Stress*, *4*, 2470547020923711. https://doi.org/10.1177/2470547020923711

Nicolas, L. B., Kolb, Y., & Prinssen, E. P. M. (2006). A combined marble burying-locomotor activity test in mice: A practical screening test with sensitivity to different classes of anxiolytics and antidepressants. *European Journal of Pharmacology*, *547*(1–3), 106–115. https://doi.org/10.1016/j.ejphar.2006.07.015

Nikolin, S., Tan, Y. Y., Schwaab, A., Moffa, A., Loo, C. K., & Martin, D. (2021). An investigation of working memory deficits in depression using the n-back task: A systematic review and meta-analysis. *Journal of Affective Disorders*, *284*, 1–8. https://doi.org/10.1016/j.jad.2021.01.084

Nishimura, K. J., Ortiz, J. B., & Conrad, C. D. (2017). Antagonizing the GABAA receptor during behavioral training improves spatial memory at different doses in control and chronically stressed rats. *Neurobiology of Learning and Memory*, *145*(Supplement C), 114–118. https://doi.org/10.1016/j.nlm.2017.09.002

Nolte, E. D., Nolte, K. A., & Yan, S. S. (2019). Anxiety and task performance changes in an aging mouse model. *Biochemical and Biophysical Research Communications*, *514*(1), 246–251. https://doi.org/10.1016/j.bbrc.2019.04.049

O'Connor, K. A., Feustel, P. J., Ramirez-Zamora, A., Molho, E., Pilitsis, J. G., & Shin, D. S. (2016). Investigation of diazepam efficacy on anxiety-like behavior in hemiparkinsonian rats. *Behavioural Brain Research*, *301*, 226–237. https://doi.org/10.1016/j.bbr.2015.12.045

Olausson, P., Kiraly, D. D., Gourley, S. L., & Taylor, J. R. (2013). Persistent effects of prior chronic exposure to corticosterone on reward-related learning and motivation in rodents. *Psychopharmacology*, *225*(3). https://doi.org/10.1007/s00213-012-2844-4

Ortiz, J. B., Anglin, J. M., Daas, E. J., Paode, P. R., Nishimura, K., & Conrad, C. D. (2018). BDNF and TrkB Mediate the Improvement from Chronic Stress-induced Spatial Memory Deficits and CA3 Dendritic Retraction. *Neuroscience*. https://doi.org/10.1016/j.neuroscience.2018.07.049

Ortiz, J. B., & Conrad, C. D. (2018). The impact from the aftermath of chronic stress on hippocampal structure and function: Is there a recovery? *Frontiers in Neuroendocrinology*, *49*, 114–123. https://doi.org/10.1016/j.yfrne.2018.02.005

Ortiz, J. B., Mathewson, C. M., Hoffman, A. N., Hanavan, P. D., Terwilliger, E. F., & Conrad, C. D. (2014). Hippocampal brain-derived neurotrophic factor mediates recovery from chronic stress-induced spatial reference memory deficits. *The European Journal of Neuroscience*, *40*(9), 3351–3362. https://doi.org/10.1111/ejn.12703

Ortiz, J. B., Taylor, S. B., Hoffman, A. N., Campbell, A. N., Lucas, L. R., & Conrad, C. D. (2015). Sex-specific impairment and recovery of spatial learning following the end of chronic unpredictable restraint stress: Potential relevance of limbic GAD. *Behavioural Brain Research*, *282*, 176–184. https://doi.org/10.1016/j.bbr.2014.12.051

Packard, M. G., Kohlmaier, J. R., & Alexander, G. M. (1996). Posttraining intrahippocampal estradiol injections enhance spatial memory in male rats: Interaction with cholinergic systems. *Behavioral Neuroscience, 110*(3), 626–632. https://doi.org/10.1037//0735-7044.110.3.626

Padival, M. A., Blume, S. R., & Rosenkranz, J. A. (2013). Repeated restraint stress exerts different impact on structure of neurons in the lateral and basal nuclei of the amygdala. *Neuroscience*, *246*, 230–242. https://doi.org/10.1016/j.neuroscience.2013.04.061

Pare, D., & Duvarci, S. (2012). Amygdala microcircuits mediating fear expression and extinction. *Current Opinion in Neurobiology*, *22*(4), 717–723. https://doi.org/10.1016/j.conb.2012.02.014

Pariante, C. M., & Lightman, S. L. (2008). The HPA axis in major depression: Classical theories and new developments. *Trends in Neurosciences*, *31*(9), 464–468. https://doi.org/10.1016/j.tins.2008.06.006

Park, C., Rosenblat, J. D., Brietzke, E., Pan, Z., Lee, Y., Cao, B., Zuckerman, H., Kalantarova, A., & McIntyre, R. S. (2019). Stress, epigenetics and depression: A systematic review. *Neuroscience & Biobehavioral Reviews*, *102*, 139–152. https://doi.org/10.1016/j.neubiorev.2019.04.010

Patel, D., Anilkumar, S., Chattarji, S., & Buwalda, B. (2018). Repeated social stress leads to contrasting patterns of structural plasticity in the amygdala and hippocampus. *Behavioural Brain Research*, *347*. https://doi.org/10.1016/j.bbr.2018.03.034

Payne, J. L. (2003). The role of estrogen in mood disorders in women. *International Review of Psychiatry*, *15*(3). https://doi.org/10.1080/0954026031000136893

Pazini, F. L., Cunha, M. P., Rosa, J. M., Colla, A. R. S., Lieberknecht, V., Oliveira, Á., & Rodrigues, A. L. S. (2016). Creatine, Similar to Ketamine, Counteracts Depressive-Like Behavior Induced by Corticosterone via PI3K/Akt/mTOR Pathway. *Molecular Neurobiology*, *53*(10). https://doi.org/10.1007/s12035-015-9580-9

Peay, D. N., Acuna, A., Reynolds, C. M., Willis, C., Takalkar, R., Bryce Ortiz, J., & Conrad, C. D. (2023). Chronic stress leads to persistent and contrasting stellate neuron dendritic hypertrophy in the amygdala of male and female rats, an effect

not found in the hippocampus. *Neuroscience Letters*, *812*, 137403. https://doi.org/10.1016/j.neulet.2023.137403

Peay, D. N., Saribekyan, H. M., Parada, P. A., Hanson, E. M., Badaruddin, B. S., Judd, J. M., Donnay, M. E., Padilla-Garcia, D., & Conrad, C. D. (2020). Chronic unpredictable intermittent restraint stress disrupts spatial memory in male, but not female rats. *Behavioural Brain Research*, *383*, 112519. https://doi.org/10.1016/j.bbr.2020.112519

Perez-Cruz, C., Müller-Keuker, J. I. H., Heilbronner, U., Fuchs, E., & Flügge, G. (2007). Morphology of pyramidal neurons in the rat prefrontal cortex: Lateralized dendritic remodeling by chronic stress. *Neural Plasticity*, *2007*. https://doi.org/10.1155/2007/46276

Perini, G., Cotta Ramusino, M., Sinforiani, E., Bernini, S., Petrachi, R., & Costa, A. (2019). Cognitive impairment in depression: Recent advances and novel treatments. *Neuropsychiatric Disease and Treatment*, *15*, 1249–1258. https://doi.org/10.2147/NDT.S199746

Pham, K., Nacher, J., Hof, P. R., & McEwen, B. S. (2003). Repeated restraint stress suppresses neurogenesis and induces biphasic PSA-NCAM expression in the adult rat dentate gyrus. *European Journal of Neuroscience*. https://doi.org/10.1046/j.1460-9568.2003.02513.x

Phan, A., Gabor, C. S., Favaro, K. J., Kaschack, S., Armstrong, J. N., MacLusky, N. J., & Choleris, E. (2012). Low doses of 17β-estradiol rapidly improve learning and increase hippocampal dendritic spines. *Neuropsychopharmacology*, *37*(10). https://doi.org/10.1038/npp.2012.82

Pitman, D. L., Ottenweller, J. E., & Natelson, B. H. (1988). Plasma corticosterone levels during repeated presentation of two intensities of restraint stress: Chronic stress and habituation. *Physiology and Behavior*, *43*(1), 47–55. https://doi.org/10.1016/0031-9384(88)90097-2

Pitsikas, N., Zisopoulou, S., Tarantilis, P. A., Kanakis, C. D., Polissiou, M. G., & Sakellaridis, N. (2007). Effects of the active constituents of Crocus sativus L., crocins on recognition and spatial rats' memory. *Behavioural Brain Research*, *183*(2), 141–146. https://doi.org/10.1016/j.bbr.2007.06.001

Planchez, B., Surget, A., & Belzung, C. (2019). Animal models of major depression: Drawbacks and challenges. *Journal of Neural Transmission*, *126*(11), 1383–1408. https://doi.org/10.1007/s00702-019-02084-y

Podgorny, O. V., & Gulyaeva, N. V. (2021). Glucocorticoid-mediated mechanisms of hippocampal damage: Contribution of subgranular neurogenesis. *Journal of Neurochemistry*, *157*(3). https://doi.org/10.1111/jnc.15265

Poling, A., Cleary, J., & Monaghan, M. (1981). Burying by rats in response to aversive and nonaversive stimuli. *Journal of the Experimental Analysis of Behavior*, *35*(1), 31–44. https://doi.org/10.1901/jeab.1981.35-31

Prakapenka, A. V., Hiroi, R., Quihuis, A. M., Carson, C., Patel, S., Berns-Leone, C., Fox, C., Sirianni, R. W., & Bimonte-Nelson, H. A. (2018). Contrasting effects of individual versus combined estrogen and progestogen regimens as working memory load increases in middle-aged ovariectomized rats: One plus one does not equal two. *Neurobiology of Aging*, *64*. https://doi.org/10.1016/j.neurobiolaging.2017.11.015

Price, M. E., & McCool, B. A. (2022). Structural, Functional, and Behavioral Significance of Sex and Gonadal Hormones in the Basolateral Amygdala: A Review of Preclinical Literature. *Alcohol (Fayetteville, N.Y.)*, *98*, 25–41. https://doi.org/10.1016/j.alcohol.2021.08.001

Prut, L., & Belzung, C. (2003). The open field as a paradigm to measure the effects of drugs on anxiety-like behaviors: A review. *European Journal of Pharmacology*, *463*(1–3), 3–33. https://doi.org/10.1016/S0014-2999(03)01272-X

Qin, D., Li, Z., Li, Z., Wang, L., Hu, Z., Lü, L., Wang, Z., Liu, Y., Yin, Y., Li, Z., & Hu, X. (2019). Chronic Glucocorticoid Exposure Induces Depression-Like Phenotype in Rhesus Macaque (Macaca Mulatta). *Frontiers in Neuroscience*, *13*. https://www.frontiersin.org/articles/10.3389/fnins.2019.00188

Quirk, G. J., Likhtik, E., Pelletier, J. G., & Paré, D. (2003). Stimulation of Medial Prefrontal Cortex Decreases the Responsiveness of Central Amygdala Output Neurons. *The Journal of Neuroscience*, *23*(25), 8800–8807. https://doi.org/10.1523/JNEUROSCI.23-25-08800.2003

Rachman, I. M., Unnerstall, J. R., Pfaff, D. W., & Cohen, R. S. (1998). Estrogen alters behavior and forebrain c-fos expression in ovariectomized rats subjected to the

forced swim test. *Proceedings of the National Academy of Sciences*, *95*(23), 13941–13946. https://doi.org/10.1073/pnas.95.23.13941

Radley, J. J., Rocher, A. B., Janssen, W. G. M., Hof, P. R., McEwen, B. S., & Morrison, J. H. (2005). Reversibility of apical dendritic retraction in the rat medial prefrontal cortex following repeated stress. *Experimental Neurology*, *196*(1). https://doi.org/10.1016/j.expneurol.2005.07.008

Rahman, M. M., Callaghan, C. K., Kerskens, C. M., Chattarji, S., & O'Mara, S. M. (2016). Early hippocampal volume loss as a marker of eventual memory deficits caused by repeated stress. *Scientific Reports*. https://doi.org/10.1038/srep29127

Renczés, E., Borbélyová, V., Steinhardt, M., Höpfner, T., Stehle, T., Ostatníková, D., & Celec, P. (2020). The Role of Estrogen in Anxiety-Like Behavior and Memory of Middle-Aged Female Rats. *Frontiers in Endocrinology*, *11*. https://www.frontiersin.org/articles/10.3389/fendo.2020.570560

Retana-Márquez, S., Bonilla-Jaime, H., Vázquez-Palacios, G., Domínguez-Salazar, E., Martínez-García, R., & Velázquez-Moctezuma, J. (2003). Body weight gain and diurnal differences of corticosterone changes in response to acute and chronic stress in rats. *Psychoneuroendocrinology*. https://doi.org/10.1016/S0306-4530(02)00017-3

Riaz, M. S., Bohlen, M. O., Gunter, B. W., Henry, Q., Stockmeier, C. A., & Paul, I. A. (2015). Attenuation of social interaction-associated ultrasonic vocalizations and spatial working memory performance in rats exposed to chronic unpredictable stress. *Physiology and Behavior*. https://doi.org/10.1016/j.physbeh.2015.09.005

Richard, J. E., López-Ferreras, L., Anderberg, R. H., Olandersson, K., & Skibicka, K. P. (2017). Estradiol is a critical regulator of food-reward behavior. *Psychoneuroendocrinology*, *78*, 193–202. https://doi.org/10.1016/j.psyneuen.2017.01.014

Richards, D. (2011). Prevalence and clinical course of depression: A review. *Clinical Psychology Review*, *31*(7). https://doi.org/10.1016/j.cpr.2011.07.004

Richardson, J. T. E. (2011). Eta squared and partial eta squared as measures of effect size in educational research. *Educational Research Review*, *6*(2), 135–147. https://doi.org/10.1016/j.edurev.2010.12.001

Roca, C. A., Schmidt, P. J., Altemus, M., Deuster, P., Danaceau, M. A., Putnam, K., & Rubinow, D. R. (2003). Differential menstrual cycle regulation of hypothalamic-pituitary-adrenal axis in women with premenstrual syndrome and controls. *Journal of Clinical Endocrinology and Metabolism*, *88*(7). https://doi.org/10.1210/jc.2002-021570

Rodgers, R. J., & Dalvi, A. (1997). Anxiety, defence and the elevated plus-maze. *Neuroscience and Biobehavioral Reviews*. https://doi.org/10.1016/S0149-7634(96)00058-9

Romano-Torres, M., & Fernández-Guasti, A. (2010). Estradiol valerate elicits antidepressant-like effects in middle-aged female rats under chronic mild stress. *Behavioural Pharmacology*, *21*(2), 104. https://doi.org/10.1097/FBP.0b013e328337bdfc

Roozendaal, B., McEwen, B. S., & Chattarji, S. (2009). Stress, memory and the amygdala. *Nature Reviews Neuroscience*, *10*(6), Article 6. https://doi.org/10.1038/nrn2651

Rubinow, D. R., Johnson, S. L., Schmidt, P. J., Girdler, S., & Gaynes, B. (2015). EFFICACY of ESTRADIOL in PERIMENOPAUSAL DEPRESSION: So MUCH PROMISE and so FEW ANSWERS. *Depression and Anxiety*, *32*(8). https://doi.org/10.1002/da.22391

Rubinow, D. R., Schmidt, P. J., & Roca, C. A. (1998). Estrogen-serotonin interactions: Implications for affective regulation. *Biological Psychiatry*, *44*(9). https://doi.org/10.1016/S0006-3223(98)00162-0

Rubinow, M. J., Drogos, L. L., & Juraska, J. M. (2009). Age-related dendritic hypertrophy and sexual dimorphism in rat basolateral amygdala. *Neurobiology of Aging*, *30*(1), 137–146. https://doi.org/10.1016/j.neurobiolaging.2007.05.006

Rubinow, M. J., Mahajan, G., May, W., Overholser, J. C., Jurjus, G. J., Dieter, L., Herbst, N., Steffens, D. C., Miguel-Hidalgo, J. J., Rajkowska, G., & Stockmeier, C. A. (2016). Basolateral amygdala volume and cell numbers in major depressive disorder: A postmortem stereological study. *Brain Structure and Function*, *221*(1). https://doi.org/10.1007/s00429-014-0900-z

Sachar, E. J., & Baron, M. (1979). The biology of affective disorders. *Annual Review of Neuroscience*, *2*. https://doi.org/10.1146/annurev.ne.02.030179.002445

Saleh, K., Carballedo, A., Lisiecka, D., Fagan, A. J., Connolly, G., Boyle, G., & Frodl, T. (2012). Impact of family history and depression on amygdala volume. *Psychiatry Research - Neuroimaging*, *203*(1). https://doi.org/10.1016/j.pscychresns.2011.10.004

SAMHSA. (2020). *Results from the 2020 National Survey on Drug Use and Health*. 2020 National Survey on Drug Use and Health. https://www.samhsa.gov/data/sites/default/files/reports/rpt35323/NSDUHDetailedTabs2020v25/NSDUHDetailedTabs2020v25/NSDUHDetTabsSect8pe2020.htm

Sánchez-Andrade, G., & Kendrick, K. M. (2011). Roles of α- and β-estrogen receptors in mouse social recognition memory: Effects of gender and the estrous cycle. *Hormones and Behavior*, *59*(1). https://doi.org/10.1016/j.yhbeh.2010.10.016

Sandi, C., Davies, H. A., Cordero, M. I., Rodriguez, J. J., Popov, V. I., & Stewart, M. G. (2003). Rapid reversal of stress induced loss of synapses in CA3 of rat hippocampus following water maze training. *European Journal of Neuroscience*. https://doi.org/10.1046/j.1460-9568.2003.02675.x

Sántha, P., Pákáski, M., Fodor, E. K., Fazekas, Ö. C., Kálmán, S., Jr, J. K., Janka, Z., Szabó, G., & Kálmán, J. (2013). Cytoskeletal Protein Translation and Expression in the Rat Brain Are Stressor-Dependent and Region-Specific. *PLOS ONE*, *8*(10), e73504. https://doi.org/10.1371/journal.pone.0073504

Santoro, N., Brown, J. R., Adel, T., & Skurnick, J. H. (1996). Characterization of reproductive hormonal dynamics in the perimenopause. *Journal of Clinical Endocrinology and Metabolism*, *81*(4). https://doi.org/10.1210/jc.81.4.1495

Sapolsky, R. M., Krey, L. C., & McEwen, B. S. (1983). The adrenocorticol stress-response in the aged male rat: Impairment of recovery from stress. *Experimental Gerontology*, *18*(1), 55–64. https://doi.org/10.1016/0531-5565(83)90051-7

Saré, R. M., Lemons, A., & Smith, C. B. (2021). Behavior Testing in Rodents: Highlighting Potential Confounds Affecting Variability and Reproducibility. *Brain Sciences*, *11*(4), Article 4. https://doi.org/10.3390/brainsci11040522

Schmidt, P. J., & Rubinow, D. R. (2009). Sex hormones and mood in the perimenopause. *Annals of the New York Academy of Sciences*, *1179*, 70–85. https://doi.org/10.1111/j.1749-6632.2009.04982.x

Schweinfurth, M. K., Neuenschwander, J., Engqvist, L., Schneeberger, K., Rentsch, A. K., Gygax, M., & Taborsky, M. (2017). Do female Norway rats form social bonds? *Behavioral Ecology and Sociobiology*, *71*(6), 98. https://doi.org/10.1007/s00265-017-2324-2

Seedat, S., Scott, K. M., Angermeyer, M. C., Berglund, P., Bromet, E. J., Brugha, T. S., Demyttenaere, K., de Girolamo, G., Haro, J. M., Jin, R., Karam, E. G., Kovess-Masfety, V., Levinson, D., Medina Mora, M. E., Ono, Y., Ormel, J., Pennell, B.-E., Posada-Villa, J., Sampson, N. A., … Kessler, R. C. (2009). Cross-National Associations Between Gender and Mental Disorders in the World Health Organization World Mental Health Surveys. *Archives of General Psychiatry*, *66*(7), 785–795. https://doi.org/10.1001/archgenpsychiatry.2009.36

Seewoo, B. J., Hennessy, L. A., Feindel, K. W., Etherington, S. J., Croarkin, P. E., & Rodger, J. (2020). Validation of Chronic Restraint Stress Model in Young Adult Rats for the Study of Depression Using Longitudinal Multimodal MR Imaging. *ENeuro*, *7*(4), ENEURO.0113-20.2020. https://doi.org/10.1523/ENEURO.0113-20.2020

Seibenhener, M. L., & Wooten, M. C. (2015). Use of the Open Field Maze to Measure Locomotor and Anxiety-like Behavior in Mice. *Journal of Visualized Experiments*, *96*. https://doi.org/10.3791/52434

Shansky, R. M., & Morrison, J. H. (2009). Stress-induced dendritic remodeling in the medial prefrontal cortex: Effects of circuit, hormones and rest. *Brain Research*. https://doi.org/10.1016/j.brainres.2009.03.062

Shansky, R. M., Rubinow, K., Brennan, A., & Arnsten, A. F. T. (2006). The effects of sex and hormonal status on restraint-stress-induced working memory impairment. *Behavioral and Brain Functions*. https://doi.org/10.1186/1744-9081-2-8

Sheldrick, A., Camara, S., Ilieva, M., Riederer, P., & Michel, T. M. (2017). Brain-derived neurotrophic factor (BDNF) and neurotrophin 3 (NT3) levels in post-mortem brain tissue from patients with depression compared to healthy individuals – a proof of concept study. *European Psychiatry*, *46*. https://doi.org/10.1016/j.eurpsy.2017.06.009

Sheline, Y. I., Gado, M. H., & Kraemer, H. C. (2003). Untreated depression and hippocampal volume loss. *American Journal of Psychiatry*, *160*(8). https://doi.org/10.1176/appi.ajp.160.8.1516

Sheline, Y. I., Liston, C., & McEwen, B. S. (2019). Parsing the Hippocampus in Depression: Chronic Stress, Hippocampal Volume, and Major Depressive Disorder. *Biological Psychiatry*, *85*(6), 436–438. https://doi.org/10.1016/j.biopsych.2019.01.011

Sholl, D. A. (1953). Dendritic organization in the neurons of the visual and motor cortices of the cat. *Journal of Anatomy*, *87*(Pt 4), 387-406.1.

Si, L., Xiao, L., Xie, Y., Xu, H., Yuan, G., Xu, W., & Wang, G. (2023). Social isolation after chronic unpredictable mild stress perpetuates depressive-like behaviors, memory deficits and social withdrawal via inhibiting ERK/KEAP1/NRF2 signaling. *Journal of Affective Disorders*, *324*, 576–588. https://doi.org/10.1016/j.jad.2022.12.092

Singh, M., Meyer, E. M., Millard, W. J., & Simpkins, J. W. (1994). Ovarian steroid deprivation results in a reversible learning impairment and compromised cholinergic function in female Sprague-Dawley rats. *Brain Research*, *644*(2). https://doi.org/10.1016/0006-8993(94)91694-2

Slattery, D. A., & Cryan, J. F. (2012). Using the rat forced swim test to assess antidepressant-like activity in rodents. *Nature Protocols*, *7*(6). https://doi.org/10.1038/nprot.2012.044

Slattery, D. A., & Cryan, J. F. (2014). The Ups and Downs of Modelling Mood Disorders in Rodents. *ILAR Journal*, *55*(2), 297–309. https://doi.org/10.1093/ilar/ilu026

Smith, A., Woodside, B., & Abizaid, A. (2022). Ghrelin and the Control of Energy Balance in Females. *Frontiers in Endocrinology*, *13*. https://www.frontiersin.org/articles/10.3389/fendo.2022.904754

Snihur, A. W. K., Hampson, E., & Cain, D. P. (2008). Estradiol and corticosterone independently impair spatial navigation in the Morris water maze in adult female rats. *Behavioural Brain Research*, *187*(1), 56–66. https://doi.org/10.1016/j.bbr.2007.08.023

Snyder, J. S., Glover, L. R., Sanzone, K. M., Kamhi, J. F., & Cameron, H. A. (2009). The effects of exercise and stress on the survival and maturation of adult-generated granule cells. *Hippocampus*. https://doi.org/10.1002/hipo.20552

Snyder, J. S., Soumier, A., Brewer, M., Pickel, J., & Cameron, H. A. (2011). Adult hippocampal neurogenesis buffers stress responses and depressive behaviour. *Nature*. https://doi.org/10.1038/nature10287

Soares, C. N. (2017). Depression and Menopause. *Psychiatric Clinics of North America*, *40*(2), 239–254. https://doi.org/10.1016/j.psc.2017.01.007

Sorge, R. E., Martin, L. J., Isbester, K. A., Sotocinal, S. G., Rosen, S., Tuttle, A. H., Wieskopf, J. S., Acland, E. L., Dokova, A., Kadoura, B., Leger, P., Mapplebeck, J. C. S., McPhail, M., Delaney, A., Wigerblad, G., Schumann, A. P., Quinn, T., Frasnelli, J., Svensson, C. I., … Mogil, J. S. (2014). Olfactory exposure to males, including men, causes stress and related analgesia in rodents. *Nature Methods*, *11*(6), 629–632. https://doi.org/10.1038/nmeth.2935

Souery, D., Amsterdam, J., De Montigny, C., Lecrubier, Y., Montgomery, S., Lipp, O., Racagni, G., Zohar, J., & Mendlewicz, J. (1999). Treatment resistant depression: Methodological overview and operational criteria. *European Neuropsychopharmacology*, *9*(1–2), 83–91. https://doi.org/10.1016/S0924-977X(98)00004-2

Sousa, N., Lukoyanov, N. V., Madeira, M. D., Almeida, O. F., & Paula-Barbosa, M. M. (2000a). Reorganization of the morphology of hippocampal neurites and synapses after stress-induced damage correlates with behavioral improvement. *Neuroscience*, *97*(2), 253–266.

Sousa, N., Lukoyanov, N. V., Madeira, M. D., Almeida, O. F. X., & Paula-Barbosa, M. M. (2000b). Reorganization of the morphology of hippocampal neurites and synapses after stress-induced damage correlates with behavioral improvement. *Neuroscience*, *97*(2), 253–266. https://doi.org/10.1016/S0306-4522(00)00050-6

Spanswick, S. C., & Sutherland, R. J. (2010). Object/context-specific memory deficits associated with loss of hippocampal granule cells after adrenalectomy in rats. *Learning & Memory (Cold Spring Harbor, N.Y.)*, *17*(5), 241–245. https://doi.org/10.1101/lm.1746710

Spiteri, T., & Ågmo, A. (2009). Ovarian hormones modulate social recognition in female rats. *Physiology and Behavior*, *98*(1–2). https://doi.org/10.1016/j.physbeh.2009.05.001

Spritzer, M. D., & Galea, L. A. M. (2007). Testosterone and dihydrotestosterone, but not estradiol, enhance survival of new hippocampal neurons in adult male rats. *Developmental Neurobiology*, *67*(10), 1321–1333. https://doi.org/10.1002/dneu.20457

Srivastava, D. P., Woolfrey, K. M., & Evans, P. D. (2013). Mechanisms underlying the interactions between rapid estrogenic and BDNF control of synaptic connectivity. *Neuroscience*, *239*. https://doi.org/10.1016/j.neuroscience.2012.12.004

Stamp, J. A., & Herbert, J. (1999). Multiple immediate-early gene expression during physiological and endocrine adaptation to repeated stress. *Neuroscience*, *94*(4), 1313–1322. https://doi.org/10.1016/S0306-4522(99)00368-1

Stanton, C. H., Holmes, A. J., Chang, S. W. C., & Joormann, J. (2019). From Stress to Anhedonia: Molecular Processes through Functional Circuits. *Trends in Neurosciences*, *42*(1), 23–42. https://doi.org/10.1016/j.tins.2018.09.008

Staufenbiel, S. M., Penninx, B. W. J. H., Spijker, A. T., Elzinga, B. M., & van Rossum, E. F. C. (2013). Hair cortisol, stress exposure, and mental health in humans: A systematic review. *Psychoneuroendocrinology*, *38*(8), 1220–1235. https://doi.org/10.1016/j.psyneuen.2012.11.015

Steffen, A., Nübel, J., Jacobi, F., Bätzing, J., & Holstiege, J. (2020). Mental and somatic comorbidity of depression: A comprehensive cross-sectional analysis of 202 diagnosis groups using German nationwide ambulatory claims data. *BMC Psychiatry*, *20*(1), 142. https://doi.org/10.1186/s12888-020-02546-8

Steimer, T. (2011). Animal models of anxiety disorders in rats and mice: Some conceptual issues. *Dialogues in Clinical Neuroscience*, *13*(4), 495–506. https://doi.org/10.31887/DCNS.2011.13.4/tsteimer

Stuart, S. A., & Robinson, E. S. J. (2015). Reducing the stress of drug administration: Implications for the 3Rs. *Scientific Reports*, *5*(1), Article 1. https://doi.org/10.1038/srep14288

Sunanda, Shankaranarayana Rao, B. S., & Raju, T. R. (2000). Chronic restraint stress impairs acquisition and retention of spatial memory task in rats. *Current Science*.

Tafet, G. E., & Nemeroff, C. B. (2016). The Links Between Stress and Depression: Psychoneuroendocrinological, Genetic, and Environmental Interactions. *The Journal of Neuropsychiatry and Clinical Neurosciences*, *28*(2), 77–88. https://doi.org/10.1176/appi.neuropsych.15030053

Talboom, J. S., Williams, B. J., Baxley, E. R., West, S. G., & Bimonte-Nelson, H. A. (2008). Higher levels of estradiol replacement correlate with better spatial memory in surgically menopausal young and middle-aged rats. *Neurobiology of Learning and Memory*, *90*(1). https://doi.org/10.1016/j.nlm.2008.04.002

Taliaz, D., Stall, N., Dar, D. E., & Zangen, A. (2010). Knockdown of brain-derived neurotrophic factor in specific brain sites precipitates behaviors associated with depression and reduces neurogenesis. *Molecular Psychiatry*, *15*(1). https://doi.org/10.1038/mp.2009.67

Tang, A. C., Nakazawa, M., Romeo, R. D., Reeb, B. C., Sisti, H., & McEwen, B. S. (2005). Effects of long-term estrogen replacement on social investigation and social memory in ovariectomized C57BL/6 mice. *Hormones and Behavior*, *47*(3). https://doi.org/10.1016/j.yhbeh.2004.10.010

Taxier, L. R., Gross, K. S., & Frick, K. M. (2020a). Oestradiol as a neuromodulator of learning and memory. *Nature Reviews Neuroscience*, *21*(10). https://doi.org/10.1038/s41583-020-0362-7

Taxier, L. R., Gross, K. S., & Frick, K. M. (2020b). Oestradiol as a neuromodulator of learning and memory. *Nature Reviews Neuroscience*, *21*(10), Article 10. https://doi.org/10.1038/s41583-020-0362-7

Teo, A. R., Nelson, S., Strange, W., Kubo, H., Katsuki, R., Kurahara, K., Kanba, S., & Kato, T. A. (2020). Social withdrawal in major depressive disorder: A case-control study of hikikomori in japan. *Journal of Affective Disorders*, *274*, 1142–1146. https://doi.org/10.1016/j.jad.2020.06.011

Ter Horst, G. J., Wichmann, R., Gerrits, M., Westenbroek, C., & Lin, Y. (2009). Sex differences in stress responses: Focus on ovarian hormones. *Physiology and Behavior*, *97*(2). https://doi.org/10.1016/j.physbeh.2009.02.036

Thase, M., Weisler, R., Manning, J., & Trivedi, M. (2017). Utilizing the DSM-5 Anxious Distress Specifier to Develop Treatment Strategies for Patients With Major Depressive Disorder: (Academic Highlights). *The Journal of Clinical Psychiatry*, *78*. https://doi.org/10.4088/JCP.ot17015ah1

Thomas, A., Burant, A., Bui, N., Graham, D., Yuva-Paylor, L. A., & Paylor, R. (2009). Marble burying reflects a repetitive and perseverative behavior more than novelty-induced anxiety. *Psychopharmacology*, *204*(2), 361–373. https://doi.org/10.1007/s00213-009-1466-y

Topic, B., Dere, E., Schulz, D., de Souza Silva, M. A., Jocham, G., Kart, E., & Huston, J. P. (2005). Aged and adult rats compared in acquisition and extinction of escape from the water maze: Focus on individual differences. *Behavioral Neuroscience*, *119*(1), 127–144. https://doi.org/10.1037/0735-7044.119.1.127

Trunnell, E. R., & Carvalho, C. (2021). The forced swim test has poor accuracy for identifying novel antidepressants. *Drug Discovery Today*, *26*(12), 2898–2904. https://doi.org/10.1016/j.drudis.2021.08.003

University of Texas Health Science Center at Houston, & Jalnapurkar, I. (2018). Sex Differences in Anxiety Disorders: A Review. *Psychiatry, Depression & Anxiety*, *4*, 1–9. https://doi.org/10.24966/PDA-0150/100011

van Eijndhoven, P., van Wingen, G., van Oijen, K., Rijpkema, M., Goraj, B., Jan Verkes, R., Oude Voshaar, R., Fernández, G., Buitelaar, J., & Tendolkar, I. (2009). Amygdala Volume Marks the Acute State in the Early Course of Depression. *Biological Psychiatry*, *65*(9). https://doi.org/10.1016/j.biopsych.2008.10.027

van Gaalen, M. M., & Steckler, T. (2000). Behavioural analysis of four mouse strains in an anxiety test battery. *Behavioural Brain Research*, *115*(1), 95–106. https://doi.org/10.1016/S0166-4328(00)00240-0

Van Londen, L., Goekoop, J. G., Zwinderman, A. H., Lanser, J. B. K., Wiegant, V. M., & De Wied, D. (1998). Neuropsychological performance and plasma cortisol, arginine vasopressin and oxytocin in patients with major depression. *Psychological Medicine*, *28*(2). https://doi.org/10.1017/S0033291797006284

Vaucher, E., Reymond, I., Najaffe, R., Kar, S., Quirion, R., Miller, M. M., & Franklin, K. B. J. (2002). Estrogen effects on object memory and cholinergic receptors in

young and old female mice. *Neurobiology of Aging*, *23*(1). https://doi.org/10.1016/S0197-4580(01)00250-0

Vázquez-Pereyra, F., Rivas-Arancibia, S., Loaeza-Del Castillo, A., & Schneider-Rivas, S. (1995). Modulation of short term and long term memory by steroid sexual hormones. *Life Sciences*, *56*(14), PL255-260. https://doi.org/10.1016/0024-3205(95)00067-g

Vega-Rivera, N. M., López-Rubalcava, C., & Estrada-Camarena, E. (2013). The antidepressant-like effect of ethynyl estradiol is mediated by both serotonergic and noradrenergic systems in the forced swimming test. *Neuroscience*, *250*, 102–111. https://doi.org/10.1016/j.neuroscience.2013.06.058

Vetter-O'Hagen, C. S., & Spear, L. P. (2012). The effects of gonadectomy on sex- and age-typical responses to novelty and ethanol-induced social inhibition in adult male and female Sprague-Dawley rats. *Behavioural Brain Research*, *227*(1), 224–232. https://doi.org/10.1016/j.bbr.2011.10.023

Viau, V., & Meaney, M. J. (1991). Variations in the hypothalamic-pituitary-adrenal response to stress during the estrous cycle in the rat. *Endocrinology*, *129*(5), 2503–2511. https://doi.org/10.1210/endo-129-5-2503

Viau, V., & Sawchenko, P. E. (2002). Hypophysiotropic neurons of the paraventricular nucleus respond in spatially, temporally, and phenotypically differentiated manners to acute vs. Repeated restraint stress. *Journal of Comparative Neurology*. https://doi.org/10.1002/cne.10178

Villarroel, M. A., & Terlizzi, E. P. (2020). Symptoms of Depression Among Adults: United States, 2019. *US Department of Health and Human Services, Centers for Disease Control and Prevention, National Center for Health Statistics*, *379*, 1–8.

Võikar, V., Vasar, E., & Rauvala, H. (2004). Behavioral alterations induced by repeated testing in C57BL/6J and 129S2/ Sv mice: Implications for phenotyping screens. *Genes, Brain and Behavior*, *3*(1), 27–38. https://doi.org/10.1046/j.1601-183X.2003.0044.x

Vyas, A., Jadhav, S., & Chattarji, S. (2006). Prolonged behavioral stress enhances synaptic connectivity in the basolateral amygdala. *Neuroscience*, *143*(2). https://doi.org/10.1016/j.neuroscience.2006.08.003

Vyas, A., Mitra, R., Shankaranarayana Rao, B. S., & Chattarji, S. (2002). Chronic stress induces contrasting patterns of dendritic remodeling in hippocampal and amygdaloid neurons. *Journal of Neuroscience*, *22*(15). https://doi.org/10.1523/jneurosci.22-15-06810.2002

Vyas, A., Pillai, A. G., & Chattarji, S. (2004). Recovery after chronic stress fails to reverse amygdaloid neuronal hypertrophy and enhanced anxiety-like behavior. *Neuroscience*, *128*(4), 667–673. https://doi.org/10.1016/j.neuroscience.2004.07.013

Walf, A. A., & Frye, C. A. (2005). Antianxiety and antidepressive behavior produced by physiological estradiol regimen may be modulated by hypothalamic-pituitary-adrenal axis activity. *Neuropsychopharmacology*, *30*(7). https://doi.org/10.1038/sj.npp.1300708

Walf, A. A., & Frye, C. A. (2006). A review and update of mechanisms of estrogen in the hippocampus and amygdala for anxiety and depression behavior. *Neuropsychopharmacology*, *31*(6). https://doi.org/10.1038/sj.npp.1301067

Walsh, R. N., & Cummins, R. A. (1976). The open-field test: A critical review. *Psychological Bulletin*, *83*(3), 482–504. https://doi.org/10.1037/0033-2909.83.3.482

Watanabe, Y., Gould, E., & McEwen, B. S. (1992). Stress induces atrophy of apical dendrites of hippocampal CA3 pyramidal neurons. *Brain Research*, *588*(2), 341–345. https://doi.org/10.1016/0006-8993(92)91597-8

Waters, P., & McCormick, C. M. (2011). Caveats of chronic exogenous corticosterone treatments in adolescent rats and effects on anxiety-like and depressive behavior and hypothalamic-pituitary-adrenal (HPA) axis function. *Biology of Mood & Anxiety Disorders*, *1*(1), 4. https://doi.org/10.1186/2045-5380-1-4

Wei, J., Yuen, E. Y., Liu, W., Li, X., Zhong, P., Karatsoreos, I. N., McEwen, B. S., & Yan, Z. (2014). Estrogen protects against the detrimental effects of repeated stress on glutamatergic transmission and cognition. *Molecular Psychiatry*. https://doi.org/10.1038/mp.2013.83

Weiser, M. J., & Handa, R. J. (2009). Estrogen impairs glucocorticoid dependent negative feedback on the hypothalamic-pituitary-adrenal axis via estrogen

receptor alpha within the hypothalamus. *Neuroscience*, *159*(2). https://doi.org/10.1016/j.neuroscience.2008.12.058

Weissman, M. M., Bland, R., Joyce, P. R., Newman, S., Wells, J. E., & Wittchen, H. U. (1993). Sex differences in rates of depression: Cross-national perspectives. *Journal of Affective Disorders*, *29*(2–3), 77–84.

Wellman, C. L., Bollinger, J. L., & Moench, K. M. (2020). Chapter Six - Effects of stress on the structure and function of the medial prefrontal cortex: Insights from animal models. In A. Clow & N. Smyth (Eds.), *International Review of Neurobiology* (Vol. 150, pp. 129–153). Academic Press. https://doi.org/10.1016/bs.irn.2019.11.007

Wellman, C. L., & Moench, K. M. (2019). Preclinical studies of stress, extinction, and prefrontal cortex: Intriguing leads and pressing questions. *Psychopharmacology*, *236*(1), 59–72. https://doi.org/10.1007/s00213-018-5023-4

WHO. (2017). *Depression and other common mental disorders: Global health estimates*.

Willi, J., & Ehlert, U. (2019). Assessment of perimenopausal depression: A review. *Journal of Affective Disorders*, *249*, 216–222. https://doi.org/10.1016/j.jad.2019.02.029

Willner, P. (1991). Animal models as simulations of depression. *Trends in Pharmacological Sciences*. https://doi.org/10.1016/0165-6147(91)90529-2

Willner, P. (2005). Chronic mild stress (CMS) revisited: Consistency and behavioural-neurobiological concordance in the effects of CMS. *Neuropsychobiology*, *52*(2). https://doi.org/10.1159/000087097

Willner, P., & Mitchell, P. J. (2002). The validity of animal models of predisposition to depression. *Behavioural Pharmacology*. https://doi.org/10.1097/00008877-200205000-00001

Willner, P., Muscat, R., & Papp, M. (1992). Chronic mild stress-induced anhedonia: A realistic animal model of depression. *Neuroscience and Biobehavioral Reviews*, *16*(4). https://doi.org/10.1016/S0149-7634(05)80194-0

Willner, P., Towell, A., Sampson, D., Sophokleous, S., & Muscat, R. (1987). Reduction of sucrose preference by chronic unpredictable mild stress, and its restoration by a

tricyclic antidepressant. *Psychopharmacology*. https://doi.org/10.1007/BF00187257

Wilson, I. A., Ikonen, S., Gureviciene, I., McMahan, R. W., Gallagher, M., Eichenbaum, H., & Tanila, H. (2004). Cognitive Aging and the Hippocampus: How Old Rats Represent New Environments. *The Journal of Neuroscience*, *24*(15), 3870–3878. https://doi.org/10.1523/JNEUROSCI.5205-03.2004

Winocur, G., & Gilbert, M. (1984). The hippocampus, context, and information processing. *Behavioral and Neural Biology*. https://doi.org/10.1016/S0163-1047(84)90146-8

Winocur, G., & Mills, J. A. (1970). Transfer between related and unrelated problems following hippocampal lesions in rats. *Journal of Comparative and Physiological Psychology*. https://doi.org/10.1037/h0030006

Winocur, G., & Salzen, E. A. (1968). HIPPOCAMPAL LESIONS AND TRANSFER BEHAVIOR IN THE RAT. *Journal of Comparative and Physiological Psychology*. https://doi.org/10.1037/h0025535

Wirt, R. A., & Hyman, J. M. (2017). Integrating spatial working memory and remote memory: Interactions between the medial prefrontal cortex and hippocampus. *Brain Sciences*, *7*(4). https://doi.org/10.3390/brainsci7040043

Wong, E. Y. H., & Herbert, J. (2006). Raised circulating corticosterone inhibits neuronal differentiation of progenitor cells in the adult hippocampus. *Neuroscience*, *137*(1). https://doi.org/10.1016/j.neuroscience.2005.08.073

World Health Organization. (1996). *Research on the menopause in the 1990s: Report of a WHO scientific group*.

Worley, N., Djerdjaj, A., & Christianson, J. (2019). *DeepLabCut Analysis of Social Novelty Preference*. https://doi.org/10.1101/736983

Wright, R. L., & Conrad, C. D. (2005). Chronic stress leaves novelty-seeking behavior intact while impairing spatial recognition memory in the Y-maze. *Stress*, *8*(2), 151–154. https://doi.org/10.1080/10253890500156663

Wright, R. L., & Conrad, C. D. (2008). Enriched environment prevents chronic stress-induced spatial learning and memory deficits. *Behavioural Brain Research*, *187*(1), 41–47. https://doi.org/10.1016/j.bbr.2007.08.025

Wright, R. L., Lightner, E. N., Harman, J. S., Meijer, O. C., & Conrad, C. D. (2006). Attenuating corticosterone levels on the day of memory assessment prevents chronic stress-induced impairments in spatial memory. *European Journal of Neuroscience*, *24*(2), 595–605. https://doi.org/10.1111/j.1460-9568.2006.04948.x

Wu, R., Jiang, X., Wu, X., Pang, J., Tang, Y., Ren, Z., Yang, F., Yang, S., & Wei, W. (2022). Interspecific differences in sociability, social novelty preference, anxiety- and depression-like behaviors between Brandt's voles and C57BL/6J mice. *Behavioural Processes*, *197*, 104624. https://doi.org/10.1016/j.beproc.2022.104624

Xie, X., Shen, Q., Ma, L., Chen, Y., Zhao, B., & Fu, Z. (2018). Chronic corticosterone-induced depression mediates premature aging in rats. *Journal of Affective Disorders*, *229*. https://doi.org/10.1016/j.jad.2017.12.073

Xu, P., Wang, K., Lu, C., Dong, L., Chen, Y., Wang, Q., Shi, Z., Yang, Y., Chen, S., & Liu, X. (2017). Effects of the chronic restraint stress induced depression on reward-related learning in rats. *Behavioural Brain Research*. https://doi.org/10.1016/j.bbr.2016.12.045

Yagi, S., & Galea, L. A. M. (2019). Sex differences in hippocampal cognition and neurogenesis. *Neuropsychopharmacology*, *44*(1), Article 1. https://doi.org/10.1038/s41386-018-0208-4

Yang, Y., & Wang, J.-Z. (2017). From Structure to Behavior in Basolateral Amygdala-Hippocampus Circuits. *Frontiers in Neural Circuits*, *11*. https://www.frontiersin.org/articles/10.3389/fncir.2017.00086

Youssef, M. M., Underwood, M. D., Huang, Y. Y., Hsiung, S. chi, Liu, Y., Simpson, N. R., Bakalian, M. J., Rosoklija, G. B., Dwork, A. J., Arango, V., & John Mann, J. (2018). Association of BDNF Val66MET polymorphism and brain BDNF levels with major depression and suicide. *International Journal of Neuropsychopharmacology*, *21*(6). https://doi.org/10.1093/ijnp/pyy008

Yuen, E. Y., Wei, J., & Yan, Z. (2016). Estrogen in prefrontal cortex blocks stress-induced cognitive impairments in female rats. *Journal of Steroid Biochemistry and Molecular Biology*. https://doi.org/10.1016/j.jsbmb.2015.08.028

Zhang, W., Hetzel, A., Shah, B., Atchley, D., Blume, S. R., Padival, M. A., & Rosenkranz, J. A. (2014). Greater Physiological and Behavioral Effects of Interrupted Stress Pattern Compared to Daily Restraint Stress in Rats. *PLoS ONE*, *9*(7). https://doi.org/10.1371/journal.pone.0102247

Zhang, X., Ge, T. tong, Yin, G., Cui, R., Zhao, G., & Yang, W. (2018). Stress-Induced Functional Alterations in Amygdala: Implications for Neuropsychiatric Diseases. *Frontiers in Neuroscience*, *12*. https://www.frontiersin.org/articles/10.3389/fnins.2018.00367

Zhao, Y., Ma, R., Shen, J., Su, H., Xing, D., & Du, L. (2008). A mouse model of depression induced by repeated corticosterone injections. *European Journal of Pharmacology*, *581*(1), 113–120. https://doi.org/10.1016/j.ejphar.2007.12.005

Zhao, Z., Fan, L., & Frick, K. M. (2010). Epigenetic alterations regulate estradiol-induced enhancement of memory consolidation. *Proceedings of the National Academy of Sciences of the United States of America*, *107*(12). https://doi.org/10.1073/pnas.0910578107

Zobel, A. W., Nickel, T., Sonntag, A., Uhr, M., Holsboer, F., & Ising, M. (2001). Cortisol response in the combined dexamethasone/CRH test as predictor of relapse in patients with remitted depression: A prospective study. *Journal of Psychiatric Research*, *35*(2). https://doi.org/10.1016/S0022-3956(01)00013-9

APPENDIX A

DISSERTATION FIGURES

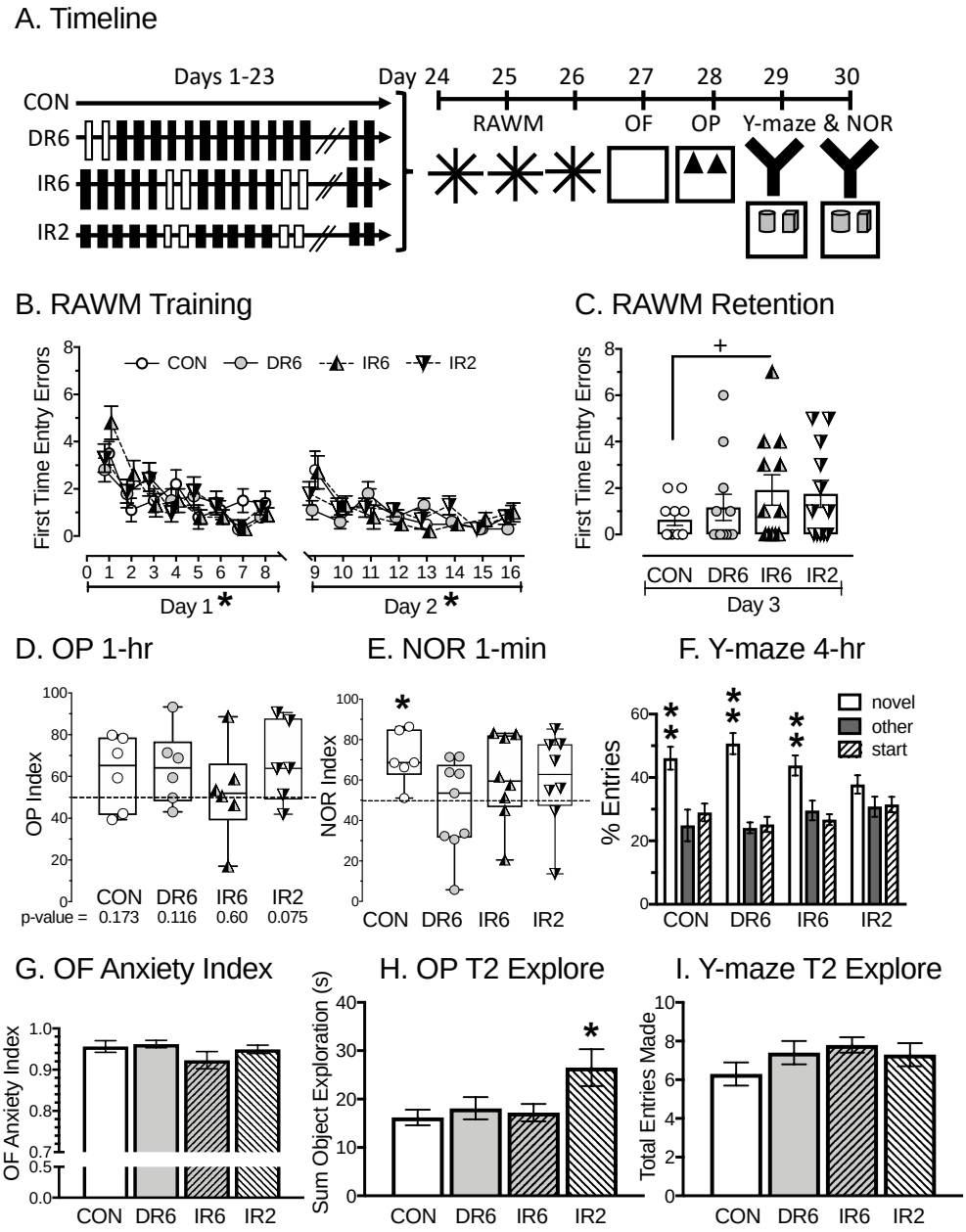

Fig. 2.1: Effect of two different IR durations on spatial ability and anxiety profiles using male rats. A) Timeline of manipulations. Rats were restrained for 6hrs (long black-filled rectangles) or 2hrs (short black-filled rectangles). White-filled rectangles indicate days off from restraint. CON = control, DR6 = daily restraint for 6-hrs, IR6 = intermittent restraint for 6-hrs., IR2 = intermittent restraint for 2-hrs. The day after restraint ended, rats were tested on the RAWM for 3 days, followed by the OF, OP and then the Y-maze

and NOR. B) First time entry errors on the RAWM during training on days 1 and 2. All groups acquired the task by decreasing first time entry errors over days. There were no group differences. C) Single retention trial on RAWM. IR6 made more first-time entry errors than did CON. D) OP Index from the second trial after a 1-hr ITI. Preference for the moved object will show values greater than 0.5 with the dashed horizontal line indicating chance levels. All groups performed at chance, with p-values listed below each group name; however, half the rats failed to explore, reducing the power of the analyses. E) NOR Index from the second trial with a 1-min ITI, which reflects minimum cognitive load. Preference for the new object will show values greater than 0.5 with the dashed horizontal line indicating chance levels. CON spent more time with the new object than the familiar object despite a low subject number (n=6). The remaining groups performed at chance levels. F) Y-maze performance showing the %Entry in each of the arms (novel, start, other) in trial 2 after a 4-hr ITI. Six to seven days after IR ended, CON, DR6 and IR6 entered (and spent more time in) the novel arm than the other arm (dwell data are not shown). IR2 performed at chance levels. Two asterisks indicate significance in both entry and dwell measures. G) OF anxiety index. All groups showed statistically similar and high anxiety profiles. H) OP total time exploring both objects during trial 2. IR2 spent more time exploring both objects in trial 2 than did CON, DR6, and IR6. There were no other group differences. I) Entries made in all three arms of the Y-maze during Trial 2. All groups made similar number of entries. *$p < 0.05$, +$p < 0.05$ with covariate, CON = control, DR6 = daily restraint for 6hrs., IR6 = intermittent restraint for 6hrs, IR2 = intermittent restraint for 2hrs. Boxes represent median and inter-quartile ranges. All other data points are mean ± S.E.M.

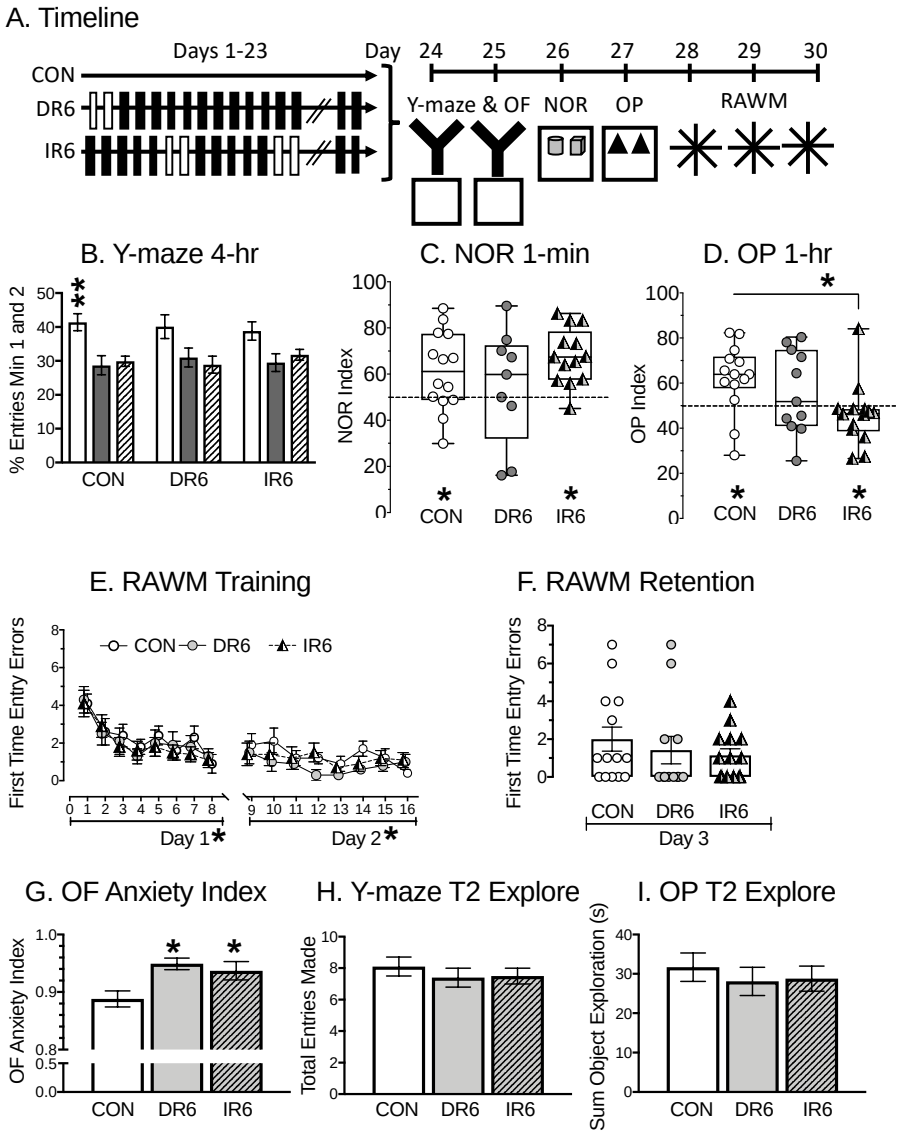

Fig. 2.2: Effects of three weeks IR6 on a behavioral battery, ordered with the least aversive task first and using male rats A) Timeline of manipulations. Solid black rectangles indicate restraint day and white-filled rectangles indicate when rats were given a day off. CON = control, DR6 = daily restraint for 6hrs., IR6 = intermittent restraint for 6hrs. The day after restraint ended, rats were tested on the Y-maze and OF, followed by NOR, OP and then the RAWM (last 3 days). B) Y-maze performance showing the %Entry in each of the arms (novel, start, other) in trial 2 after a 4-hr ITI. CON entered (and spent more time in) the novel arm than the other arm, whereas DR6 and IR6 performed at chance levels. Two asterisks indicate significance for each assessment in

entry and dwell (dwell data are not shown). C) NOR Index from trial 2 with a 1-min ITI when cognitive load should be minimal. Preference for the new object will show values greater than 0.5 with the dashed horizontal line indicating chance levels. CON and IR6 spent more time with the new object than the familiar object when cognitive load was minimal (1-min ITI). DR6 performed at chance levels. D) OP Index from the second trial after a 1-hr ITI. Preference for the moved object will show values greater than 0.5 with the dashed horizontal line indicating chance levels. CON preferred the object in the novel location. DR6 performed at chance while IR6 preferred the object in the familiar location. IR6 significantly differed from CON in OP index. E) First time entry errors on the RAWM during training on days 1 and 2. All groups acquired the task by decreasing first time entry errors over days. There were no group differences. F) Single retention trial on RAWM. There were no group differences. G) OF anxiety index. DR6 and IR6 showed greater anxiety profiles compared to CON. H) Entries made in all three arms of the Y-maze during Trial 2. All groups made similar number of entries. I) OP total object exploration time. All groups spent similar time exploring both objects. *$p < 0.05$, CON = control, DR6 = daily restraint for 6hrs., IR6 = intermittent restraint for 6hrs.

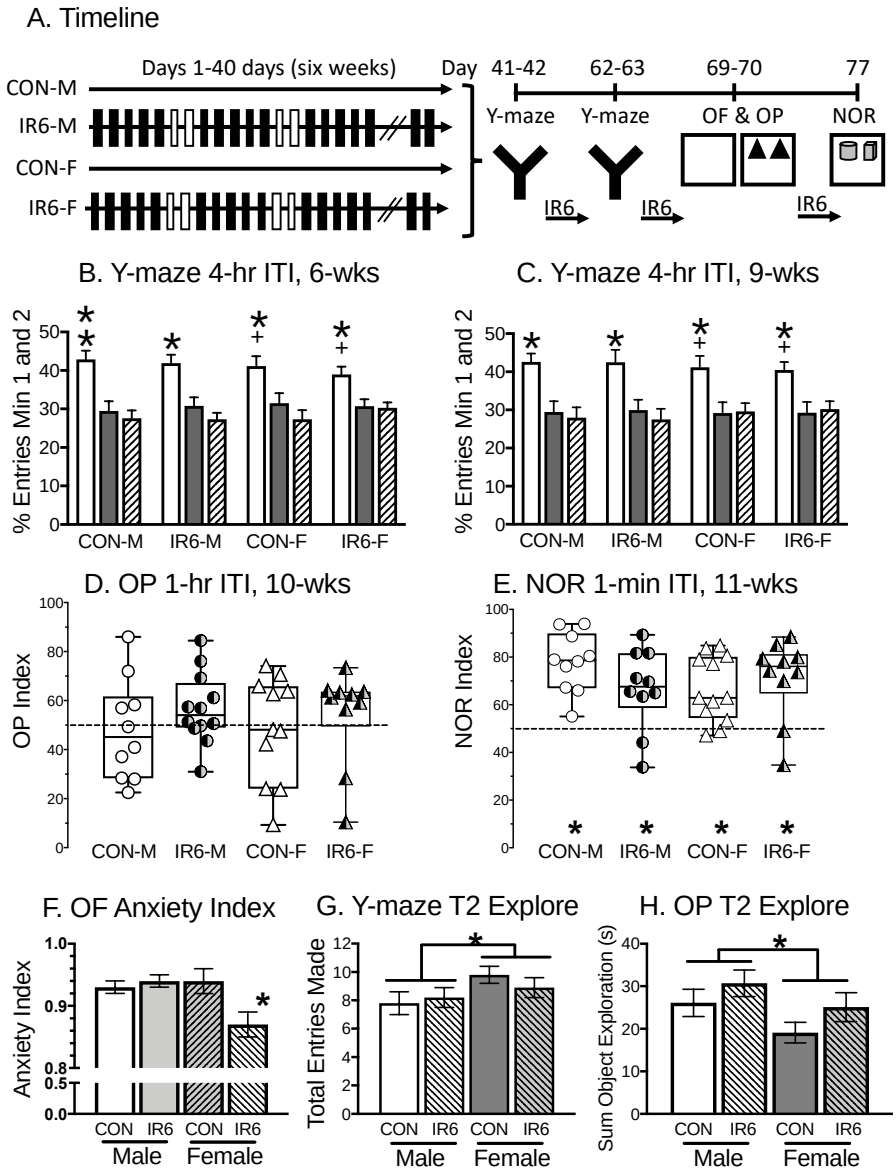

Fig. 2.3: Effects of an extended IR6 paradigm on spatial memory in male and female rats. A) Timeline of manipulations. Black-filled rectangles indicate restraint was performed and white-filled rectangles indicate when rats were given a day off. After 6-weeks of restraint, rats were tested on the Y-maze and then returned to the restraint paradigm for an additional 3-weeks. Behavioral testing then occurred weekly on days without restraint, starting with the Y-maze, followed by OF/OP (consecutive days) and ending with NOR. B) Y-maze performance on trial 2 after 6-weeks of IR for entries in the first two minutes. CON-M entered (and spent more time in, dwell data not shown) the novel arm than the

other arm. IR6-M, CON-F and IR6-F entered the novel arm more than the other arm. Two symbols indicate significance or tendency toward significance in both entry and dwell measures with entry measure listed first, followed by dwell. C) Y-maze performance on trial 2 after 9-weeks of IR for entries in the first two minutes. CON-M, IR6-M and CON-F entered the novel arm more than the other arm. IR6-F showed a tendency to enter the novel arm more than the other arm. Two symbols indicate significance or a tendency toward significance in both entry and dwell measures with entry measure listed first, followed by dwell (dwell data are not shown). D) OP performance after 10 weeks of IR. The OP Index represents data from the second trial after a 1-hr ITI. Preference for the moved object will show values greater than 0.5 with the dashed horizontal line indicating chance levels. All groups performed at chance. E) NOR performance after 11 weeks of IR. The OP Index represents data from the second trial with a 1-min ITI to reflect a low cognitive load. Preference for the new object will show values greater than 0.5 with the dashed line indicating chance levels. All groups spent more time with the new object than the familiar object to show that they would seek the novel object when cognitive demand was low. G) OF anxiety index used from the same arena that rats were given acclimation before OP and NOR. IR6-F had a significantly lower anxiety profile compared to CON-F, CON-M, and IR6-M, but this did not seem to change performance on OP or NOR. H) Assessment of the total number of entries made in all three arms of the Y-maze as a measure of motivation. Data were taken from the second Y-maze assessment after 9 weeks of IR and reflect activity during the first two minutes from trial 2. Female rats made more total entries compared to male rats, but no other group differences were found. I) The total time spent exploring both objects in OP during trial 2 after 10 weeks of IR. Male rats spent significantly more time exploring both objects compared to female rats, but this fails to explain why none of the groups showed object placement recognition. *$p < 0.05$, + $p < 0.1$, CON-M = control males, IR6-M = intermittent restraint for 6hrs. males, CON-F = control females, IR6-F = intermittent restraint for 6hrs females.

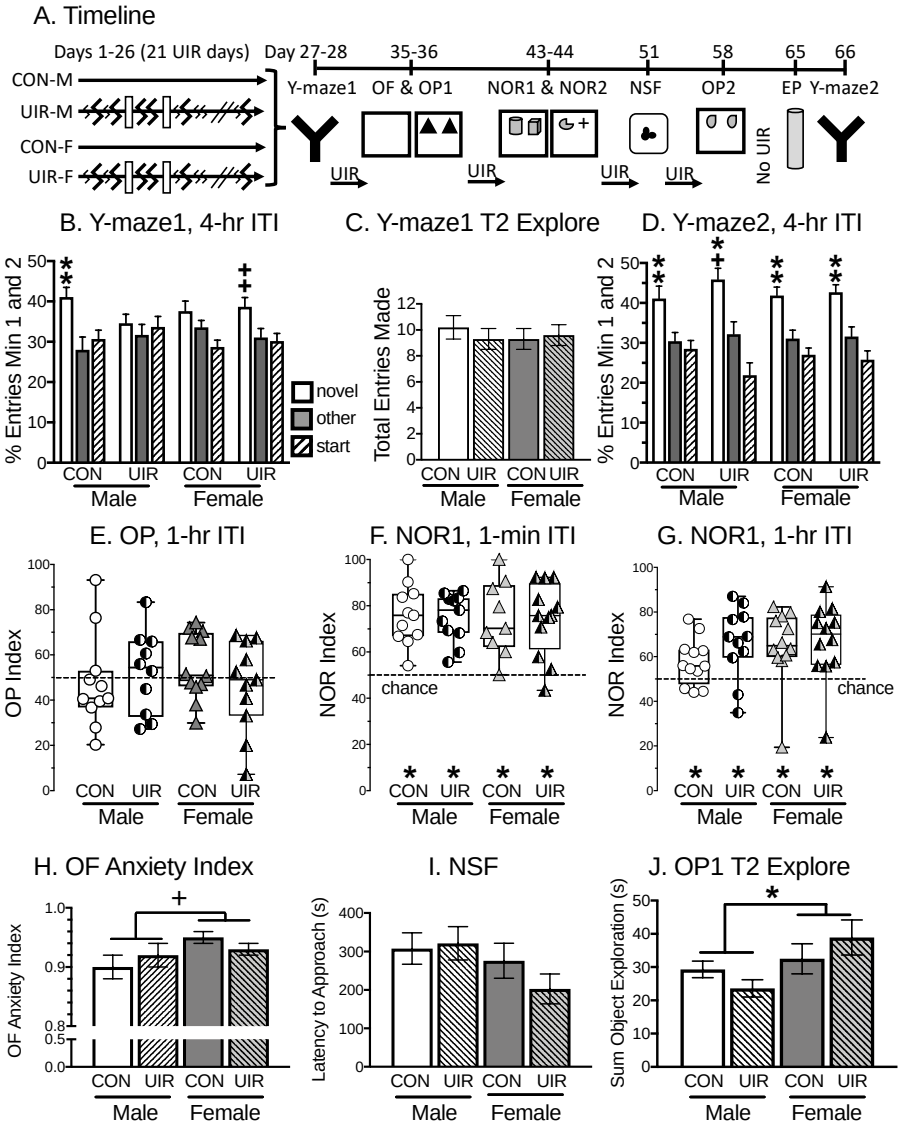

Fig. 2.4: Effects of a novel UIR paradigm on spatial ability and anxiety profiles in both male and female rats. A) Timeline of manipulations. The stressor was 30-min (small zigzag) or 60-min (large zigzag) restraint with gentle shaking. White-filled rectangles indicate when rats were given a day off. After 28 days of UIR (which was 21 days of actual restraint), rats were tested on the Y-maze and then returned to the UIR paradigm as indicated. At the end of UIR, rats were given seven days before being placed on the EP for 30-min and then tested one day later on the Y-maze. B) Y-maze1 performance on trial 2 after 26 days of UIR for entries in the first two minutes after a 4-hr ITI. CON entered (and spent more time in, data not shown) the novel arm than the other arm. UIR-F tended

to enter (and spend more time in, data not shown) the novel arm than the other arm. UIR-M and CON-F performed at chance levels. Two symbols indicate significance or tendency toward significance in both entry and dwell measures. C) Entries made in all three arms of the Y-maze1 during Trial 2 for the first two minutes. The groups made a similar number of total entries during trial 2 of the Y-maze to demonstrate similar motivation to explore. D) Y-maze2 performance following seven days after the end of UIR and one day after EP (post-EP) for entries made in minutes 1 and 2 during trial 2 after a 4-hr ITI. CON-M, UIR-M, CON-F and UIR-F entered (and spent more time in, data not shown) the novel arm more than the other arm. Chance is denoted by the dashed horizontal line. Two symbols indicate significance (or tendency toward significance) in both entry and dwell measures. E) OP1 performance after 36 days of UIR. The OP Index reflects time spent with the moved object compared to total time spent with both objects in the second trial after a 1-hr ITI. Preference for the moved object will show values greater than 0.5 with the dashed horizontal line indicating chance levels. All groups performed at chance. F) NOR performance after 43 days of UIR. The NOR Index reflects time spent with the novel object compared to total time with both objects in the second trial with a 1-min ITI. Preference for the new object will show values greater than 0.5 with the dashed horizontal line indicating chance levels. All groups spent more time with the new object than the familiar object to demonstrate that they were able to recognize the new object when cognitive load was minimal. G) NOR performance after 44 days of UIR, with the NOR Index from the second trial with a 1-hr ITI. All groups spent more time with the new object than the familiar object even with a longer delay than the day before. H) OF anxiety index. Female rats tended to have an elevated anxiety profile compared to male rats, but this does not explain the poor performance on OP for all groups. I) NSF latency to feed. The time it took for rats to approach the chow was similar across groups. J) The total time spent exploring both objects in OP1 during trial 2 after 36 days of UIR. Female rats spent significantly more time exploring both objects compared to male rats. There were no other group differences. *$p < 0.05$, +$p < 0.1$, CON-M = control males, UIR-M = unpredictable intermittent restraint males, CON-F = control females, UIR-F = unpredictable intermittent restraint females.

Chapter 3 Figures

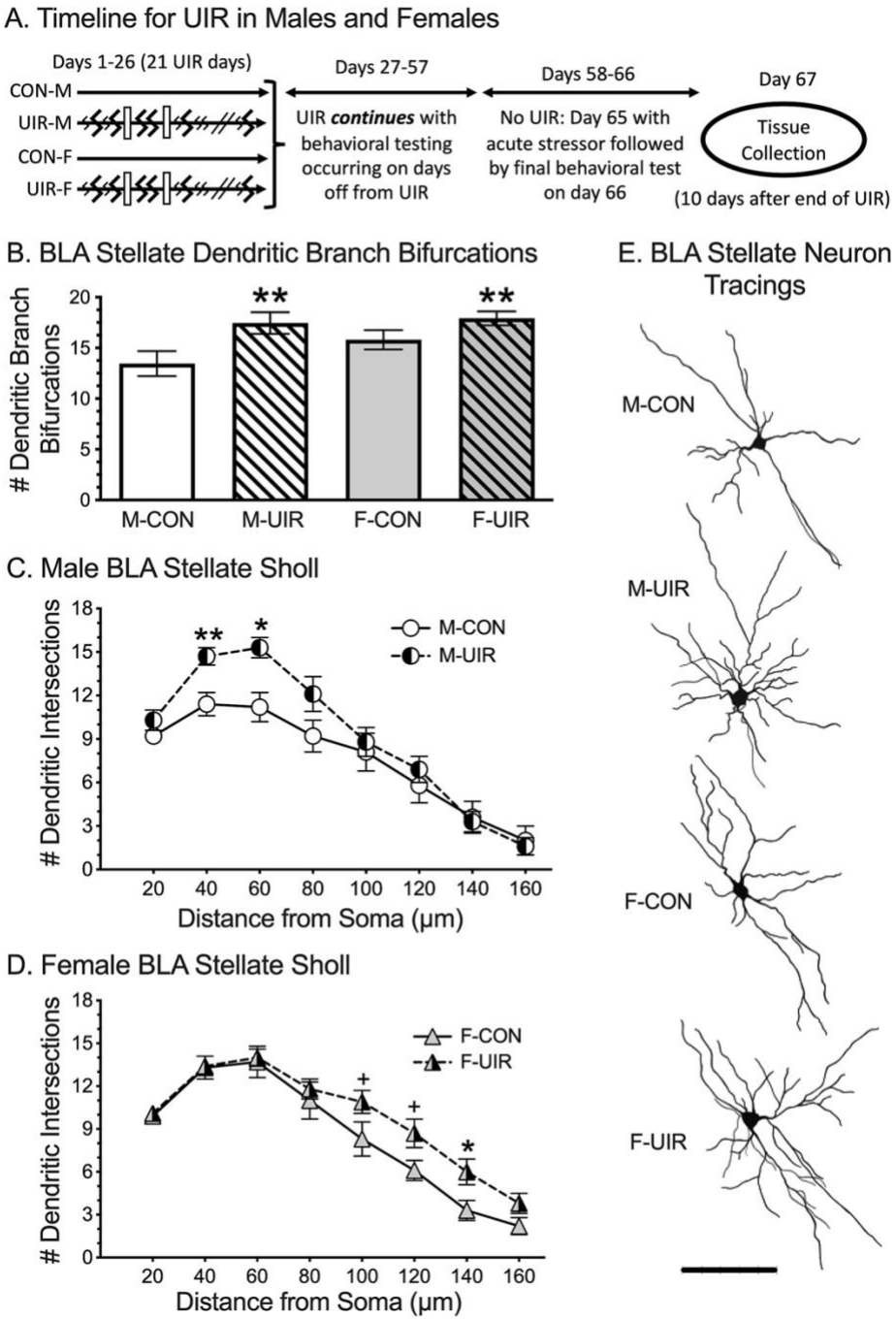

Figure 3.1: UIR Effects on BLA Stellate Dendrites in Male and Female Rats. A) Timeline with brains collected 10-days following the end of UIR (see (Peay et al., 2020)

for behavioral tasks). B) UIR enhanced male and female stellate dendritic branch numbers. C) In males, UIR enhanced stellate dendritic complexity at distances proximal from the soma. D) In females, UIR enhanced BLA stellate dendritic complexity at distances distal from the soma. E) Examples of BLA stellate neuronal tracings. **$p < 0.01$, *$p < 0.05$, +$p < 0.10$. M=Male, F=Female, CON=Control, UIR=Unpredictable Intermittent Restraint. Data are represented as mean ± S.E.M. M-CON: 7 rats, 2.6 neurons/rat. M-UIR: 6 rats 2.3 neurons/rat. F-CON: 5 rats 3.2 neurons/rat. F-UIR: 6 rats 2.3 neurons/rat. Scale bar = 125µm.

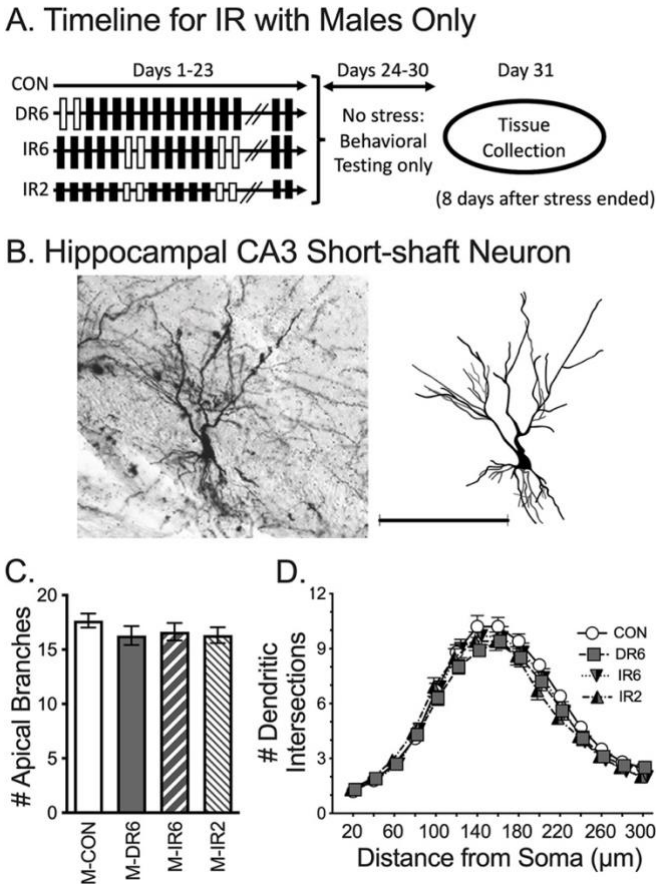

Figure 3.2: Different Restraint Paradigm Effects on Hippocampal CA3 Apical Dendrites in Male Rats. A) Timeline with brains collected 8-days following the end of restraint (see (Peay et al., 2020) for behavioral tasks). B) Example of Golgi-stained hippocampal CA3 neuron (short-shaft) and its representative tracing. C) Various restraint manipulations failed to alter CA3 total apical branch points. D) CA3 apical dendritic complexity was similar across treatments using Sholl analysis. M=Male, C=Control, DR6=Daily restraint 6h/d, IR6=intermittent restraint for 6h/d, IR2=intermittent restraint for 2h/d. M-CON: 10 rats, 9.1 neurons/rat. M-DR6: 9 rats, 10.0 neurons/rat. M-IR6: 12 rats, 9.3 neurons/rat. M-IR2: 12 rats, 10.2 neurons/rat. Scale bar = 125µm.

Chapter 4 Figures

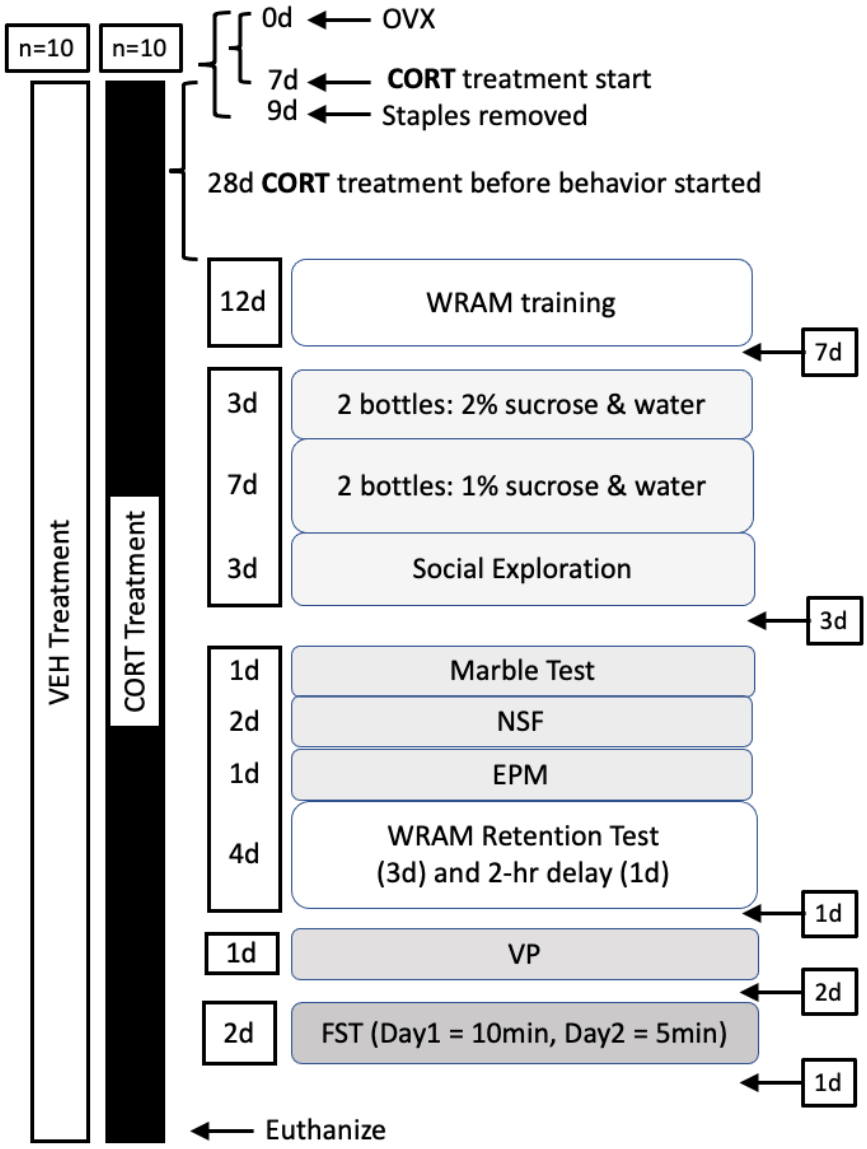

Fig 4.1: Study Timeline. All rats were OVX and received 28 days of daily CORT or VEH treatment prior to beginning behavioral testing and treatment continued throughout. Rats first completed 12 days of RAWM training before undergoing SP, social exploration, defensive marble burying, NSF and EPM. After EPM testing, rats completed 4 days of RAWM testing and the 4th day included a 2-hour delay between trials 2 and 3. Group sizes were n = 10 for both CORT and VEH treated rats. Following RAWM testing, rats completed VP and FST assessments prior to being euthanized. Blocked arrows indicate the delay between assessments.

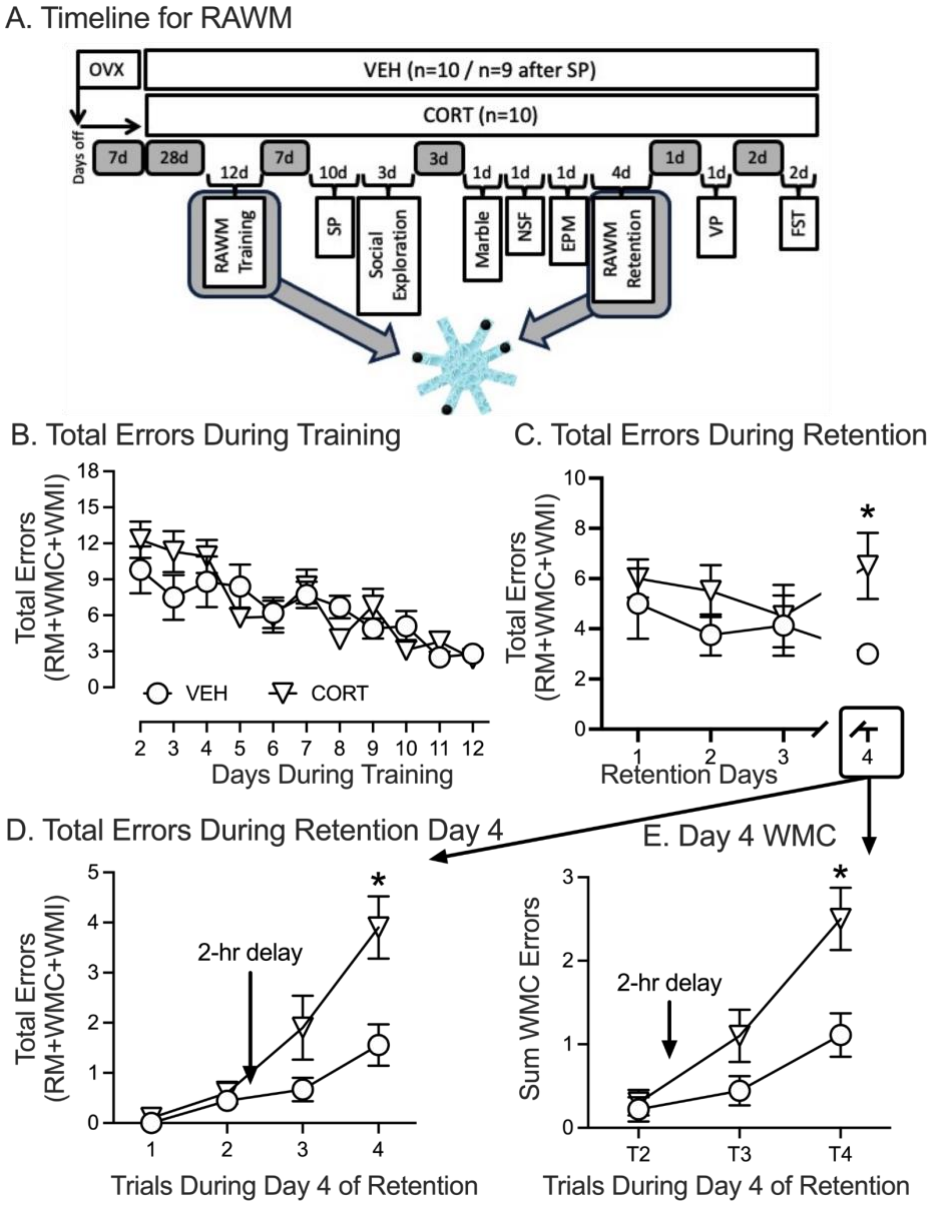

Fig 4.2: Radial Arm Water Maze (RAWM) Training and Retention Testing. A) Timeline for RAWM training and retention testing. RAWM training was the 1st behavioral assessment, RAWM retention testing occurred 26 days later after multiple behavioral tests. B) Total daily errors in Training. Across the 12 days of RAWM training, CORT and VEH rats made a similar number of errors. There was an effect of day, which showed that both groups made fewer errors as training progressed. C) Total daily errors made during retention testing. CORT and VEH treated rats made a similar number of errors

during the first 3 days of retention testing. On day 4 of retention testing, CORT treated rats made significantly more errors than VEH treated rats. D) Total errors by trial retention testing day 4. Retention testing day 4 included a 2-hour delay between trials 2 and 3. On retention testing day 4, CORT treated rats made significantly more errors than VEH treated rats on trial 4. E) Retention testing day 4 WMC errors by trial. CORT treated rats made significantly more WMC errors on trial 4 of retention testing day 4. *p< 0.05, VEH = Daily Vehicle Treatment, CORT = Daily Corticosterone Treatment.

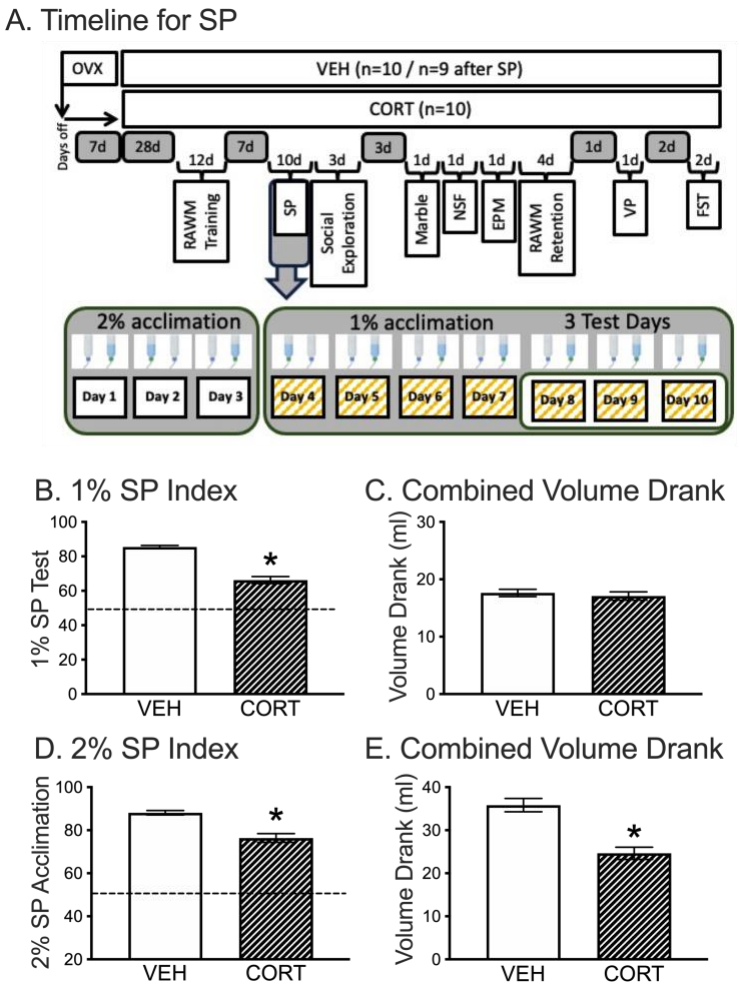

Fig. 4.3. Sucrose Preference (SP). A) Ten-day 2-bottle choice SP protocol timeline. Rats were single-housed and given ad libitum access to food during SP. Rats had access to 2% sucrose or water for 3 days during acclimation before reducing the sucrose concentration to 1% sucrose for the following 7 days. The final 3 days of 1% SP were the test days and averages from the 3 days were used. B) During the 3-day 1% SP testing period, CORT treated rats showed a significantly lower SP index compared to VEH rats. C) During 1% SP testing, CORT and VEH treated rats consumed similar volumes of fluid (1% sucrose and RO water) per day. D) During the 3-day 2% sucrose acclimation period, CORT treated rats showed a significantly lower SP index compared to VEH rats. E) During 2% SP acclimation, CORT treated rats consumed significantly less total fluid (2% sucrose and RO water) compared to VEH rats. *p< 0.05, VEH = Daily Vehicle Treatment, CORT = Daily Corticosterone Treatment

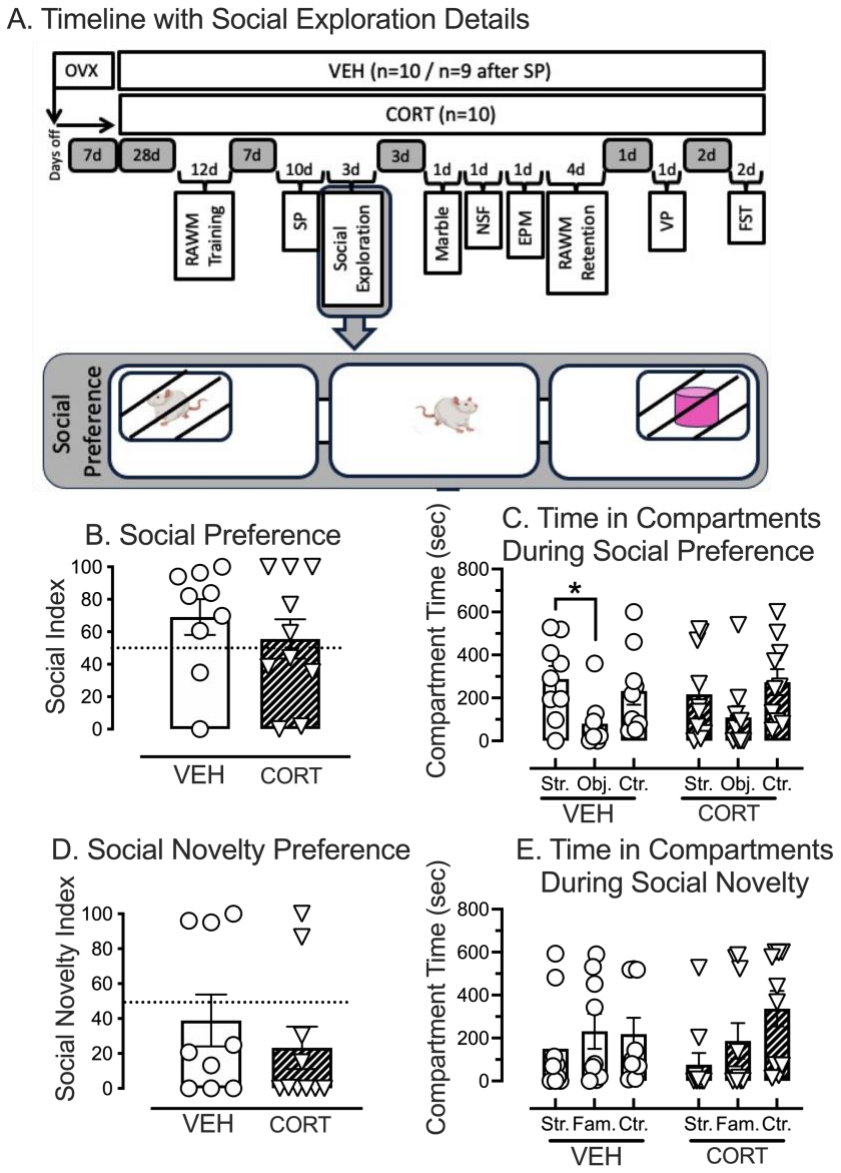

Fig 4.4. Social Exploration. A) Timeline as to when social exploration was assessed. Social exploration assessment includes 2 trials, Trial 1 is a sociability task involving a stranger conspecific and a novel object. Trial 2 assesses social novelty which involves the rat from trial 1 (familiar) and a novel stranger conspecific. B) During trial 1 of sociability testing, CORT and VEH treated rats showed similar sociability indexes. C) During sociability testing, VEH treated rats spent significantly more time in the conspecific chamber compared to the object chamber, whereas CORT treated rats spent a similar amount of time in the stranger and object chambers. D) CORT and VEH treated rats

showed similar social novelty indexes. E) During sociability testing, both CORT and VEH rats spent similar time in novel conspecific and familiar conspecific chambers. Individual animals represented by circles (VEH) and triangles (CORT). *$p < 0.05$, VEH = Daily Vehicle Treatment, CORT = Daily Corticosterone Treatment. Str. = Stranger rat chamber, Obj = Object chamber, Fam. = Familiar rat chamber, Ctr. = Center chamber.

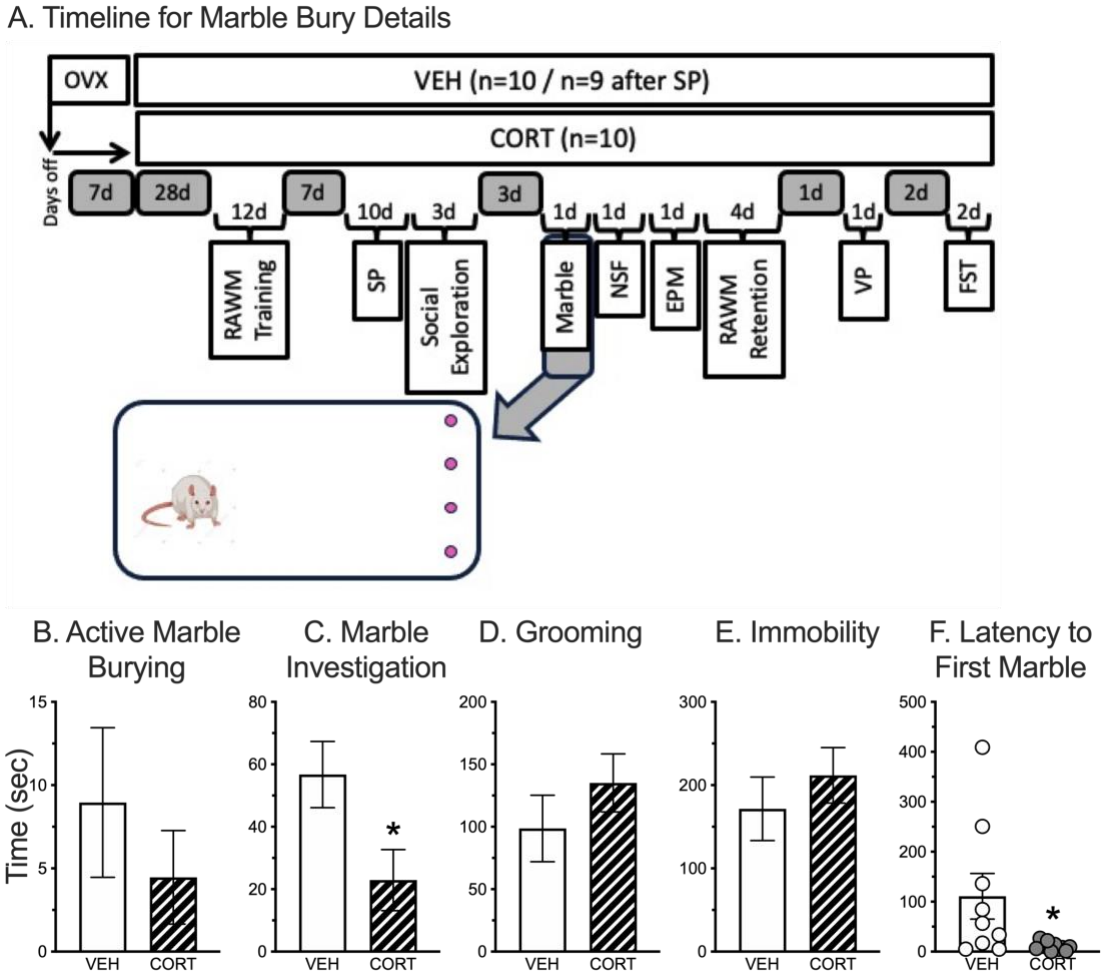

Fig 4.5. Defensive Marble Burying. A) Timeline for when defensive marble burying was performed. B) CORT and VEH rats spent a similar amount of time burying marbles. C) CORT treated rats spent more time investigating marbles compared to VEH treated rats. D) CORT and VEH treated rats spent a similar amount of time grooming. E) CORT and VEH treated rats spent a similar amount of time immobile. F) CORT treated rats took less time to approach marbles compared to VEH treated rats. Circles represent individual animals. *p< 0.05, VEH = Daily Vehicle Treatment, CORT = Daily Corticosterone Treatment.

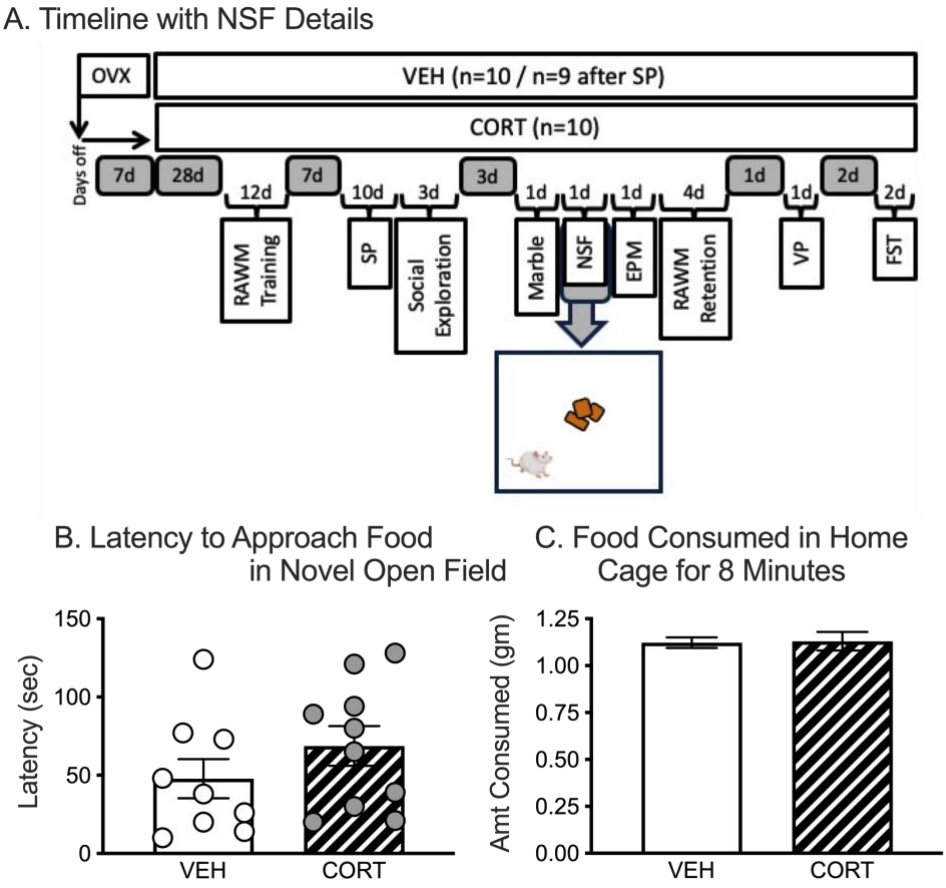

Fig. 4.6 Novelty Suppressed Feeding **(**NSF). A) The timeline for when NSF was performed. Prior to NSF assessment, rats were food-deprived for 24-hours. B.) CORT and VEH treated rats showed similar latency to approach the food in the center. C.) Immediately upon completing the NSF, the amount of food consumed during 8 minutes in the rats' home cage was assessed. CORT and VEH treated rats consumed similar amounts of food. Circles represent individual animals. VEH = Daily Vehicle Treatment, CORT = Daily Corticosterone Treatment.

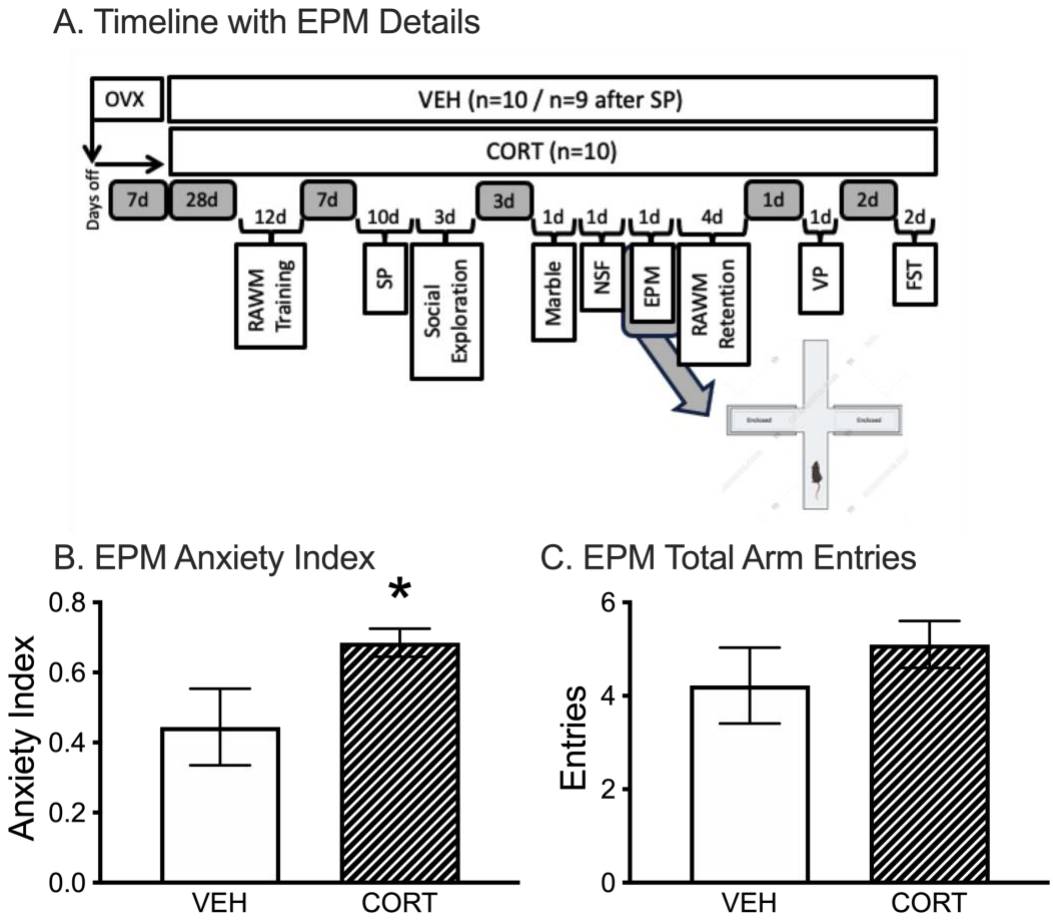

Fig. 4.7 Elevated Plus Maze **(**EPM). A) The timeline for when EPM was performed. EPM involves a one trial, 5-minute exposure. B) CORT treated rats showed a significantly higher anxiety index compared to VEH rats during EPM testing. C) EPM total arm entries. CORT and VEH treated rats made a similar number of overall arm entries during EPM testing. *p< 0.05, VEH = Daily Vehicle Treatment, CORT = Daily Corticosterone Treatment.

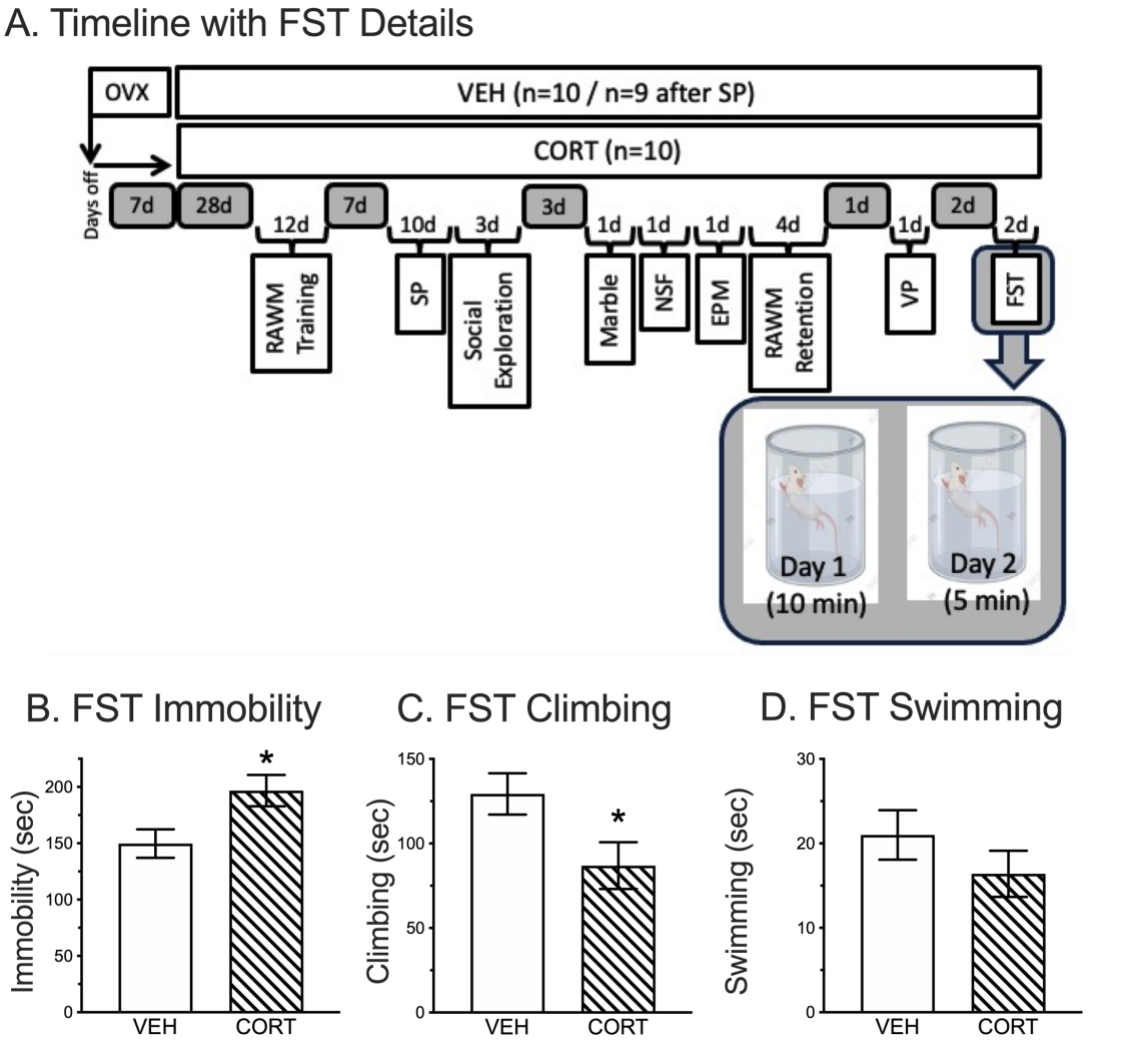

Fig. 4.8 Forced Swim Test (FST). A) The timeline for when FST was performed. Tne FST involves 2-days, the 1st day included a 10-min water exposure, and the 2nd day was the formal test (5-min). B) CORT treated rats spent significantly more time immobile than VEH-treated rats. C) CORT treated rats spent significantly more time climbing than the VEH-treated rats. D) CORT and VEH treated rats spent similar amounts of time swimming. *p< 0.05, VEH = Daily Vehicle Treatment, CORT = Daily Corticosterone Treatment.

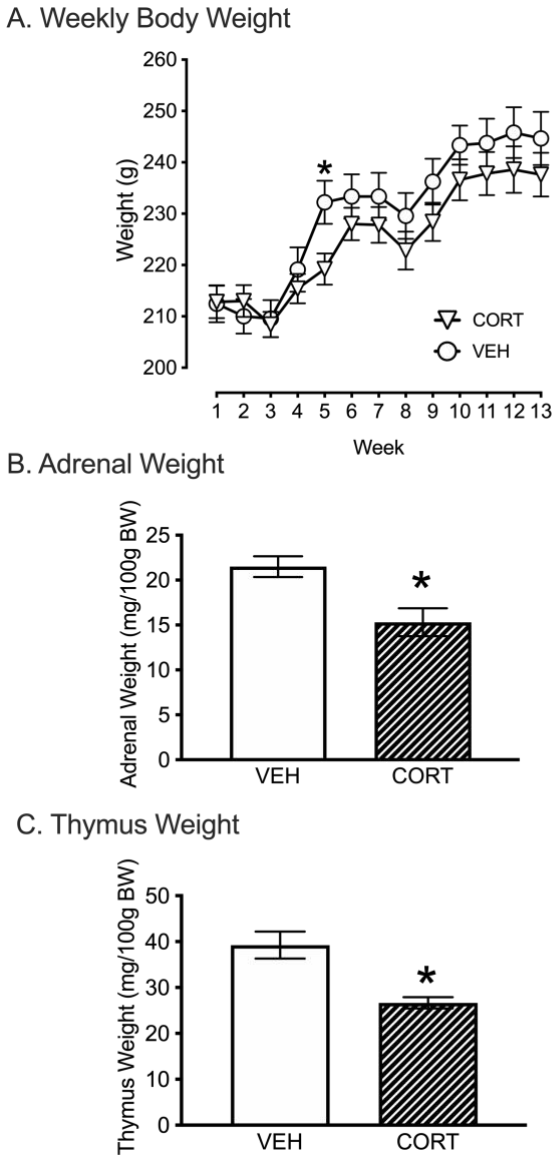

Fig. 4.9 Effects of CORT on Body, Adrenal and Thymus Weights. A) CORT treated rats had significantly lower body weights compared to VEH treated rats at week 5 of the experiment. Group weights were similar at all other points of the experiment. B) CORT treated rats had significantly lower adrenal weights at euthanasia compared to VEH treated rats. C) CORT treated rats had significantly lower thymus weights at euthanasia compared to VEH treated rats. *p< 0.05, VEH = Daily Vehicle Treatment, CORT = Daily Corticosterone Treatment.

Chapter 5 Figures

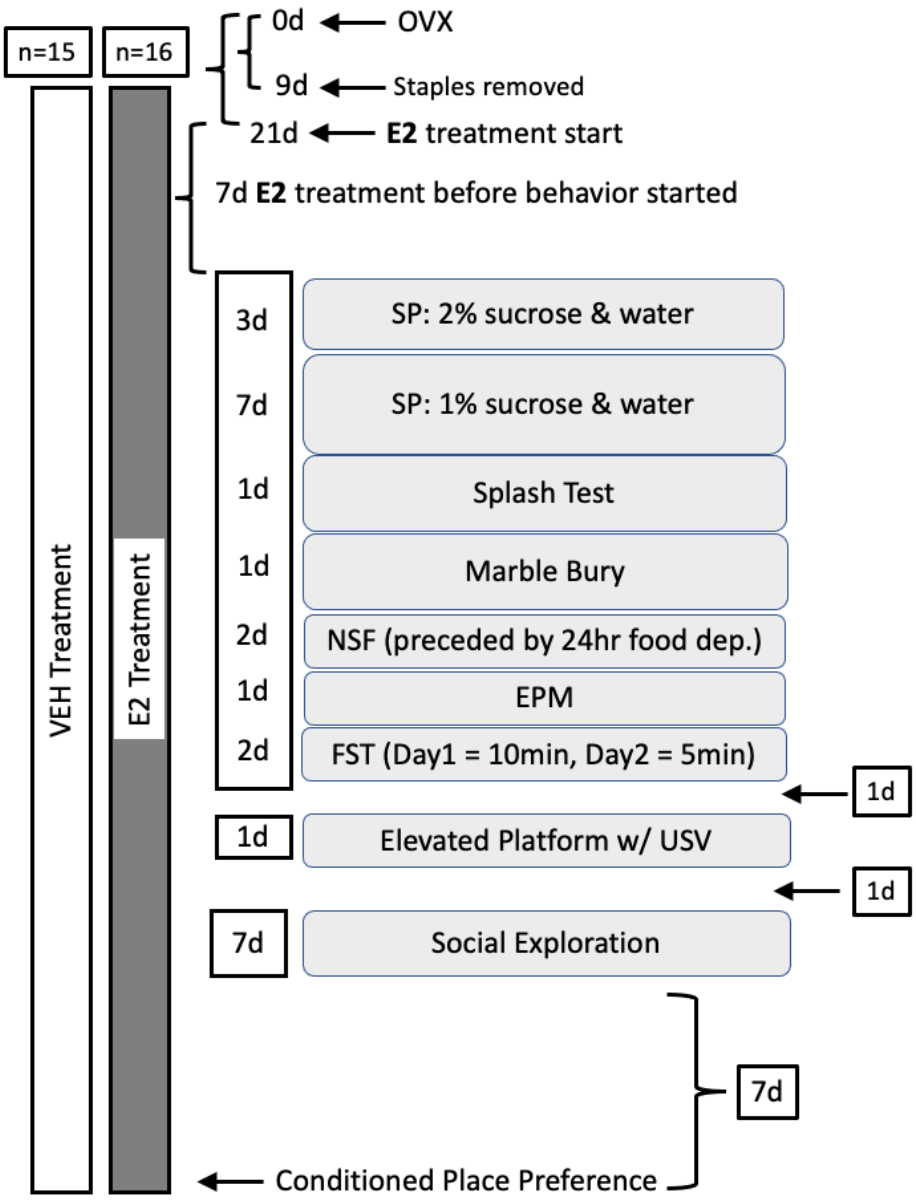

Fig 5.1: Chapter 5 Study Timeline. All rats were OVX and received 7 days of daily E2 (or VEH) injections prior to the beginning of behavioral testing and injections continued throughout the experiment. Rats first completed 10 days of SP testing before undergoing sucrose splash, defensive marble burying, NSF, EPM, FST, elevated platform and social exploration testing. EPM and elevated platform assessments were not included in this chapter. Group sizes were n = 16 for E2 treated rats and n= 15 for VEH treated rats.

Following WRAM testing, rats completed VP and FST assessments prior to being euthanized. Blocks with arrows indicate the delay between assessments.

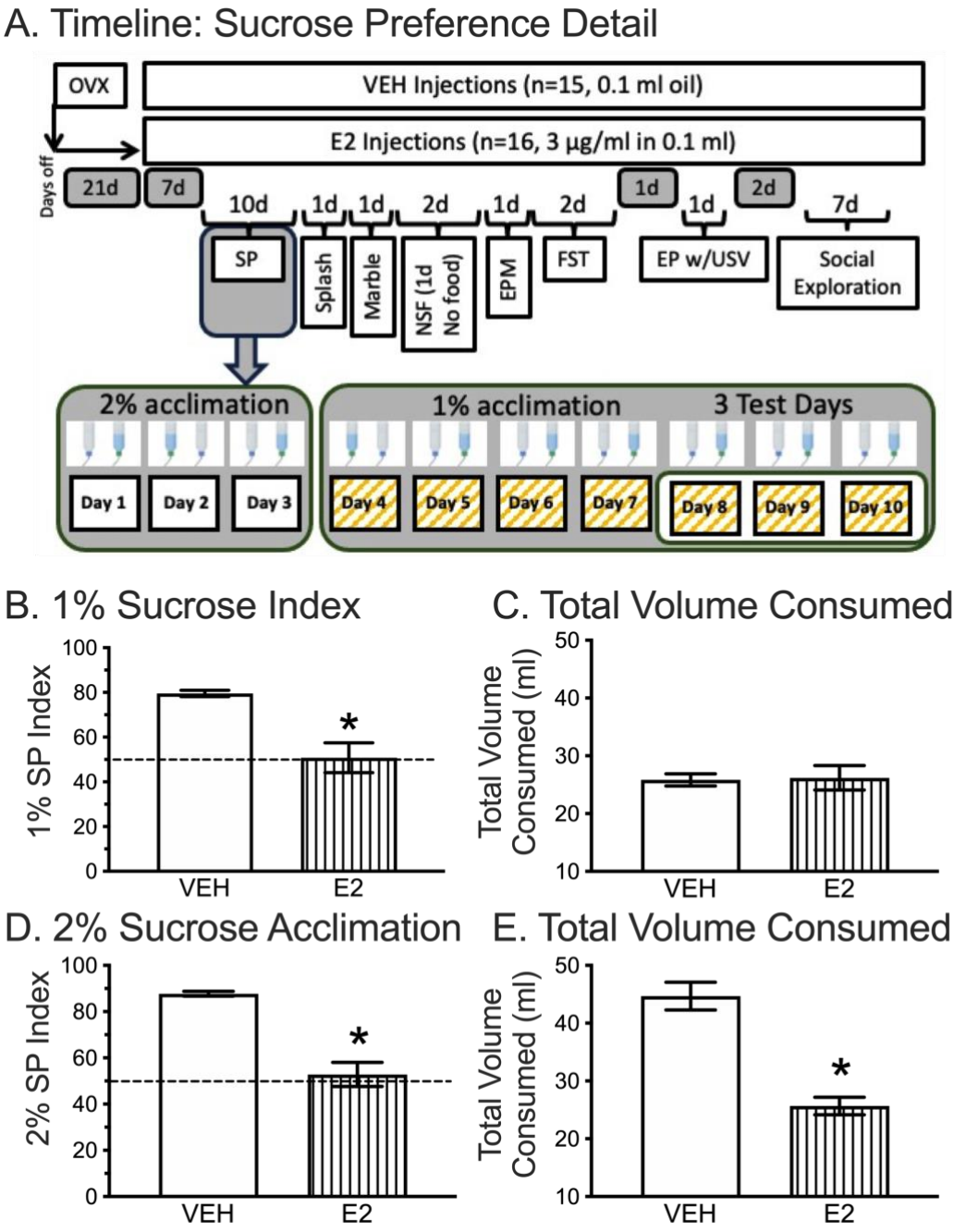

Fig 5.2: Sucrose Preference (SP). A) Ten days of a 2-bottle choice SP was given and shown on the timeline. B) During the 3-day 1% SP testing period, E2 treated rats showed a significantly lower SP index compared to VEH treated rats. C) During 1% SP testing,

E2 and VEH treated rats consumed similar volumes of fluid (1% sucrose and water) per day. D) During the beginning when the rats were first individually housed and given a 3-day exposure to 2% sucrose and water, E2 treated rats showed a significantly lower 2% SP index compared to VEH treated rats. E) During the 2% SP acclimation, E2 treated rats consumed significantly less total fluid (2% sucrose and water) compared to VEH rats. *$p < 0.05$, VEH = Daily Vehicle Treatment, E2 = Daily Estradiol Treatment.

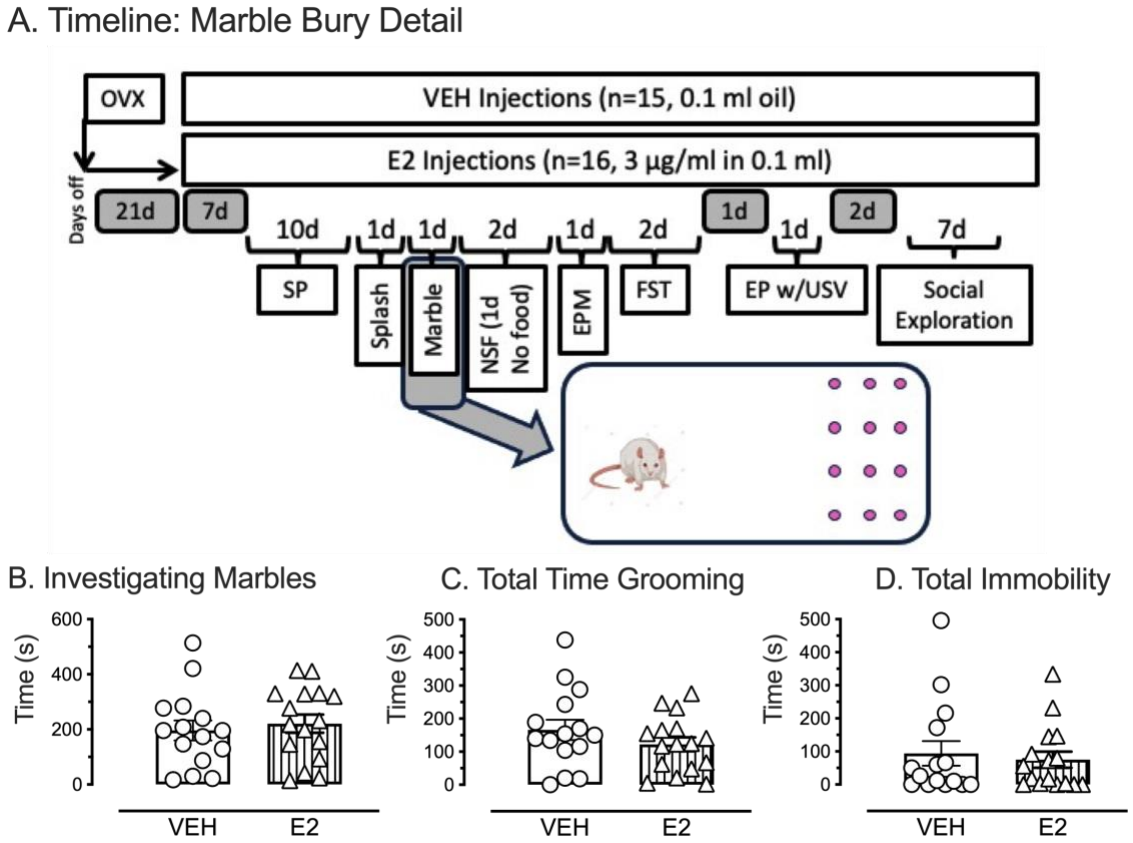

Fig 5.3. Defensive Marble Burying. A) Timeline for when defensive marble burying was performed. B) E2 and VEH treated rats spent a similar amount of time investigating marbles during the first 10-min of the 15-min marble burying task. C) E2 and VEH treated rats spent a similar amount of time grooming during the first 10-min. D) E2 and VEH treated rats spent a similar amount of time immobile during the first 10-min. Individual animals represented by circles (VEH) and triangles (E2). VEH = Daily Vehicle Treatment, E2 = Daily Estradiol Treatment.

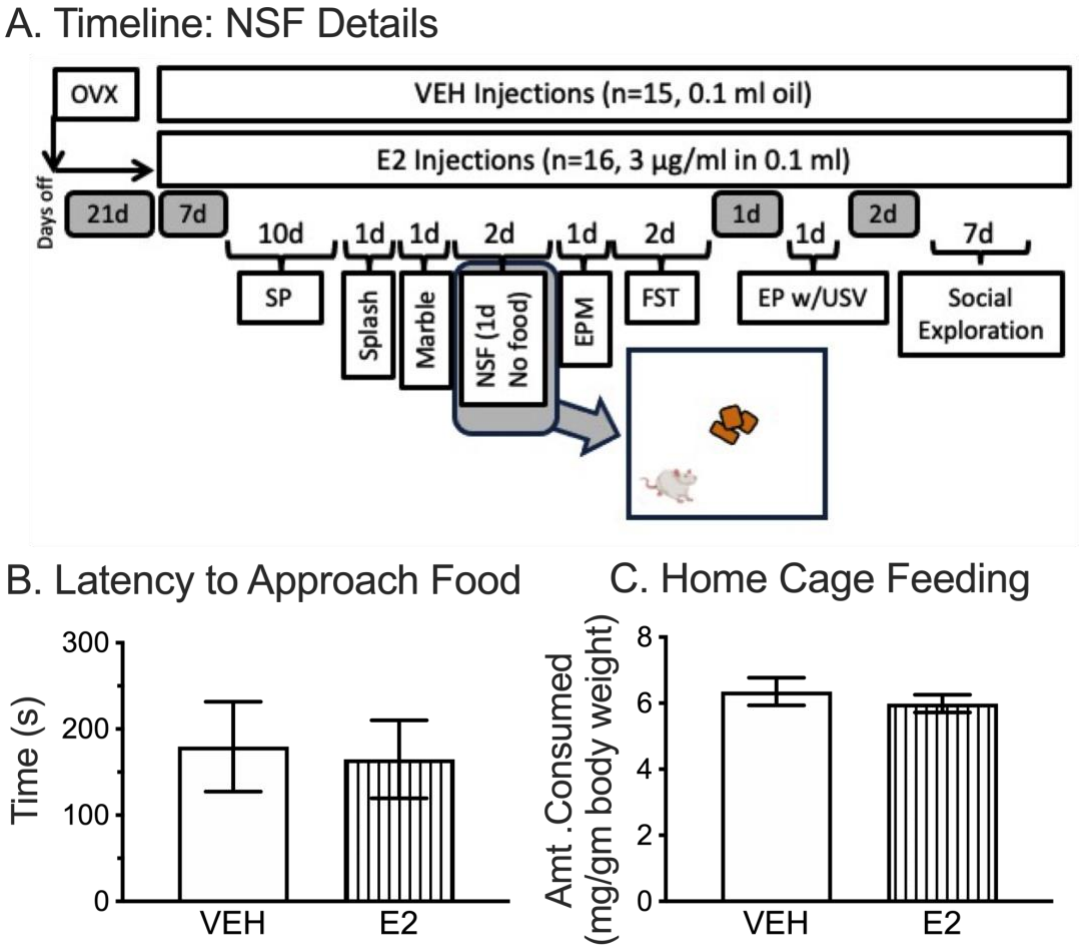

Fig 5.4: Novelty Suppressed Feeding (NSF). A) For the NSF, the rats are exposed to a 24-hour food deprivation period. B) E2 and VEH treated rats showed a similar latency to approach food in the center. C) Immediately upon conclusion of the NSF, the amount of food consumed in the rat's home cage was assessed for 8 minutes and E2 and VEH treated rats consumed similar amounts of food. VEH = Daily Vehicle Treatment, E2 = Daily Estradiol Treatment.

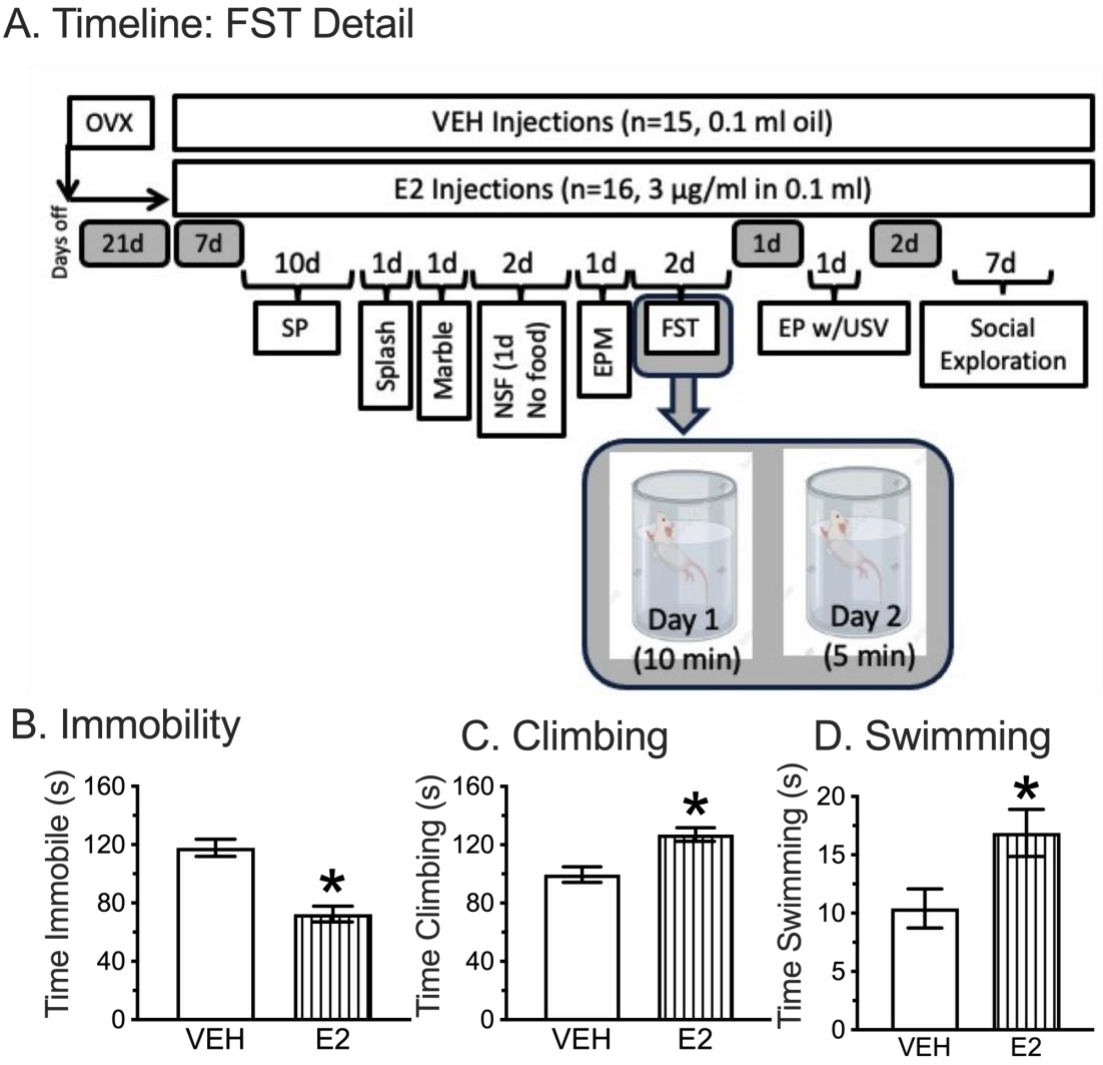

Fig 5.5: Forced Swim Task (FST). A) The timeline for when the FST was performed is shown. FST involved 2-days, the 1st included a 10-min water exposure, and the 2nd day was the test for 5-min. B) E2 treated rats spent significantly less time immobile than did the VEH-treated rats. C) E2 treated rats spent significantly more time climbing than did the VEH-treated rats. D) E2 treated rats spent significantly more time swimming than did the VEH-treated rats. *p< 0.05, VEH = Daily Vehicle Treatment, E2 = Daily Estradiol Treatment.

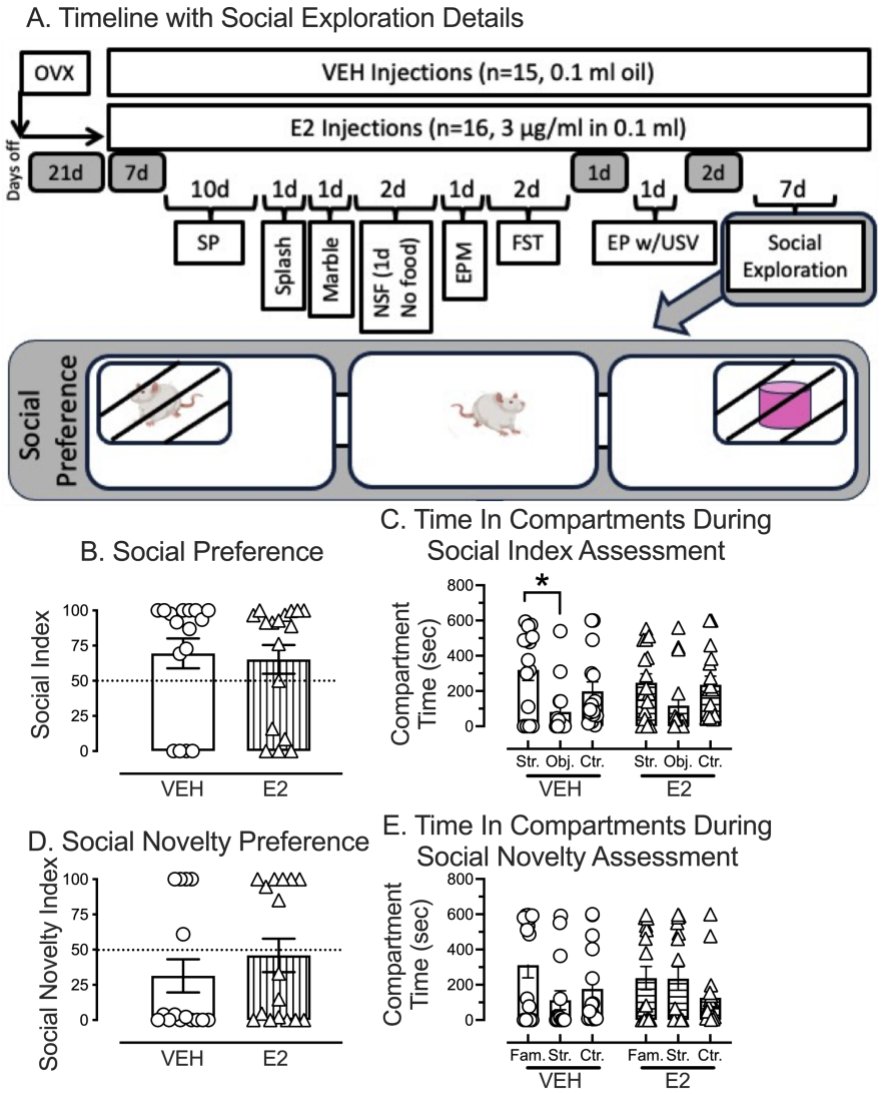

Fig 5.6: Social Exploration. A) The timeline for when social exploration was performed. Social exploration assessment included 2 trials, Trial 1 was a sociability task involving a stranger conspecific and a novel object. Trial 2 assessed social novelty which involved the stranger from trial 1 and a novel stranger conspecific. B) During trial 1 of sociability testing, E2 and VEH treated rats showed similar sociability indexes. C) Also, during trial 1 of sociability testing, VEH treated rats spent significantly more time in the conspecific chamber compared to the object chamber, whereas E2 treated rats spent a similar amount of time in the stranger and object chambers. D) E2 and VEH treated rats showed similar social novelty indexes. E) Also, during trial 2 of social novelty testing, both E2 and VEH treated rats spent similar time in the novel conspecific chamber as they did with the

familiar conspecific chamber. Individual animals represented by circles (VEH) and triangles (E2). *p< 0.05, VEH = Daily Vehicle Treatment, E2 = Daily Estradiol Treatment. Str. = Stranger rat chamber, Obj = Object chamber, Fam. = Familiar rat chamber, Ctr. = Center chamber

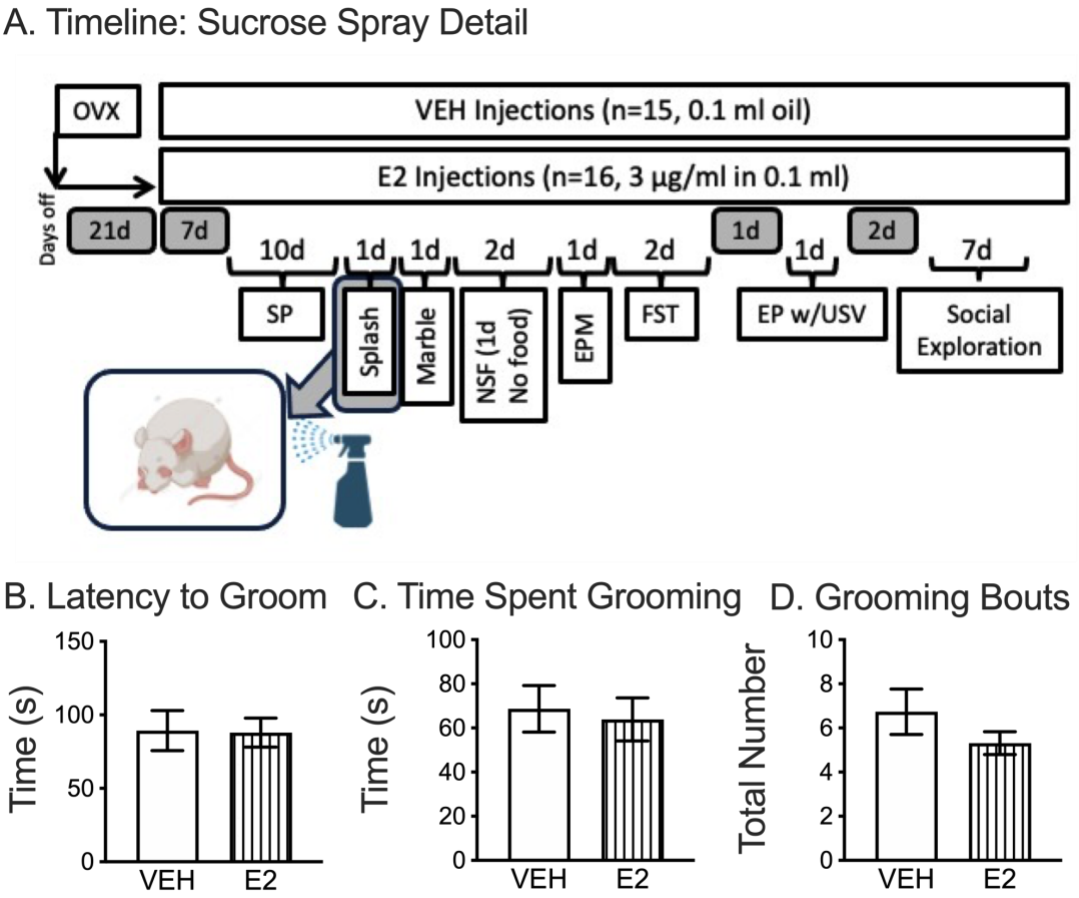

Fig 5.7: Sucrose Splash Test. A) Timeline for when sucrose splash was performed. B) E2 and VEH treated rats showed a similar latency to groom following a spray of 10% sucrose on their backside. C) E2 and VEH treated rats spent a similar amount of time grooming following a spray of sucrose. D) E2 and VEH treated rats performed a similar number of grooming bouts following the sucrose spray. VEH = Daily Vehicle Treatment, E2 = Daily Estradiol Treatment.

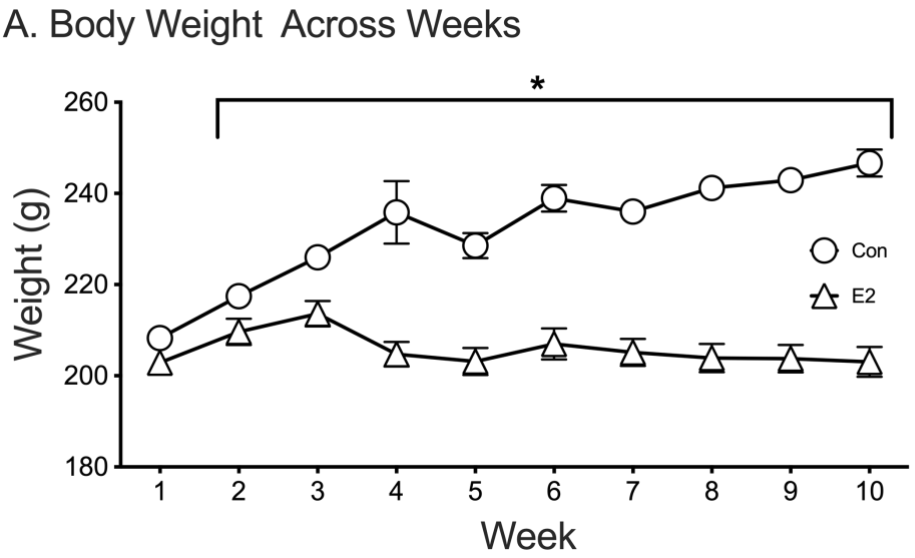

Fig 5.8: Effects of E2 on Weekly Body Weights. A) Starting at week 2, E2 treated rats weighed significantly less than CON and this difference continued throughout the experiment. *p< 0.05, VEH = Daily Vehicle Treatment, E2 = Daily Estradiol Treatment.

Chapter 6 Figures

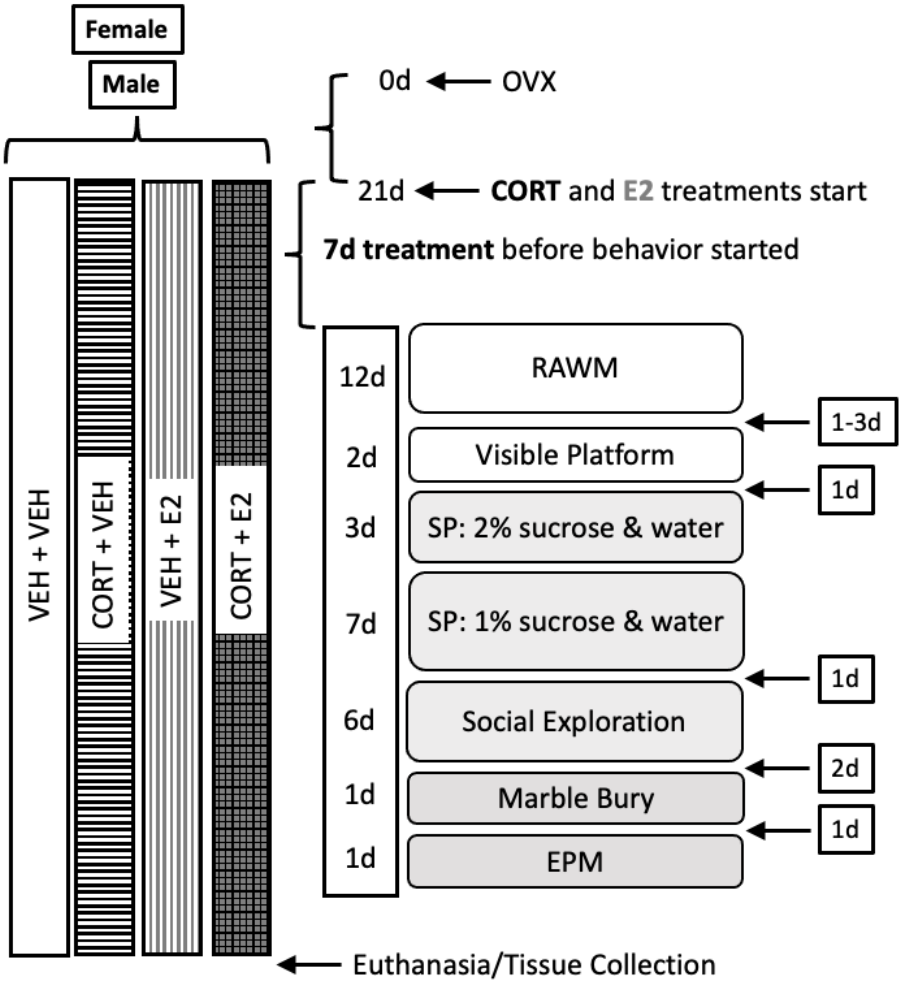

Fig 6.1: Chapter 6 Study Timeline. All rats were OVX and after 21 days, received 7 days of daily CORT (or VEH), E2 (or VEH) or a combination thereof prior to and continuing throughout behavioral testing. Rats completed a 12-day RAWM, a VP, a 10-day SP test, social exploration, marble bury and ended with EPM. The experiment was conducted in 2 cohorts. Group sizes were as follows: F-V-V n=10, F-C-V n=10, F-V-E n=10, F-C-E n=11, M-V-V n=11, M-C-V n=12, M-V-E n=13, M-C-E n=14t. Female = F, Male = M, CORT = C, E2 = E, VEH = V, Order of listing is Sex-CORT-E2. Blocks with arrows indicate the delay between assessments.

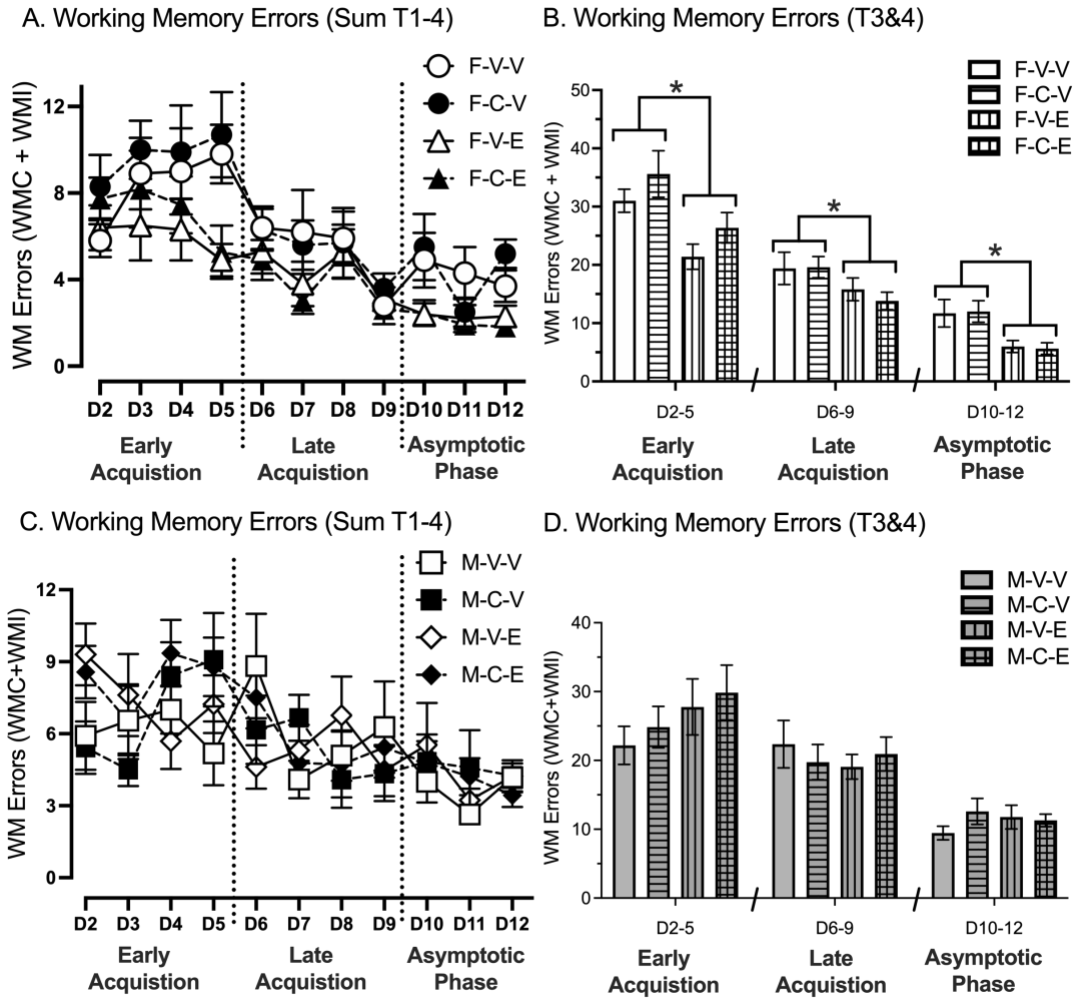

Fig 6.2: Radial Arm Water Maze (RAWM) Total Working Memory (WMC+WMI) Errors. A) For the total number of daily working memory errors committed throughout RAWM testing in females, all groups made fewer working memory errors as days progressed. B) The trials with the highest working memory load (T3, T4) were compared and revealed that for females, E2 treated female rats made significantly fewer working memory errors compared to VEH treated female rats during each learning phase (early acquisition, late acquisition, and final testing). C) In males, all groups decreased the number of working memory errors made as days progressed and all groups performed similarly. D) The trials with the highest working memory load (T3,T4) were compared and revealed that all treatments made similar total working memory errors during each learning phase (early acquisition, late acquisition, and final testing). Dotted lines indicate learning phases (Early Acquisition, Late Acquisition, and Final Testing). *p< 0.05, F-V-V = Female Vehicle stress hormone + Vehicle ovarian hormone, F- C-V = Female Stress

hormone + Vehicle ovarian hormone, F-V-E = Female Vehicle stress hormone + E2, F-C-E = Female CORT + E2, M-V-V = Male Vehicle stress hormone + Vehicle ovarian hormone, M- C-V = Male Stress hormone + Vehicle ovarian hormone, M-V-E = Male Vehicle stress hormone + E2, M- C-E = Male CORT + E2

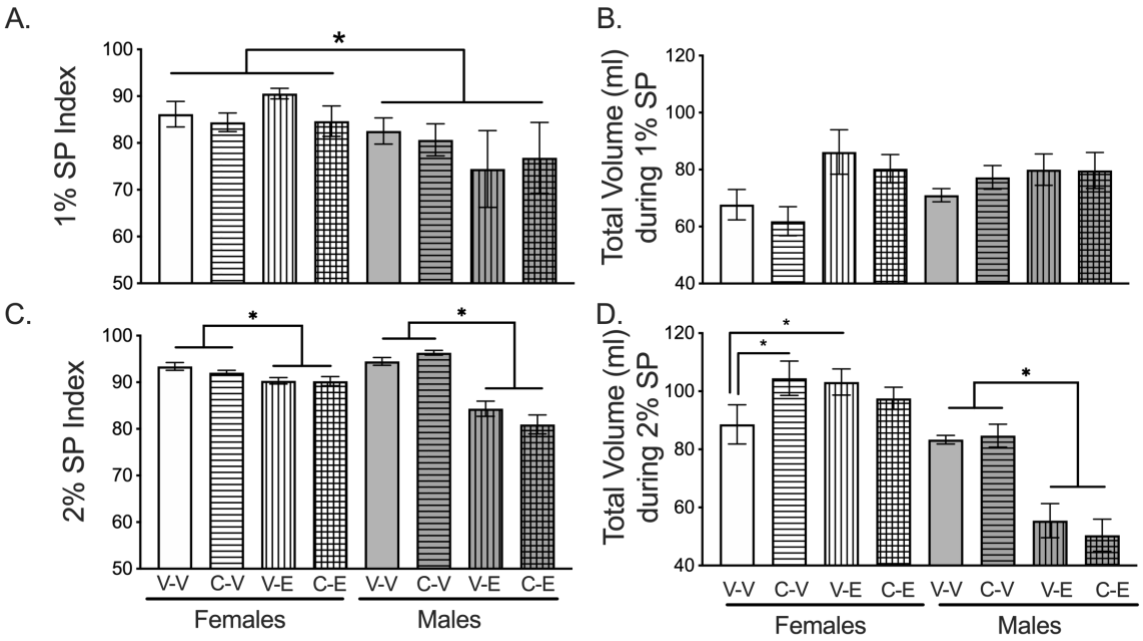

Fig 6.3: Sucrose Preference (SP). A) 1% SP testing, SP index. During the 3-day 1% SP testing period, male rats showed a significantly lower SP index compared to female rats. There were no other significant group differences during the 1% SP testing period. B.) SP testing, total volume consumed. During SP testing, groups consumed similar volumes of fluid per day. C) 2% Sucrose Acclimation, SP index. During the 3-day 2% sucrose acclimation period, E2 treatment significantly lowered SP index compared to VEH treatment in both male and female rats. D) SP acclimation, total volume consumed. During SP acclimation, female rats treated with either CORT or E2 consumed significantly more total fluid compared to rats that received only VEH treatment. Male rats treated with E2 consumed significantly less total fluid compared to males that did not receive E2 treatment. *p< 0.05, V-V = Vehicle stress hormone + Vehicle ovarian hormone, C-V = Stress hormone + Vehicle ovarian hormone, V-E = Vehicle stress hormone + E2, C-E = CORT + E2

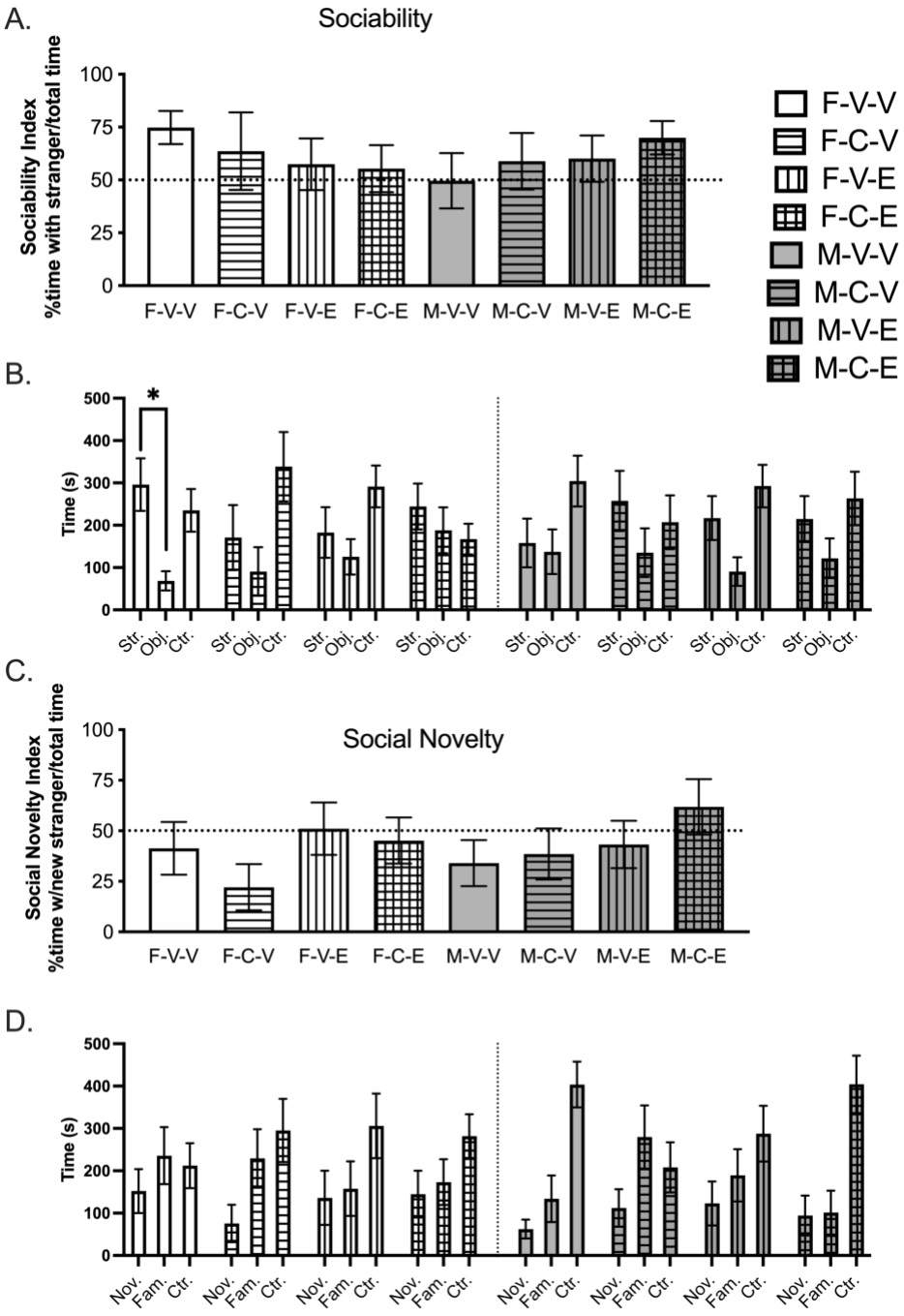

Fig 6.4: Social Exploration. A) Social exploration trial 1, sociability index. During trial 1, all groups showed similar sociability indexes. B) Time spent per chamber during social exploration trial 1. During sociability testing, only females treated with both VEH

treatments spent significantly more time in the conspecific chamber compared to the object chamber. The remaining groups spent a similar amount of time in the conspecific chamber compared to the object chamber. C) Social exploration trial 2, social novelty index. During trial 2, groups showed similar social novelty indexes. D) Time spent per chamber during social exploration trial 2. During social novelty testing, all groups spent a similar amount of time in each conspecific chamber. *$p < 0.05$, F-V-V = Female Vehicle stress hormone + Vehicle ovarian hormone, F- C-V = Female Stress hormone + Vehicle ovarian hormone, F-V-E = Female Vehicle stress hormone + E2, F- C-E = Female CORT + E2, M-V-V = Male Vehicle stress hormone + Vehicle ovarian hormone, M- C-V = Male Stress hormone + Vehicle ovarian hormone, M-V-E = Male Vehicle stress hormone + E2, M- C-E = Male CORT + E2

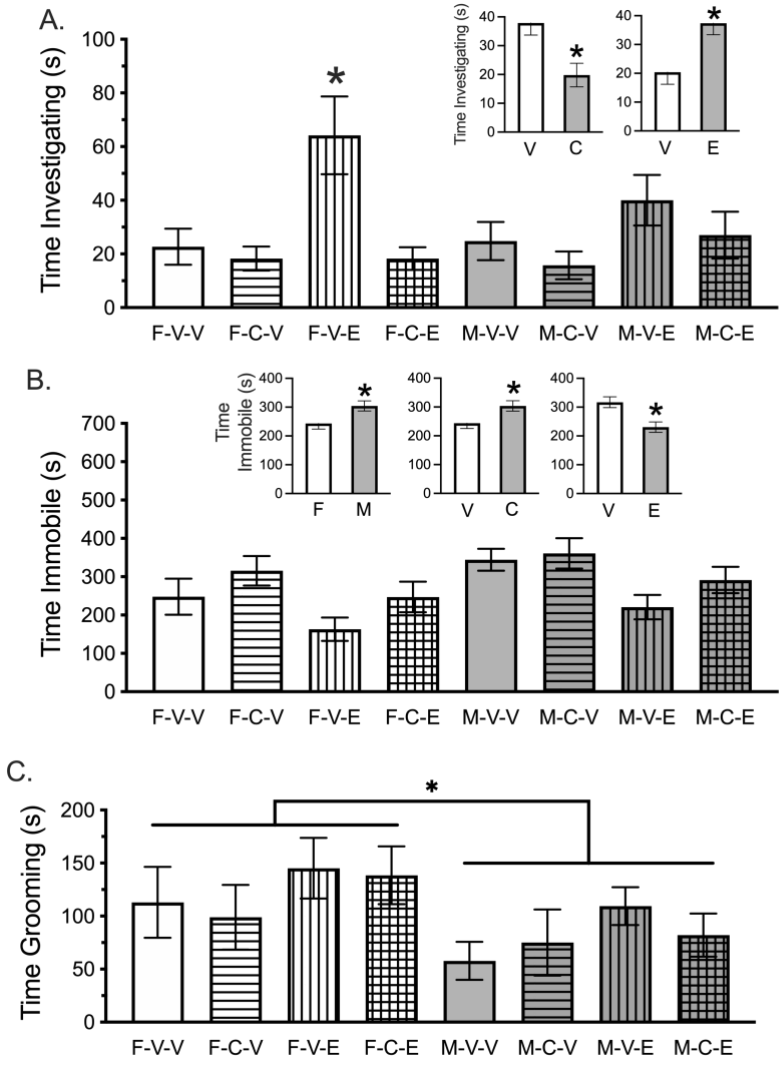

Fig 6.5: Defensive Marble Burying. A) Time spent investigating marbles. B) Time spent immobile during defensive marble burying. 1% SP testing, SP index. During the 3-day 1% SP testing period, E2 treated rats showed a significantly lower SP index compared to VEH rats. C) Time spent grooming during defensive marble burying. SP testing, total volume consumed daily. During SP testing, E2 and VEH treated rats consumed similar volumes of fluid per day. *p< 0.05, F-V-V = Female Vehicle stress hormone + Vehicle ovarian hormone, F- C-V = Female Stress hormone + Vehicle ovarian hormone, F-V-E = Female Vehicle stress hormone + E2, F- C-E = Female CORT + E2, M-V-V = Male Vehicle stress hormone + Vehicle ovarian hormone, M- C-V = Male Stress hormone + Vehicle ovarian hormone, M-V-E = Male Vehicle stress hormone + E2, M- C-E = Male CORT + E2

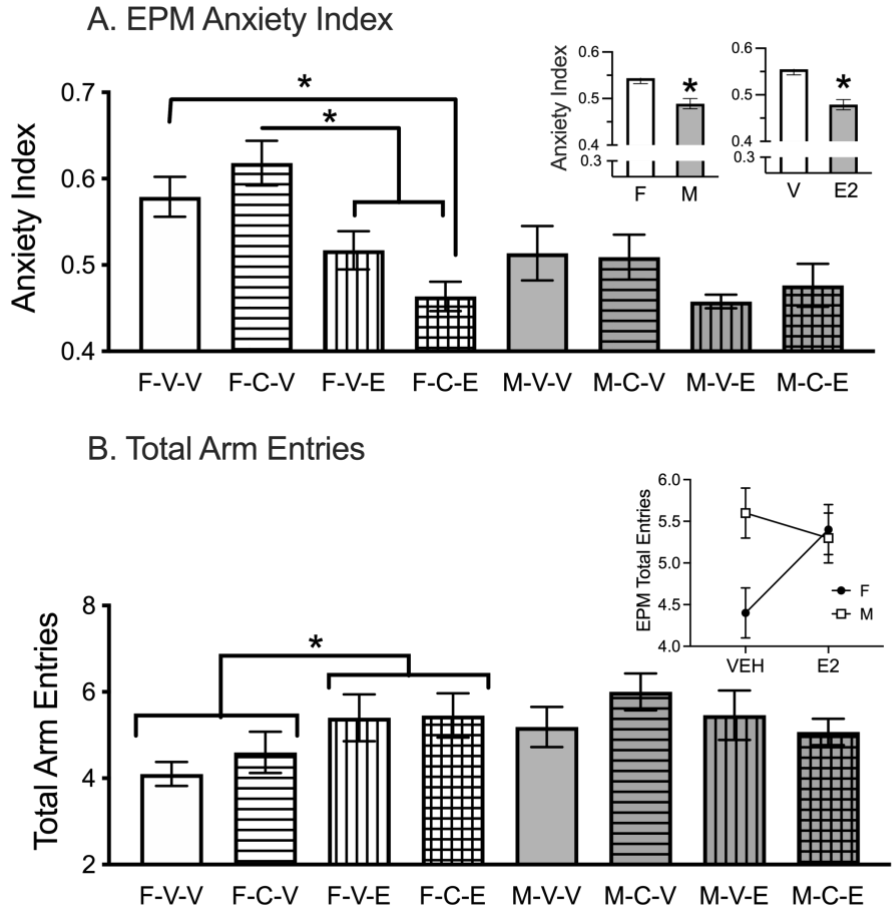

Fig 6.6: Elevated Plus Maze (EPM). A) Female and male EPM anxiety index. E2 treated rats had significantly higher anxiety indexes compared to VEH treated rats and female rats had significantly higher anxiety indexes compared to male rats. B) EPM total arm entries. E2 treated female rats made significantly more entries compared to VEH treated female rats. E2 treatment showed no effect on male total arm entries. *$p < 0.05$, F-V-V = Female Vehicle stress hormone + Vehicle ovarian hormone, F- C-V = Female Stress hormone + Vehicle ovarian hormone, F-V-E = Female Vehicle stress hormone + E2, F-C-E = Female CORT + E2, M-V-V = Male Vehicle stress hormone + Vehicle ovarian hormone, M- C-V = Male Stress hormone + Vehicle ovarian hormone, M-V-E = Male Vehicle stress hormone + E2, M- C-E = Male CORT + E2

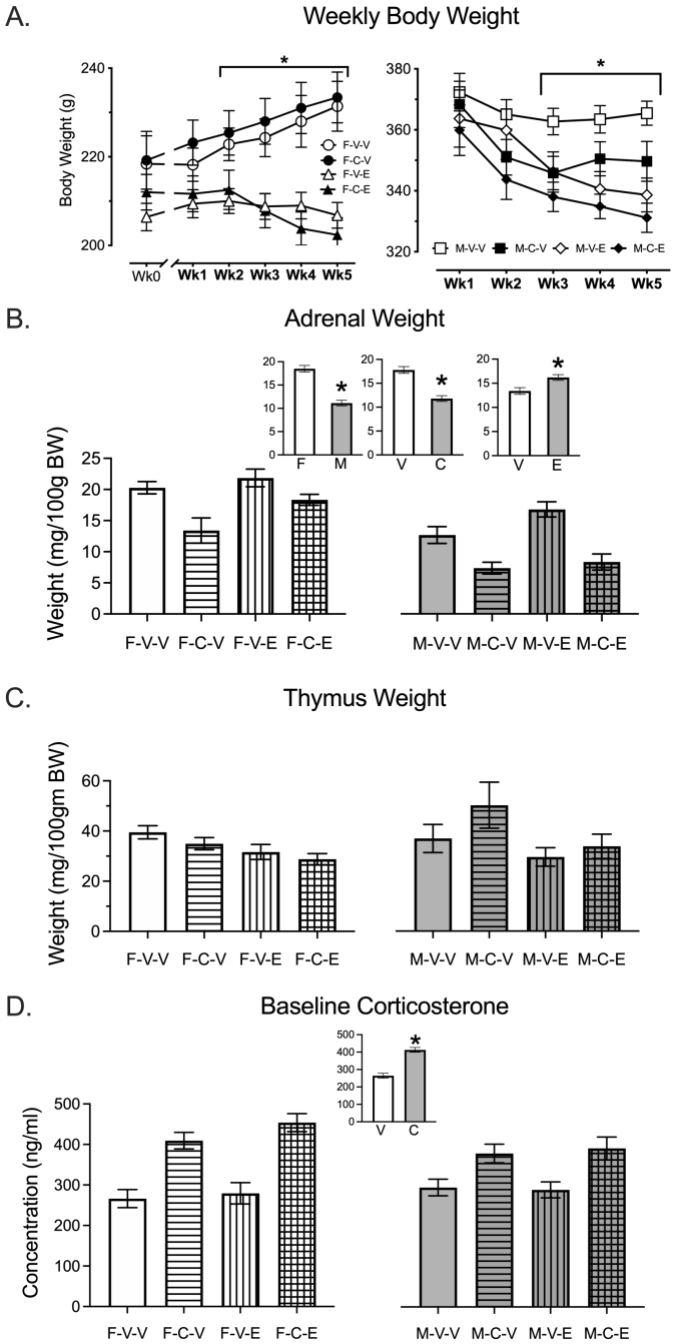

Fig 6.7: Effects of CORT and E2 on Body Weight, Adrenal Weight, Thymus Weight and Serum CORT. A) Female and male weekly body weight E2 treatment significantly lowered weight in females at week 2 and throughout the experiment and in males starting at week 3 compared to VEH treated rats. Female surgery occurred after week 1 and

treatment occurred during week 1 for both males and females (post-treatment weeks in bold). B) Female and male adrenal weight per body weight. CORT treated rats had significantly lower adrenal weights than VEH treated rats, E2 treated rats had significantly greater adrenal weights compared to VEH treated rats and females had higher adrenal weight compared to males. C) Female and male thymus weight per body weight. There were no group differences in thymus weight. D) Female and male baseline serum corticosterone. CORT treated rats had higher baseline CORT compared to VEH treated rats. *$p < 0.05$, F-V-V = Female Vehicle stress hormone + Vehicle ovarian hormone, F- C-V = Female Stress hormone + Vehicle ovarian hormone, F-V-E = Female Vehicle stress hormone + E2, F- C-E = Female CORT + E2, M-V-V = Male Vehicle stress hormone + Vehicle ovarian hormone, M- C-V = Male Stress hormone + Vehicle ovarian hormone, M-V-E = Male Vehicle stress hormone + E2, M- C-E = Male CORT + E2

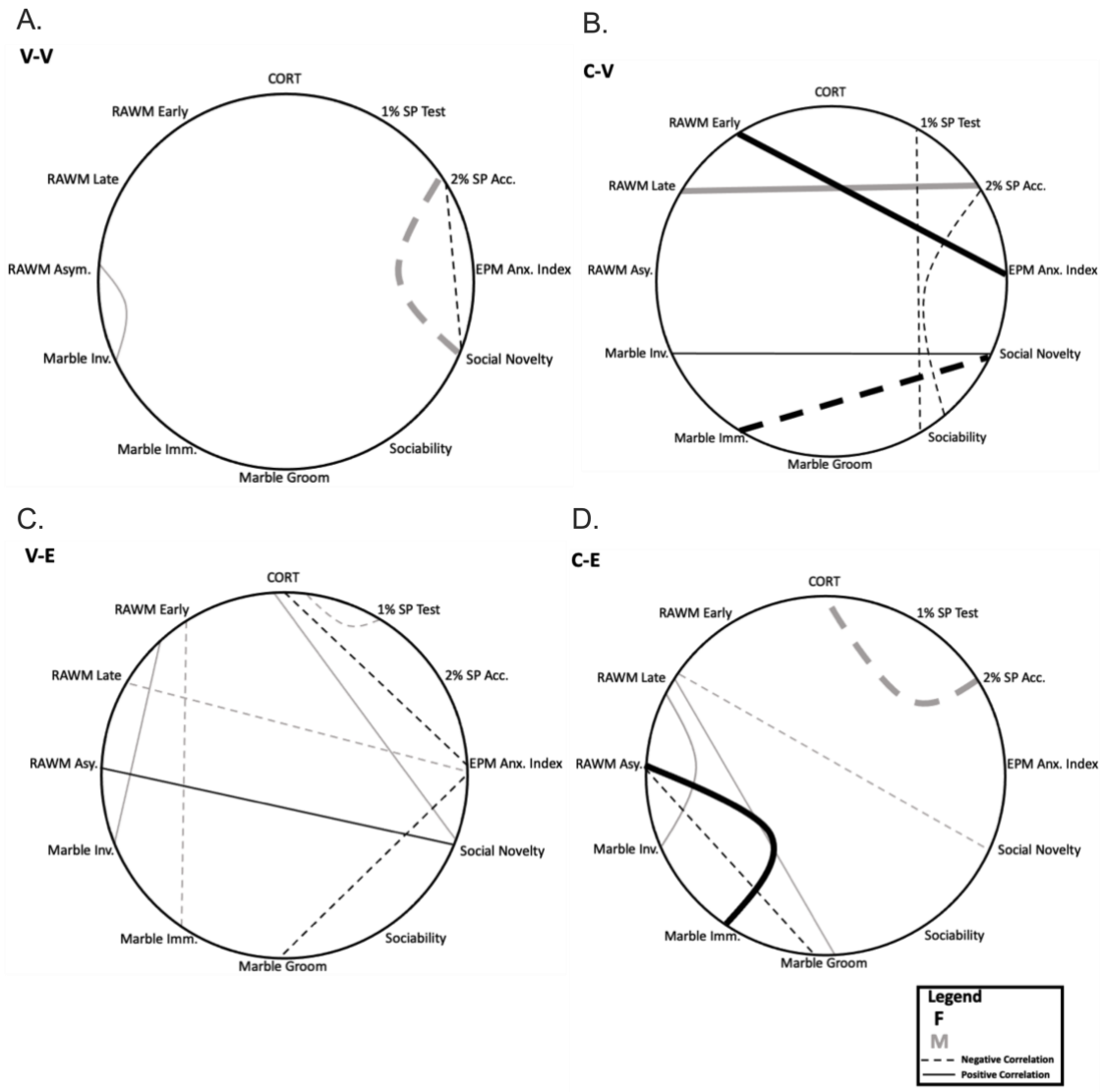

Fig 6.8: Spearman Correlation Circle Diagrams. A) Spearman correlations for rats treated with stress hormone vehicle and ovarian hormone vehicle. Female rats treated with both stress hormone vehicle and ovarian hormone vehicle showed a significant negative correlation for social novelty index with 2% acclimation SP index. For male rats treated with both stress hormone vehicle and ovarian hormone vehicle there was a significant positive correlation for RAWM asymptotic phase working memory errors with defensive marble bury investigation as well as a negative correlation for social novelty index with 2% acclimation SP index. B) Spearman correlations for rats treated with CORT and ovarian hormone vehicle. Female rats treated with CORT and ovarian hormone vehicle, there were significant positive correlations for RAWM early acquisition errors with EPM

anxiety index and marble bury investigation with social novelty index. There were significant negative correlations for marble bury immobility and social novelty index, sociability index with 1% testing SP index, and sociability index with 2% acclimation SP index. For male rats treated with CORT and ovarian hormone vehicle, there was a significant positive correlation for RAWM late acquisition working memory errors with 2% acclimation SP index. C) Spearman correlations for rats treated with stress hormone vehicle and E2. For female rats treated with stress hormone vehicle and E2, there was a significant positive correlation for RAWM asymptotic phase working memory errors with social novelty index. There were significant negative correlations for baseline CORT with EPM anxiety index and marble bury grooming and EPM anxiety index. For male rats treated with stress hormone vehicle and E2, there were significant positive correlations for RAWM early acquisition working memory errors with marble bury investigation and social novelty index with baseline CORT. There were significant negative correlations for RAWM early acquisition working memory errors with marble bury immobility, RAWM late acquisition working memory errors with EPM anxiety index, and 1% testing SP index with baseline CORT. D) Spearman correlations for rats treated with CORT and E2. For female rats treated with CORT and E2, there was a significant positive correlation for RAWM asymptotic phase working memory errors with marble bury immobility and there was a significant negative correlation for RAWM asymptotic phase working memory errors with marble bury grooming. For male rats treated with CORT and E2, there were significant positive correlations for RAWM late acquisition working memory errors with marble bury investigation and RAWM late acquisition working memory errors with marble bury grooming. There were significant negative correlations for RAWM late acquisition working memory errors with social novelty index and 2% acclimation SP index with baseline CORT. Positive correlations shown with solid line, negative correlations shown with dashed line. Female correlations shown in black, and male correlations shown in grey. Highly significant ($P \leq 0.01$) shown with thicker line.

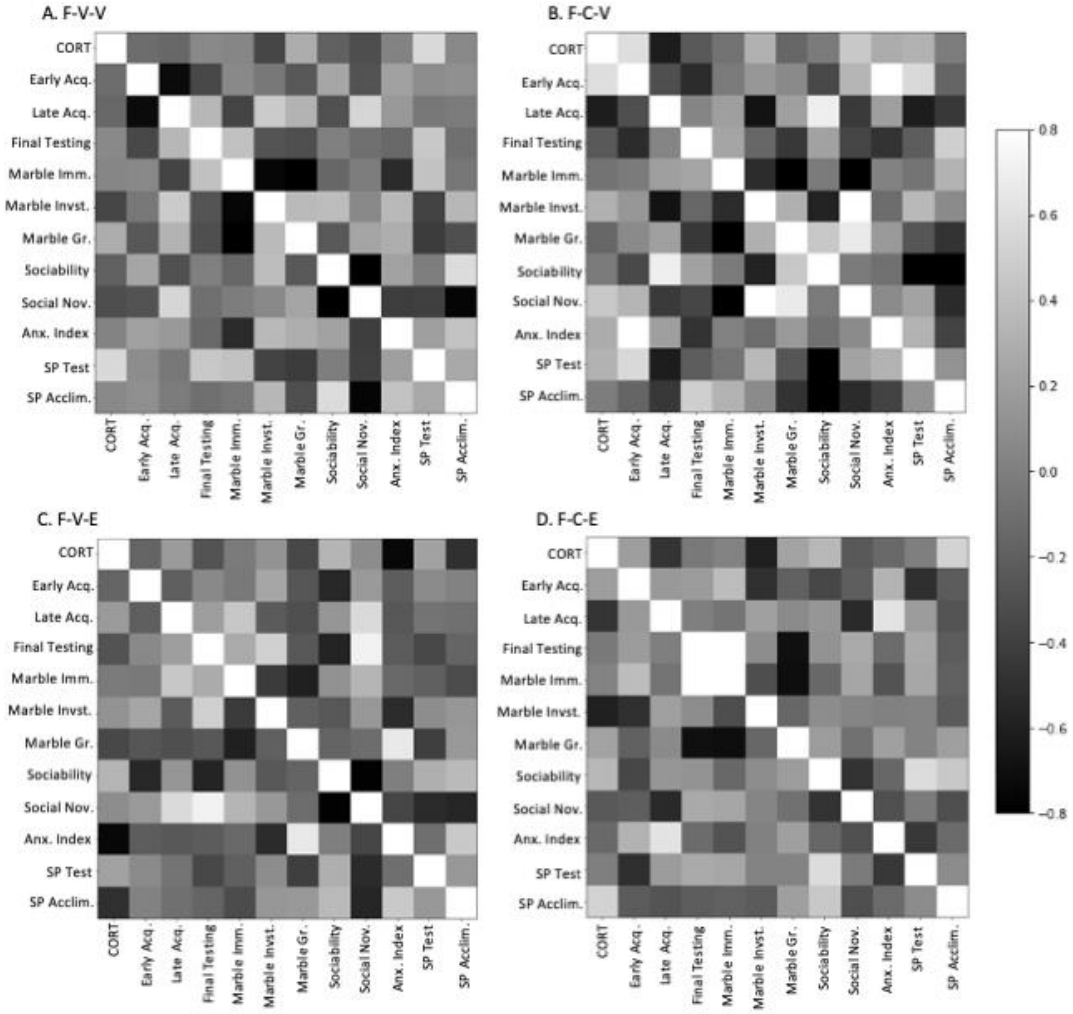

Fig 6.9: Female Spearman Correlation Heatmap Diagrams. A) Females treated with stress hormone vehicle and ovarian hormone vehicle. B) Females treated with CORT and ovarian hormone vehicle. C) Females treated with stress hormone vehicle and E2. D) Females treated with CORT and E2. Order of behaviors is CORT, RAWM early acquisition errors, RAWM late acquisition errors, RAWM final testing phase errors, Marble burying immobility, Marble burying investigation, Marble burying grooming, Sociability index, Social novelty index, EPM anxiety index, 1% SP, 2% acclimation SP. Scale bar ranges from spearman coefficient of -0.8 (black) to 0.8. (white).

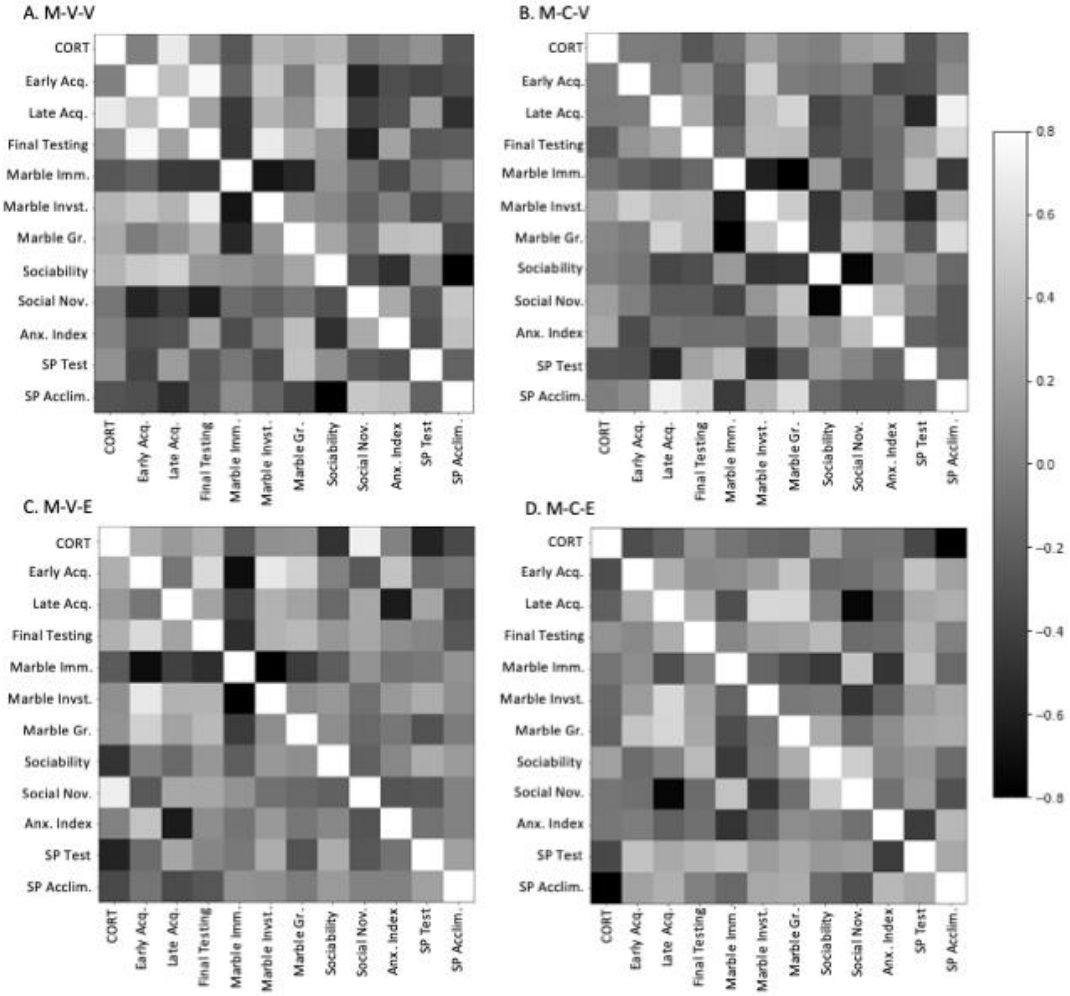

Fig 6.10: Male Spearman Correlation Heatmap Diagrams. A) Males treated with stress hormone vehicle and ovarian hormone vehicle. B) Males treated with CORT and ovarian hormone vehicle. C) Males treated with stress hormone vehicle and E2. D) Males treated with CORT and E2. Order of behaviors is CORT, RAWM early acquisition errors, RAWM late acquisition errors, RAWM final testing phase errors, Marble burying immobility, Marble burying investigation, Marble burying grooming, Sociability index, Social novelty index, EPM anxiety index, 1% SP, 2% acclimation SP. Scale bar ranges from spearman coefficient of -0.8 (black) to 0.8. (white).

APPENDIX A

INSTITUTIONAL ANIMAL USE APPROVAL

Institutional Animal Care and Use Committee (IACUC)
Office of Research Integrity and Assurance
Arizona State University
660 South Mill Avenue, Suite 315
Tempe, Arizona 85287-6111
Phone: (480) 965-4387 *FAX:* (480) 965-7772

Animal Protocol Review

ASU Protocol Number:	**17-1520R**
Protocol Title:	**Recovery from Chronic Stress**
Principal Investigator:	**Cheryl Conrad**
Date of Action:	**7/28/2016**

The animal protocol review was considered by the Committee and the following decisions were made:

The protocol was approved by Full Committee Review as modified.

If you have not already done so, documentation of Level III Training (i.e., procedure-specific training) will need to be provided to the IACUC office before participants can perform procedures independently. For more information on Level III requirements see https://researchintegrity.asu.edu/training/animals/levelthree.

Total # of Animals:	**1,008**	
Species:	**Rats**	**Pain Level: C-264; D-24; E-720**
Protocol Approval Period:	**7/28/2019 – 7/27/2019**	
Sponsor:	**N/A**	
ASU Proposal/Award #:	**N/A**	
Title:	**N/A**	

Signature: Augustowski for C Johnson Date: 8/2/2016
IACUC Chair or Designee

Cc: IACUC Office
IACUC Chair

Institutional Animal Care and Use Committee (IACUC)
Office of Research Integrity and Assurance
Arizona State University
660 South Mill Avenue, Suite 312
Tempe, Arizona 85287-6111
Phone: (480) 965-6788 *FAX:* (480) 965-7772

Animal Protocol Review

ASU Protocol Number: **19-1722R**
Protocol Title: **Chronic Stress and Cognition**
Principal Investigator: **Cheryl Conrad**
Date of Action: **6/27/2019**

The animal protocol review was considered by the Committee and the following decisions were made:

The protocol was approved.

If you have not already done so, documentation of Level III Training (i.e., procedure-specific training) will need to be provided to the IACUC office before participants can perform procedures independently. For more information on Level III requirements see https://researchintegrity.asu.edu/animals/training.

Total # of Animals: **220**
Species: **Rats** **Unalleviated Pain/Distress: Yes**

Protocol Approval Period: **6/27/2019 – 6/26/2022**

Sponsor: **N/A**
ASU Proposal/Award #: **N/A**
Title: **N/A**

Signature: _____ for C. Shalley Date: 7/8/2019
IACUC Chair or ***Designee***

Cc: IACUC Office
IACUC Chair