

**OVARIAN HORMONES AS DETERMINANTS OF RISK AND RESILIENCE TO
STRESS: BEHAVIOURAL, NEUROPLASTIC, AND NEUROIMMUNE OUTCOMES**

by

Rand Mahmoud

B.Sc. (Hons.), The University of British Columbia, 2011

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The following individuals certify that they have read, and recommend to the Faculty of Graduate and Postdoctoral Studies for acceptance, the dissertation entitled:

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submitted by Rand Mahmoud in partial fulfillment of the requirements for
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Examining Committee:

Dr. Liisa Galea
Supervisor

Dr. Joanne Weinberg
Supervisory Committee Member

Dr. Rebecca Todd
University Examiner

Dr. Yvonne Lamers
University Examiner

Additional Supervisory Committee Members:

Dr. Victor Viau
Supervisory Committee Member

Dr. Catharine Winstanley
Supervisory Committee Member

Abstract

Major Depressive Disorder (MDD) affects twice more women than men, yet little attention is paid to female-specific factors that may contribute to this disparity. Ovarian hormones regulate mood and influence MDD, but their roles are complex and sometimes contradictory. Importantly, ovarian hormones regulate processes and systems that are compromised in MDD, including neural plasticity, stress response, and immune systems. To clarify the complex roles of ovarian hormones in MDD models, this thesis examined ovarian hormones at the intersection of stress, neuroplasticity, and neuroinflammation. Chapter 2 investigated the effects of long-term ovariectomy on the expression of depressive-like phenotypes and antidepressant efficacy in middle-aged female rats exposed to chronic unpredictable stress (CUS). Under CUS conditions, long-term ovariectomy increased depressive- and anxiety-like behaviour and impaired hypothalamic-pituitary-adrenal (HPA) axis negative feedback function. The selective serotonin reuptake inhibitor fluoxetine had limited behavioural efficacy, but significant efficacy on neural and endocrine measures, as seen by increased adult hippocampal neurogenesis, reduced microglial number, and enhanced HPA axis negative feedback function. Chapter 3 investigated the effects of long-term ovariectomy on the neuroinflammatory and behavioural consequences of sub-chronic stress exposure in middle-aged mice. Under non-stress conditions, long-term ovariectomy modestly increased depressive-like behaviour, but robustly modified the central cytokine milieu, as evidence by reduced concentrations in the frontal cortex and increased concentrations in the hippocampus. Interestingly, intact mice showed a greater behavioural susceptibility to the depressive-like effects of sub-chronic stress exposure, and this was coupled with an exaggerated neuroinflammatory response in the frontal cortex and hippocampus. Chapter 4 dissected the role of estrogen receptor (ER) α and β in regulating depressive-like phenotypes

under CUS conditions in young-adult female mice. CUS exposure increased depressive-like behaviour, increased cytokine concentrations and reduced postsynaptic density protein-95 expression in the hippocampus and frontal cortex, effects that were largely driven by groups treated with ER α and ER β agonists. Further, in a CUS-independent manner, 17 β -estradiol increased neurogenesis in the dorsal hippocampus, blunted the corticosterone response to an acute stressor, and increased anxiety-like behaviour. Collectively, these findings shed light on the complex roles of ovarian hormones in regulating depressive-like behaviour, and in modulating neuroplastic and neuroimmune signatures.

Lay Summary

Women are approximately twice as likely as men to be diagnosed with Major Depressive Disorder (MDD). Moreover, the postpartum period and the transition to menopause carry an increased risk for depression in women. This suggests that fluctuations or reductions in sex hormones may contribute to depression in women. Using rodent models of depression based on stress exposure, the experiments presented in this thesis explored the role of ovarian hormones in the development of behavioural and brain changes that parallel depression symptoms in humans. We found that ovarian hormones influence depression-like behaviour in a complex manner, providing protection or increasing risk, depending on several factors including age. Further, ovarian hormones influenced the effects of stress exposure on the brain, including inflammation and neuroplasticity. Collectively, this work suggests that ovarian hormones are important determinants of depression-like behaviour, and influence brain mechanisms that may contribute to MDD.

Preface

Please note that I have published under the surname “Eid” starting in 2019, and “Mahmoud” prior to that.

Distinct portions of **Chapter 1** were published in two review papers: 1) Eid, R.S, Gobinath A.R., Galea L.A.M. (2019). Sex differences in depression: Insights from clinical and preclinical studies. *Progress in Neurobiology*. 176: 86-102. This review was conceived, planned, and written by all three authors. 2) Mahmoud, R., Wainwright, S.R., Galea, L.A.M. (2016). Sex hormones and adult hippocampal neurogenesis: Regulation, implications, and potential mechanisms. *Frontiers in Neuroendocrinology*. 41:129-152. This review was conceived and planned by all three authors. Mahmoud and Dr. Wainwright wrote the review with supervision and feedback from Dr. Galea.

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All animal studies presented in this thesis were conducted in accordance with ethical guidelines set by the Canadian Council on Animal Care and were approved by the Animal Care Committee at the University of British Columbia (certificates A12-0201 and A16-0252).

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List of Abbreviations

ABC	avidin-biotin complex
ACTH	adrenocorticotropic hormone
ANOVA	analysis of variance
AVP	arginine vasopressin
BDNF	brain-derived neurotrophic factor
BrdU	5-bromo-2-deoxyuridine
CMS	chronic mild stress
CORT	corticosterone
CRH	corticotropin-releasing hormone
CUS	chronic unpredictable stress
CXCL1	chemokine (C-X-C motif) ligand 1
DAB	diaminobenzidine
DCX	doublecortin
DEX	dexamethasone
dIPFC	dorsolateral prefrontal cortex
DPN	diarylpropionitrile
DSM-5	diagnostic and statistical manual of mental disorders, fifth edition
E2	17 β -estradiol
ECLIA	electrochemiluminescence immunoassay
ER	estrogen receptor
ERE	estrogen response element
FLX	fluoxetine

FSH	follicle stimulating hormone
FST	forced swim test
GCL	granule cell layer
GM-CSF	granulocyte-macrophage colony-stimulating factor
GnRH	gonadotropin releasing hormone
GPER	G-protein coupled estrogen receptor
GR	glucocorticoid receptor
HPA	hypothalamic-pituitary-adrenal
HPG	hypothalamic-pituitary-gonadal
Iba-1	Ionized calcium binding adaptor molecule-1
IFN- γ	interferon- γ
IHC	immunohistochemistry
IL	interleukin
IR	immunoreactive
LH	luteinizing hormone
LLOD	lower limit of detection
LPS	lipopolysaccharide
MAPK	mitogen-activated protein kinase
MDD	major depressive disorder
ML	molecular layer
mPFC	medial prefrontal cortex
MR	mineralocorticoid receptor
NDS	normal donkey serum

NGS	normal goat serum
NHS	normal horse serum
NPC	neural progenitor cell
NSF	novelty suppressed feeding
OVX	ovariectomized
PB	phosphate buffer
PBS	phosphate-buffered saline
PC	principal component
PCA	principal component analysis
pERK	phosphorylated extracellular signal-regulated kinase
PFA	paraformaldehyde
PFC	prefrontal cortex
pJNK	phosphorylated c-Jun NH ₂ -terminal kinase
PMDD	premenstrual dysphoric disorder
PPD	postpartum depression
PPT	propylpyrazole-triol
PSA-NCAM	polysialylated neuronal cell adhesion molecule
PSD-95	postsynaptic density protein 95
PVN	paraventricular nucleus
RIA	radioimmunoassay
SERM	selective estrogen receptor modulators
SGZ	subgranular zone
SSRI	selective serotonin reuptake inhibitor

ST	splash test
TBS	Tris-phosphate buffer
TCA	Tricyclic antidepressant
TNF- α	tumor necrosis factor- α
TSPO	translocator protein
TST	tail suspension test
VEGF	vascular endothelial growth factor
vmPFC	ventromedial prefrontal cortex

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Chapter 1: Introduction

1.1 Major depressive disorder

Major depressive disorder (MDD) is a significant contributor to the global burden of disease, and the leading cause of disability worldwide (Ferrari et al., 2013; World Health Organization, 2017; James et al., 2018). Lifetime prevalence estimates of MDD vary across populations (Kessler and Bromet, 2013), but reach approximately 20% in some national epidemiological surveys (Hasin et al., 2018). MDD is a serious and often recurrent disorder that, beyond diminishing quality of life (Rapaport et al., 2005), is a significant predictor of all-cause mortality (Chesney et al., 2014), is associated with increased risk for a variety of chronic physical conditions (Scott et al., 2016), predicts poorer prognosis and more rapid progression of other illnesses (De Groot et al., 2001; Modrego and Ferrández, 2004; Carney and Freedland, 2017), and in severe cases leads to suicide (Bostwick and Pankratz, 2000). The economic burden of depression is also substantial, where it is estimated that lost productivity due to MDD costs the Canadian economy \$32.2 billion/year (Conference Board of Canada, 2016). Unfortunately, despite ongoing research efforts that have improved our understanding of the etiology and pathophysiology of MDD, significant gaps in treatment remain. Currently available antidepressants are efficacious in alleviating depression symptoms in only a subpopulation of individuals (Trivedi et al., 2006; Akil et al., 2017), and efficacy eventually declines in some responders (Byrne and Rothschild, 1998), underscoring the need for continued research.

MDD is characterized by two primary symptoms, depressed mood and anhedonia, at least one of which must be present for a minimum of two weeks for an individual to meet diagnostic criteria of the fifth edition of the Diagnostic and Statistical Manual of Mental Disorders (DSM–

5)(American Psychiatric Association, 2013). The diagnostic criteria also include other core symptoms such as significant changes in weight or sleep, psychomotor agitation or retardation, fatigue or reduced energy, feelings of guilt or worthlessness, reduced ability to think or concentrate, and suicidal ideation (American Psychiatric Association, 2013). Importantly, MDD is a highly heterogeneous disorder in clinical presentation, which is reflected by specifiers outlined in the DSM-5, including but not limited to MDD “with atypical features”, “with melancholic features”, “with mixed features”, and “with anxious distress”. The heterogeneity in clinical presentation is also echoed by differences in biomarkers (Drysdale et al., 2017; Strawbridge et al., 2017) and suggests differences in etiology.

In the next sections I overview sex differences in MDD and the role of ovarian hormones in the disorder. I then discuss the link between stress and MDD, immune dysregulation in MDD, and the neurobiology of MDD with a focus on the hippocampus and prefrontal cortex. When the evidence is available, these topics are discussed through a lens of biological sex and the influence of ovarian hormones. The series of experiments presented in chapters 2-4 of this thesis explore the role of ovarian hormones in stress-based rodent models of MDD, with the overarching goal of elucidation how ovarian hormones can influence the behavioural outcomes of stress exposure, antidepressant efficacy, and markers of neuroplasticity and neuroinflammation.

1.1.1 Sex differences in major depressive disorder

Women are approximately twice as likely as men to be diagnosed with MDD (Bromet et al., 2011; Kessler and Bromet, 2013), an effect that was corroborated by a recent meta-analysis of representative national samples (Salk et al., 2017). In addition to marked differences in

prevalence rates, the clinical presentation of MDD also differs between the sexes. Compared to men, women with MDD are more likely to experience higher symptom severity (Marcus et al., 2005, 2008), and are more likely to report somatic symptoms such as pain and excessive fatigue (Silverstein, 2002; Marcus et al., 2008; Penninx et al., 2013). Women are also more likely than men to experience MDD with atypical features, which include hypersomnia and weight gain (Angst et al., 2002; Blanco et al., 2012; Marcus et al., 2005; Schuch et al., 2014). Sex also influences comorbidities in individuals with a primary diagnosis of MDD, as women have higher rates of comorbid anxiety (Schuch et al., 2014), whereas men have higher rates of comorbid substance use disorders (Marcus et al., 2008; Schuch et al., 2014).

Sex is also a factor that may influence the therapeutic response to currently available antidepressants (reviewed in Sramek et al., 2016). For example, several studies found that women respond more favourably to selective serotonin reuptake inhibitors (SSRIs) (Kornstein et al., 2000; Khan et al., 2005; Berlanga and Flores-Ramos, 2006; Young et al., 2009; Yang et al., 2011). Further, this effect appears to be more salient in younger, premenopausal women (Kornstein et al., 2000; Naito et al., 2007), perhaps pointing to synergistic effects between serotonin and ovarian hormones. On the other hand, men were found to respond more favourably to tricyclic antidepressants (TCAs) (Kornstein et al., 2000). Importantly, the literature on sex differences in antidepressant efficacy remains largely inconclusive, as several other reports indicate similar efficacy of TCAs and SSRIs between men and women (Parker et al., 2003; Wohlfarth et al., 2004; Kornstein et al., 2014). Few clinical studies have integrated sex as a variable in their analyses of the rapid-acting antidepressant actions of the N-methyl-D-aspartate antagonist ketamine, however, one study did not find sex to be associated with treatment response (Niciu et al., 2014). In general, sex differences in the pharmacokinetics and

pharmacodynamics of antidepressants can lead to differences in efficacy and tolerability (Bigos et al., 2009; Damoiseaux et al., 2014). The continued investigation of the role of sex in the therapeutic response to currently available antidepressants is warranted in order to reconcile the abovementioned inconsistencies in the literature, with a careful controlling of age, dose and duration of treatment, as well as diagnostic scales as factors (Sramek et al., 2016). Overall, sex differences in the manifestation of MDD suggest a need for dissimilar treatments. The literature discussed ahead with regards to the molecular signature of MDD further indicates that biological sex should be considered in efforts surrounding the discovery and development of new pharmacotherapies.

Recent insight into sex differences in the molecular signature of MDD may shed light on why men and women show differences in disease prevalence, presentation, and possibly treatment efficacy. Notably, a recent RNA-sequencing study examined MDD-related transcriptional changes across six brain regions in both men and women (Labonté et al., 2017). The authors found that in both sexes and across all brain regions, MDD was associated with significant changes in transcriptional patterns. More intriguingly, they detected only 5-10% overlap in MDD-related transcriptional profiles between the sexes across all brain regions (Labonté et al., 2017). Next, Labonté and colleagues employed co-expression network analyses to examine MDD-associated gene modules. Interestingly, in men, MDD-associated modules were enriched for genes expressed in several cell types including neurons, astrocytes, and microglia, whereas in women, modules were enriched predominantly for neuronal genes (Labonté et al., 2017). These findings suggest that the transcriptional changes associated with MDD may affect different cell types in the brains of males and females, pointing to differences in disease mechanisms. Another study performed a large-scale gene expression meta-analysis of

corticolimbic regions from individuals with MDD and matched controls (Seney et al., 2018), and largely recapitulated the findings of Labonté and colleagues. Seney et al. (2018) reported little overlap in MDD-related transcriptional changes between the sexes, and further found that a large number of genes displayed expression changes in opposite directions between males and females with MDD (Seney et al., 2018). Further gene-ontology analyses suggested that the expression of synapse-related genes was reduced in males but increased in females (Seney et al., 2018). On the other hand, expression of microglia- and oligodendrocyte-related genes was increased in males but decreased in females (Seney et al., 2018). There were some notable differences in brain regions examined between the two studies which likely contributes to differences in findings (Labonté et al., 2017; Seney et al., 2018). Nonetheless, these findings collectively point to sex differences in disease mechanisms, underscoring the need for investigating sex-specific factors that may contribute to MDD, and ultimately the development of sex-based pharmacotherapies.

1.1.2 Ovarian hormones in major depressive disorder

Sex differences in MDD point to a role of sex hormones in the disease mechanisms. Indeed, the onset of sex differences in prevalence rates coincides with puberty (Twenge and Nolen-Hoeksema, 2002; Salk et al., 2017), a time when concentrations of sex steroids rise in both sexes (Abreu and Kaiser, 2016)(Figure 1.1). Further, sex differences in prevalence rates are most pronounced during the reproductive years of women (Gutiérrez-Lobos et al., 2002). The overall higher prevalence of MDD in women, and the pattern of sex differences in prevalence rates across the lifespan, support the notion that ovarian hormones may increase risk for MDD in women. On the other hand, events or periods that are characterized by large fluctuations or reductions in ovarian hormones are associated with a heightened risk for depression in women,

supporting the contrasting notion that ovarian hormones may confer resilience against MDD. For example, the postpartum period is a time of increased risk, as approximately 10-15% of mothers develop postpartum depression (PPD; (Goodman, 2007; Wisner et al., 2013; Woody et al., 2017). It is hypothesized that the rapid drop in sex steroids after parturition underlies the etiology of PPD, and this is supported by evidence from human and rodent studies (Bloch et al., 2000; Galea et al., 2001; Green et al., 2009). Even in women that are not pregnant, pharmacologically-induced ovarian hormone-withdrawal increased depressive symptoms in a manner that correlated with the net reduction in 17 β -estradiol (Frokjaer et al., 2015). Additional support for the notion that ovarian hormones may protect against MDD is derived from evidence of premenstrual exacerbation of depressive symptoms in women with MDD (Kornstein et al., 2005), and from Premenstrual Dysphoric Disorder (PMDD) in which affective symptoms are typically restricted to the late luteal phase of the menstrual cycle, when estradiol and progesterone levels are declining (Reed and Carr, 2000; American Psychiatric Association, 2013)(Figure 1.1). The transition to menopause is also associated with an increased risk for first onset and recurrent MDD (Freeman et al., 2004, 2006, 2014; Cohen et al., 2006; Bromberger et al., 2007), and it is postulated that declining ovarian function during that time could precipitate depression, at least in a subpopulation of susceptible women. This is supported by a randomized clinical trial in which estradiol withdrawal after three weeks of treatment significantly increased depressive symptoms only in women with a history of perimenopausal MDD (Schmidt et al., 2015). Ovarian hormones may also be implicated in antidepressant efficacy. For example, some studies find SSRIs to be less efficacious in post- compared to pre-menopausal women (Kornstein et al., 2000; Naito et al., 2007), and efficacy is enhanced in postmenopausal women prescribed

hormone therapy (Thase et al., 2005). In addition, estradiol shows some efficacy as a standalone or adjunct treatment in women with MDD (Rasgon et al., 2007; Moses-Kolko et al., 2009).

Animal studies investigating the effects of ovarian hormone deprivation on depressive-like phenotypes have produced somewhat equivocal findings, especially within the context of valid animal models of MDD. There is general consensus that ovariectomy, without other manipulations, increases depressive-like behaviour in rats and mice (Bernardi et al., 1989; Rachman et al., 1998; Bekku and Yoshimura, 2005; Bekku et al., 2006; de Chaves et al., 2009; Li et al., 2014). What is less clear, however, is whether this effect is transient or persistent, as some studies find a restoration of depressive-like behaviour after longer durations of ovarian hormone deprivation whereas others do not (de Chaves et al., 2009; Walf et al., 2009b; Estrada-Camarena et al., 2011). Furthermore, studies investigating how ovariectomy alters depressive-like behaviour within validated animal models of MDD have reported contrasting effects. Specifically, in response to various stress paradigms, ovariectomy has been shown to either increase (Nakagawasaki et al., 2009; Lagunas et al., 2010) or mitigate (LaPlant et al., 2009; Finnell et al., 2018) the depressive-like outcomes of stress exposure. Interestingly, recent studies shed light on genetic factors that may contribute to enhanced sensitivity to fluctuations in ovarian hormones (Bath et al., 2012; Marrocco et al., 2018). Female mice carrying the Brain-Derived Neurotrophic Factor (BDNF) Valine66Methionine polymorphism show increased anxiety-like behaviour coinciding with puberty, increased anxiety-like behaviour during the estrus phase when estradiol concentrations decline (Bath et al., 2012), and increased anxiety- and depressive-like behaviour in response to 17 β -estradiol replacement after ovariectomy (Marrocco et al., 2018).

Taken together, ovarian hormones are certainly implicated in MDD and in depressive-like behaviour, yet their roles are complex and in some cases contradictory. These complexities could be clarified by considering the well-established effects of ovarian hormones in regulating processes and systems that are compromised in MDD, including stress response, immune systems, and neuroplasticity – topics that are reviewed in later sections of this chapter and are central to the experiments presented in this thesis. The complex roles of ovarian hormones could also be due, in part, to the diversity of estrogen signaling, which is briefly described below and revisited in Chapter 4.

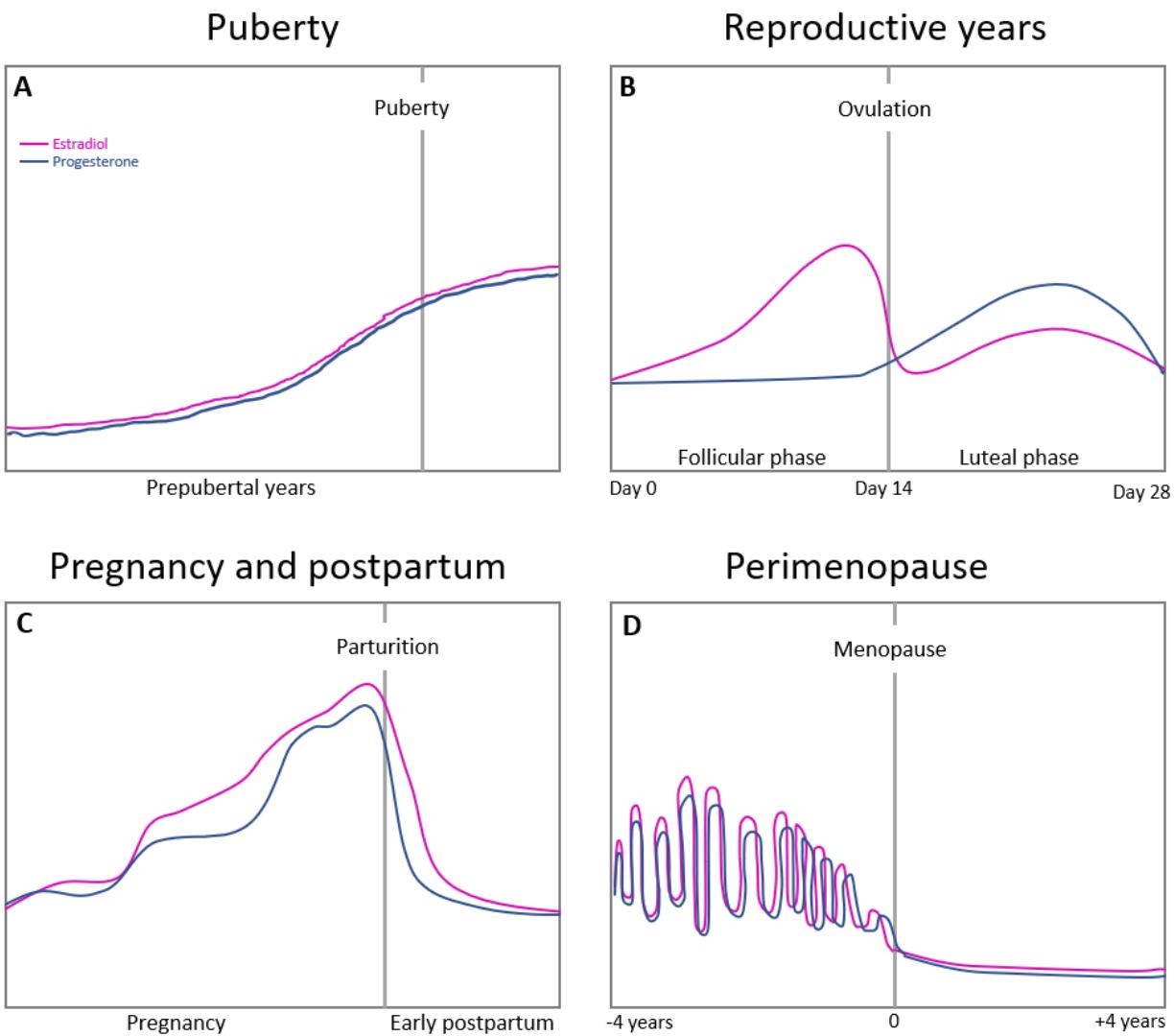


Figure 1.1 Patterns of circulating estradiol and progesterone in female humans during puberty (A), reproductive years (B), pregnancy and postpartum (C), and perimenopause (D). Note: y-axes do not represent equivalent concentrations between boxes A-C, and illustrations depict general patterns of hormone secretion and not accurate concentrations. Illustrations are based on data from Brett and Baxendale, 2001; Brunton and Russell, 2010; Burger 2008; Abreu and Kaiser, 2016.

1.1.3 Estrogens: classes and signaling mechanisms

Estradiol, estrone, and estriol are the major naturally occurring estrogens. In premenopausal women, estradiol, the most potent of the three major estrogens, is also the most abundant (Rannevik et al., 1986). After the menopausal transition, and while all circulating estrogens decline, the ratio of estrone to estradiol is gradually reversed and estrone becomes the more abundant estrogen (Rannevik et al., 1986). Estradiol exists in two optical isomers, 17α -estradiol and 17β -estradiol, with 17β -estradiol being the more potent of the two (Shappell et al., 2010).

Estrogen signaling is highly complex and mediated via at least three types of estrogen receptors (Hewitt and Korach, 2018). Estrogens can bind with the “classical” estrogen receptors (ERs), ER α and ER β , which are typically located in the cytoplasm or nucleus. The bound receptors act as transcription factors to regulate gene expression; they form homo- or hetero-dimers that translocate to the nucleus, interact with various co-regulators, and bind to estrogen response elements (EREs) located in the promoter regions of target genes (Hewitt and Korach, 2018). ER α and ER β are therefore considered to be the mediators of “genomic” actions of estrogens. However, approximately one third of genes regulated by ERs do not include ERE sequences (O’Lone et al., 2004), therefore it is becoming increasingly clear that bound ERs can exert their actions through other mechanisms such as protein–protein interactions with other transcription factors and their respective response elements (Björnström and Sjöberg, 2005). On the other hand, more rapid, “non-genomic” effects of estrogens are mediated through membrane

associated receptors, such as the G-protein coupled estrogen receptor (GPER) (Revankar et al., 2005; Olde and Leeb-Lundberg, 2009). Further, plasma membrane localization of ER α and ER β has also been reported (Levin and Hammes, 2016). Interactions with membrane receptors trigger rapid signaling via the activation of intracellular signaling pathways (e.g. ERK/MAPK, p38/MAPK, PI3K/AKT). Although the terms “genomic” and “non-genomic” are used, it is important to note that even “non-genomic” signaling pathways can result in gene transcription indirectly through downstream cascades, and these genes may differ from those directly regulated through EREs (Marino et al., 2006).

ER α and ER β are in general expressed throughout the brain, with both overlapping and unique distribution patterns depending on region and species. For example, in the cortex, ER β is the predominant receptor in mice and rats, whereas in the hippocampus, the predominant receptor is ER α in mice but ER β in rats (Shughrue et al., 1997; Laflamme et al., 1998; Österlund et al., 1998; Shughrue and Merchenthaler, 2001; Mitra et al., 2003). Further, ER β is highly expressed in the paraventricular nucleus (PVN) of the hypothalamus, whereas ER α is highly expressed in the peri-PVN (McCormick et al., 2002; Weiser and Handa, 2009). GPER is also expressed throughout the brain in female rats, with high densities in the hypothalamus and hippocampus (Brailoiu et al., 2007). Importantly, estrogen receptor expression is likely dynamic as it can be altered by a variety of factors. For example, classical ERs are regulated by age, hormone exposure, and reproductive experience (Jin et al., 2005; Pawluski et al., 2010), and GPER expression in the granule cell layer of the hippocampus is reduced by 17 β -estradiol in female rats (Duarte-Guterman et al., 2015).

1.2 Neurobiology of Stress

Stress can be broadly defined as any real or perceived challenge to homeostasis (Chrousos, 2009). Exposure to a physiological or psychological stressor recruits neural and endocrine systems in an integrated response that facilitates adaptation to the challenge and the return to homeostasis (Ulrich-Lai and Herman, 2009). The endocrine stress response is primarily mediated by the hypothalamic-pituitary-adrenal (HPA) axis, which orchestrates a sequential release of hormones (Herman et al., 2016)(Figure 1.2). Briefly, exposure to a stressor can activate neurons in the parvocellular region of the PVN in the hypothalamus, resulting in the release of corticotropin-releasing hormone (CRH) and arginine vasopressin (AVP) into the median eminence. These peptides are transported by the hypophyseal portal system to the anterior lobe of the pituitary gland and in turn trigger the release of adrenocorticotrophic hormone (ACTH) into the general circulation. In the adrenal gland, ACTH stimulates the synthesis and release of glucocorticoids into the general circulation. The main glucocorticoids are cortisol and corticosterone in humans and rodents, respectively. Along with norepinephrine and epinephrine, glucocorticoids promote the adaptation to stress by mobilizing energy reserves, promoting cardiovascular and pulmonary function, and suppressing digestive, immune, and reproductive function (Herman et al., 2016). Collectively, these responses facilitate survival in the face of a challenge and promote the return to homeostasis. Glucocorticoids bind to glucocorticoid receptors (GRs) and mineralocorticoid receptors (MRs), both of which are expressed in the brain but with different regional distributions (De Kloet et al., 2005). GRs bind glucocorticoids with tenfold lower affinity than MRs (Reul and De Kloet, 1985), therefore extensive GR binding occurs at times of stress- or circadian-induced glucocorticoid peaks. Glucocorticoids terminate the HPA axis stress response primarily via actions on GRs,

at various levels of the HPA axis and upstream corticolimbic brain regions including the hippocampus and medial prefrontal cortex (mPFC) (Diorio et al., 1993; McKlveen et al., 2015; Herman et al., 2016). This glucocorticoid-mediated termination of the stress response is essential, serving to prevent prolonged exposure to elevated concentrations of glucocorticoids, which would be detrimental to virtually all tissues and systems.

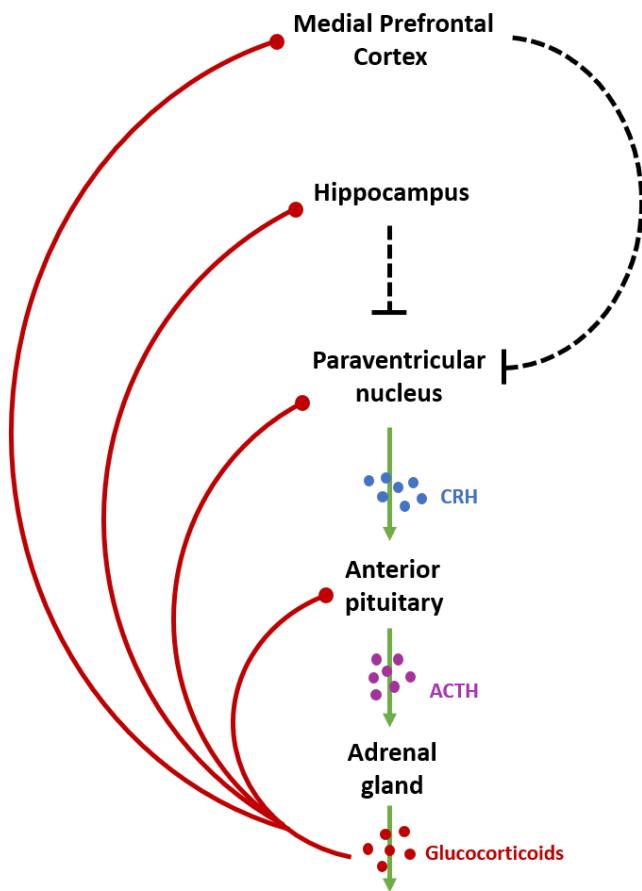


Figure 1.2 Schematic diagram of the hypothalamic-pituitary-adrenal axis. Green arrows indicate stimulatory effects, red lines indicate glucocorticoid-dependent negative feedback, and dashed black lines indicate corticolimbic inhibitory control.

1.2.1 Stress and major depressive disorder

The relationship between stressful life experiences and MDD is well established (Kessler, 1997; Kendler et al., 1999). Indeed, 90% of individuals suffering from MDD cite stressful life events as antecedent to the disorder (Angst et al., 2002; Bale, 2006). Further, the HPA axis is dysregulated

on a number of levels in a subset of individuals with MDD. This is supported by meta-analyses and evidenced by elevated circulating cortisol concentrations, abnormalities in the circadian rhythm of cortisol secretion, and impairments in HPA axis negative feedback function (Ising et al., 2007; Lopez-Duran et al., 2009; Stetler and Miller, 2011; Sher et al., 2013). Successful antidepressant treatment can normalize HPA axis function in MDD in a manner that coincides temporally with symptoms alleviation (Ising et al., 2007). Further, the effects of antidepressants in normalizing HPA axis negative feedback dysregulation are more tied to remission in women than in men (Binder et al., 2009).

It has been suggested that HPA axis dysregulation may vary across MDD subtypes (Porter and Gallagher, 2006; Gold, 2015). For example, several studies report that MDD with melancholic features is associated with HPA axis hyperactivity, as seen by increased basal cortisol concentrations in comparison to healthy controls or to individuals with non-melancholic MDD (Guechot et al., 1988; Wong et al., 2000; Paslakis et al., 2011). On the other hand, individuals suffering from MDD with atypical features are more likely to show basal cortisol concentrations that are equivalent to, or lower than, healthy controls (Asnis et al., 1995; McGinn et al., 1996; Anisman et al., 1999; Stewart et al., 2005). Further, while HPA axis negative feedback function is impaired in a substantial proportion of individuals with melancholic MDD, individuals with atypical MDD are more likely to present with unaffected or even enhanced negative feedback inhibition (Carroll, 1982; Banki et al., 1986; Evans and Nemeroff, 1987; Levitan et al., 2002). Differences in the presentation of HPA axis perturbations between MDD subtypes may be linked to sex differences in MDD. Indeed, it is well established that atypical features are more prevalent in women than in men, whereas some reports indicate that melancholic features are similarly prevalent between the sexes, or using within-sex percentages,

that more men will present with melancholic features than women (Hildebrandt et al., 2003; Marcus et al., 2005; Bogren et al., 2017). Future studies should therefore directly examine whether sex interacts with MDD subtype to affect HPA axis function.

Given that HPA axis dysregulation is commonly observed in MMD, pharmacological interventions that target the HPA axis have been explored, with overall mixed results (Menke, 2019). For example, the competitive GR antagonist mifepristone has shown some efficacy specifically for psychotic depression (Block et al., 2018), a more severe form of MDD that is characterized by delusions and/or hallucinations. However, not all trials support better efficacy of mifepristone over placebo (Schatzberg, 2015). In light of evidence for CRH hyperactivity in MDD (Waters et al., 2015b), CRH receptor antagonism has also been explored as a potential treatment. One clinical trial reported significant symptom alleviation after treatment with the CRH₁ receptor antagonist R121919 (Zobel et al., 2000). However, the clinical development of R121919 was later discontinued due to elevations in liver enzymes (Chen and Grigoriadis, 2005). Other trials using different CRH₁ receptor antagonist have generally failed to show significant symptom alleviation for MDD (Binneman et al., 2008; Spierling and Zorrilla, 2017). Overall, the limited success of agents targeting HPA axis dysfunction in MDD may have resulted from insufficient consideration of patient heterogeneity, including sex differences. It is plausible that only those individuals that already show signs of HPA axis dysregulation will benefit from treatments targeting the HPA axis. Taking this approach to personalized treatment may prove fruitful in future trials.

1.2.2 Interactions between the HPA and hypothalamic-pituitary-gonadal axes

The hypothalamic-pituitary-gonadal (HPG) axis is responsible for the growth and maturation of germ cells and the production and regulation of gonadal hormones in both sexes (Plant, 2015).

Briefly, gonadotropin releasing hormone (GnRH) is released from hypophysiotropic GnRH neurons in the hypothalamus and stimulates the secretion of luteinizing hormone (LH) and follicle stimulating hormone (FSH) from the anterior pituitary, which subsequently control the production of gonadal steroids (Plant, 2015). Androgens and estrogens in turn act via feedback loops to regulate HPG activity, much in the same manner as glucocorticoids function in the HPA axis.

There are robust sex differences in the HPA axis, and extensive bi-directional interactions occur between the HPA and HPG axes in both sexes (reviewed in Goel et al., 2014), but for the purposes of this thesis I will focus on females. Increased HPA axis activity can exert inhibitory effects on all levels of the females HPG axis (Kalantaridou et al., 2004), likely representing an evolutionary mechanism serving to suppress reproductive function in times of increased stress. On the other hand, the female HPG axis also influences HPA function on several levels. Rodent studies indicate that compared to adult males, adult females have higher basal corticosterone concentrations throughout the circadian cycle and mount a greater ACTH and corticosterone response to acute stressors (Atkinson and Waddell, 1997; Aloisi et al., 1998; Viau et al., 2005; Sterrenburg et al., 2012). This greater responsiveness of the HPA axis in females is driven by sex differences at various levels of the axis. For example, studies generally report greater expression of CRH and AVP in the PVN of females, both at baseline and in response to acute stressors (Seale et al., 2004; Viau et al., 2005;

Iwasaki-Sekino et al., 2009). Absolute and relative adrenal mass is also greater in females than in males, and this effect is driven by a larger number of cells in the zona fasciculata of the adrenal cortex (Malendowicz, 1980). This indicates a greater capacity for glucocorticoid synthesis, which is corroborated by greater corticosterone concentrations in response to ACTH administration (Kitay, 1961).

Sex differences in the HPA axis are due, in part, to the influence of sex hormones. The general consensus is that androgens exert inhibitory effects on the HPA axis in males, whereas estrogens exert stimulatory effects on the HPA axis in females (reviewed in Goel et al., 2014). Estrogens, and 17 β -estradiol in particular, stimulate basal HPA activity and potentiate activation of the HPA axis in response to acute stressors (Viau and Meaney, 1991; Seale et al., 2004; Figueiredo et al., 2007). Furthermore, estrogens regulate HPA axis negative feedback function, where in general 17 β -estradiol impairs negative feedback inhibition (Goel et al., 2014). For example, in a study using ovariectomized rats, 17 β -estradiol treatment prolonged the ACTH and corticosterone responses to an acute foot shock stressor (Burgess and Handa, 1992). The same study found that the GR agonist RU 28326 significantly suppressed corticosterone and ACTH responses to an acute ether stressor in vehicle but not 17 β -estradiol treated rats, suggesting that estradiol impairs glucocorticoid-dependent negative feedback inhibition (Burgess and Handa, 1992). Similarly, in ovariectomized rats, the GR agonist dexamethasone reduced the corticosterone response to an acute stressor and the diurnal peak of corticosterone, effects that were not observed after estradiol benzoate treatment (Weiser and Handa, 2009). In another study, corticosterone administration reduced the ACTH response to an acute stressor in intact male but not female rats, indicating reduced sensitivity to glucocorticoid feedback in females (Young, 1996). This effect was partially driven by ovarian hormones, as

ovariectomized females showed intermediate levels of ACTH suppression (Young, 1996). The effects of estrogens on HPA negative feedback function may result, at least in part, from the modulation of GR expression or signaling. Indeed, this is supported by studies showing that GR binding and mRNA in the PVN are reduced by 17 β -estradiol (Turner, 1990; Burgess and Handa, 1993). Further, the effects of estradiol to impair HPA negative feedback inhibition appear to be mediated via interactions with ER α at the level of the PVN (Weiser and Handa, 2009). Although less studied, progesterone may counteract some of the stimulatory effects of estradiol on the HPA axis (Viau and Meaney, 1991). It is important to note that HPA-HPG interactions in females have been primarily investigated at baseline or in response to acute stressors, and not within animal models of depression. To that end, how ovarian hormones can influence HPA axis function under conditions of repeated or chronic stress is not well known and as such investigated in experiments outlined in this thesis.

1.2.3 Stress-based animal models of depression

MDD is a uniquely human condition. However, some features of the disease can be modeled in animals, and despite their limitations, animal models of depression serve as valuable tools in our understanding of the disease and its treatment. Because of the abovementioned link between stress and MDD, most animal models of depression rely on exposure to stressors to produce depressive-like phenotypes (Gobinath et al., 2015; Willner, 2017). Chronic stress models emerged in the early 1980s, following the observation that chronic exposure to severe stressors reduced the consumption of a saccharin solution in male rats, an effect that was interpreted to be akin to anhedonia in MDD (Katz, 1982). The procedure was later adapted to include less severe stressors and termed Chronic Mild Stress (CMS; Willner et al., 1987). In the first publication

using this modified procedure, Willner and colleagues demonstrated that reduced preference for sucrose in response to 5-9 weeks of CMS was restored by chronic treatment with a TCA in male rats (Willner et al., 1987). Since then, CMS and modified versions have been used as a model of depression in thousands of publications (Willner, 2017). In general, stress-based models of depression are not standardized. Substantial differences exist between and within laboratories with respect to the nature of stressors, stress regimen, and the duration of stress exposure. This is also reflected by differences in naming conventions of these models, which include chronic unpredictable stress (CUS) and chronic variable stress (CVS), amongst others. Newer paradigms have also employed sub-chronic exposure to variable stressors, ranging in duration from 3-6 days (LaPlant et al., 2009; Hodes et al., 2015a). In addition, there are commonly used stress-based models that do not rely on exposure to variable stressors, including learned helplessness and chronic social defeat stress (Vollmayr and Henn, 2001; Hollis and Kabbaj, 2014), in addition to chronic administration of corticosterone (Sterner and Kalynchuk, 2010). Overall, this is not to say that differences in stress-based models is a limitation. On the contrary, different protocols could produce different depressive-like phenotypes, and this can be exploited for a better understanding of the underlying biology of different aspects of the human condition.

It can be argued that stress-based models of MDD have strong predictive, face, and construct validity. Predictive validity in animal models of MDD is generally concerned with the response to antidepressant agents or manipulations. Several studies, primarily conducted in males, demonstrate alleviation of depressive-like phenotypes after chronic but not acute treatment with a variety of antidepressants, and no alleviation by agents that do not show clinical efficacy in MDD (reviewed in (Willner, 1997)). Construct validity refers to strong theoretical rationale, which could refer to underlying neurobiological mechanisms or theories of etiology.

Construct validity of stress-based models is supported by strong theoretical rationale that links stress exposure to MDD, and by evidence of similar neurobiological underpinnings to the human condition (Kessler, 1997; Kendler et al., 1999; Willner, 2017). Finally, in support of the face validity of these models, exposure to stressors produces a variety of behavioural and physiological alterations that parallel symptoms and biomarkers of MDD. To name a few, the effects of chronic stress include increased anhedonia-like behaviour, cognitive impairments, changes in body mass, reduced hippocampal volume, dysregulation of HPA axis function, and elevated markers of inflammation in the brain and periphery (reviewed in Krishnan and Nestler, 2011; Vollmayr et al., 2007; Wainwright and Galea, 2013; Willner, 2017). It is critical to note that stress-based models of depression were developed using male rodents, and for decades thereafter have almost exclusively used male subjects. Therefore, it is imperative to keep in mind that our current knowledge surrounding the outcomes of stress exposure remains to be primarily derived from male subjects, and thus may not always translate to females.

1.3 Immune Dysregulation in Major Depressive Disorder

The evidence of immune dysregulation in MDD is extensive (Miller and Raison, 2016; Felger, 2019). At least a subset of individuals with MDD present with hallmarks of increased inflammation, both systemically and in the cerebral spinal fluid, and this is supported by several meta-analyses of the literature (Dowlati et al., 2010; Liu et al., 2012; Haapakoski et al., 2015; Wang and Miller, 2018). These meta-analyses indicate that interleukin-1 β (IL-1 β), interleukin-6 (IL-6) and, tumor necrosis factor- α (TNF- α) are reliably increased in individuals with MDD. Intriguingly, increased systemic inflammation can be predictive of future depression, as one study found that elevated IL-6 concentrations in children at age 9 predicted MDD at age 18

(Khandaker et al., 2014). Further, increased depression symptoms or MDD are commonly observed in response to interferon therapy in a number of diseases, including hepatitis, multiple sclerosis, and malignant melanoma (Franscina Pinto and Andrade, 2016), supporting the hypothesis that inflammation may precede and drive MDD. Based on the collective evidence, it is thought that chronic low-grade inflammation is implicated in the etiology of a subtype of MDD. Inflammation may also be linked to antidepressant efficacy, as poor treatment response is associated with the inability of antidepressants to normalize dysregulated inflammatory profiles (Syed et al., 2018).

Microglia, the resident immune cells of the brain and the primary orchestrators of the neuroinflammatory response (Kettenmann et al., 2013), have also received attention in MDD research (Setiawan et al., 2015, 2018). Microglia are highly dynamic cells that are capable of structural remodeling in response to pathological events or environmental perturbations such as stress exposure (Figure 1.3) (Kreutzberg, 1996; Walker et al., 2013). In the healthy adult brain, microglia typically assume a ramified morphology, characterized by thin, highly branched processes that continuously survey the surrounding parenchyma for signs of pathology (Wu et al., 2015; Wolf et al., 2017). Emerging evidence indicates that beyond surveillance, microglia participate in healthy brain functions including activity-dependent synaptic plasticity and learning and memory (Parkhurst et al., 2013; Schafer et al., 2013; Sipe et al., 2016). Microglia are activated in response to pathological events or homeostatic perturbations, a process characterized by substantial morphological changes including retraction of processes and enlargement of the soma, and this is coupled with increased phagocytic activity and enhanced secretion of pro-inflammatory cytokines (Wolf et al., 2017; Dubbelaar et al., 2018). At the extreme end of the spectrum, microglia can assume an amoeboid morphology completely void of

processes (Figure 1.3). This tight structure-function link has allowed researchers to utilize microglial morphology or protein expression as proxies for functional or activational state (Karperien et al., 2013). In humans, positron emission tomography (PET) radiotracers have been used to measure the binding of translocator protein (TSPO), which is overexpressed in microglia that are activated under inflammatory conditions (Rupprecht et al., 2010). An approximately 30% increase TSPO density was found in several brain regions in individuals with MDD during a Major Depressive Episode (MDE), and symptom severity was significantly associated with increased TSPO density in the anterior cingulate cortex (ACC) (Setiawan et al., 2015). More recently, the same group reported that longer durations of untreated MDD were associated with increased TSPO density (Setiawan et al., 2018). Thus, that the evidence collectively indicates that inflammation is involved in both the onset and progression of MDD.

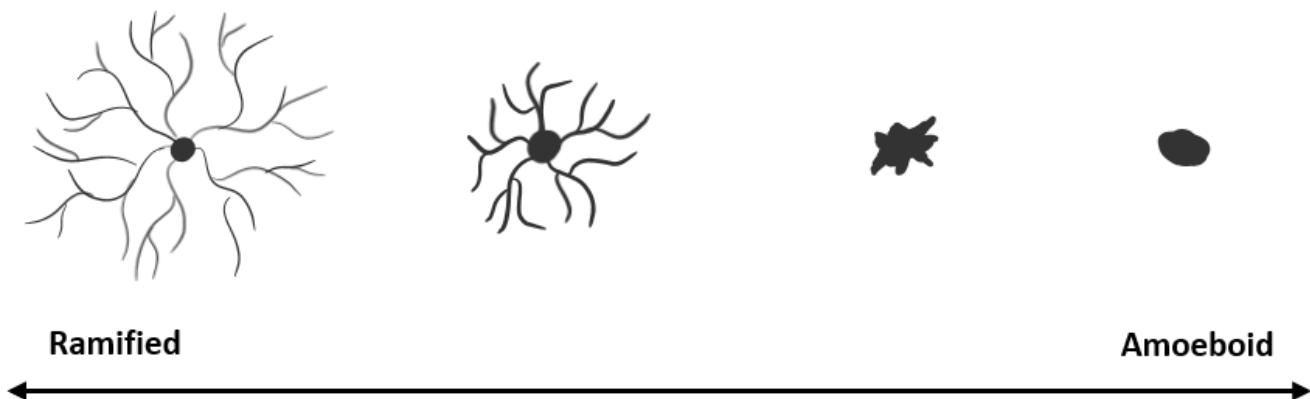


Figure 1.3 Microglia morphology in the adult brain. Microglia assume a wide spectrum of morphologies, ranging from ramified microglia with highly branched thin processes in the healthy brain, to amoeboid microglia completely void of processes under inflammatory conditions.

The link between inflammation and MDD is also substantiated by animal models.

Although mostly derived from male subjects, there is evidence that neuroimmune processes are altered in animal models of MDD (Hodes et al., 2015b; Calcia et al., 2016) and that neuroimmune signaling may underlie stress-induced depressive- or anxiety-like behaviours (Hodes et al., 2014; McKim et al., 2018). For example, indices of increased microglial activation are often reported in stress-based animal models of depression, as supported by a systematic review of the literature (Calcia et al., 2016). Stress-based models also report increased concentrations of inflammatory cytokines in the brain and periphery, although correlations between the two are not always observed (Farooq et al., 2012; Weber et al., 2017). Notable roles of microglia in stress-induced behavioural changes have also been described. For example, in male mice and rats, blocking the CUS-induced alterations in microglia rescued depressive-like behaviour (Kreisel et al., 2014). In male mice, increased anxiety-like behaviour after repeated social defeat stress was driven by the trafficking of peripheral inflammatory monocyte into the brain (Wohleb et al., 2013), and microglia were later found to be responsible for this recruitment of monocytes (McKim et al., 2018). Recently, the same group demonstrated similar effects of social defeat stress to increase microglial activation and brain monocyte accumulation in female mice (Yin et al., 2019). Thus, stress-based animal models provide further evidence that immune dysregulation can be a product and a driver of depressive-like pathology.

1.3.1 Immunomodulatory actions of estrogens

Sex differences in immune functions are well documented and found across the lifespan, mediated in part by chromosomal sex and sex hormones (Klein and Flanagan, 2016). These differences in immune responses are thought to contribute to sex differences in the prevalence

and course of various diseases (Klein and Flanagan, 2016). For example, when compared to males, females are more susceptible to autoimmune diseases but less susceptible to infectious diseases (Van Lunzen and Altfeld, 2014). In the central nervous system, the anti-inflammatory effects of estrogens have been documented in models of stroke and brain injury, which robustly engage the inflammatory response (Pozzi et al., 2006). For example, in ischemic stroke models, estradiol reduces infarct volume and improves behavioral outcomes (Suzuki et al., 2009; Villa et al., 2016). In further support of its anti-inflammatory actions, estradiol or selective estrogen receptor modulators significantly ameliorate microglia inflammatory activity and the production of inflammatory cytokines in response to acute inflammatory challenges, both *in vitro* and *in vivo* (Vegeto et al., 2001, 2008; Baker et al., 2004; Brown et al., 2010; Ishihara et al., 2015). More recently, studies have also unveiled substantial sex differences in microglia, both during development and in adulthood (reviewed in (Villa et al., 2019)). In the adult brain, intriguing sex differences are found in microglial transcriptome and neuroprotective functions (Villa et al., 2018). Female microglia are neuroprotective in a model of ischemia, and this feature is maintained when microglia are transplanted into male brains, suggesting that some of the sex-specific features of microglia are independent of circulating ovarian hormones (Villa et al., 2018).

Estradiol can also produce pro-inflammatory effects, indicating that its immunomodulatory actions are complex (Straub, 2007). Although beyond the focus of this thesis, many factors can determine the immunomodulatory actions of estradiol, including age, the nature of the inflammatory challenge, the dose and timing of estradiol administration in relation to disease, and the target tissue examined (reviewed in (Straub, 2007)). For example, in aged females, a neurotoxic rather than neuroprotective effect of estradiol is observed in ischemic

stroke (Johnson and Sohrabji, 2005; Nordell et al., 2003; Selvamani and Sohrabji, 2010), suggesting that the immunomodulatory and neuroprotective effects of estrogens may vary across the lifespan. Overall, given that biological sex influences immune function and that estrogens have immunomodulatory properties, it is conceivable that these properties could be implicated in MDD- or stress-related inflammation, but as discussed below, this topic remains understudied.

1.3.2 Sex differences in MDD-related inflammation

The current knowledge surrounding sex and sex hormone differences in inflammation and immune processes within the context of MDD is sparse. Relatively few studies to date have examined whether biological sex influences the relationship between peripheral inflammatory markers and depression, and the available evidence remains equivocal, likely because age, menstrual phase, and time of day are not consistently considered. One study reported that MDD was associated with altered inflammatory profiles in women, including increased IL-8 and IFN- γ , and reduced IL-5 (Birur et al., 2017). On the other hand, no significant differences in inflammatory profiles were found between men with or without MDD (Birur et al., 2017). These findings are in contrast with a large cohort study that found elevated inflammatory mediators to be associated with MDD in men but not women, including increased concentrations of the acute phase reactant C-reactive protein (CRP) (Ramsey et al., 2016). Indeed, several other studies also report higher CRP levels to be associated with MDD in men but not women (Elovainio et al., 2009; Vogelzangs et al., 2012; Vetter et al., 2013). On the other hand, another study found that higher levels of CRP were associated with specific depression symptoms and greater depression severity in women with MDD, but no such associations were found in men with MDD (Köhler-Forsberg et al., 2017). The apparent inconsistencies in the literature may point to an insufficient

consideration of factors such as age and steroid hormones, which can influence both MDD and the inflammatory profile. For example, across the menstrual cycle, higher levels of estradiol are associated with reduced CRP levels (Gaskins et al., 2012), indicating an anti-inflammatory effect of estradiol. Both androgens and glucocorticoids also possess immunomodulatory properties (Coutinho and Chapman, 2011; Trigunaite et al., 2015) and circulating levels of androgens display circadian variation in men (Brambilla et al., 2009), as do glucocorticoids in both sexes (Dickmeis, 2009). Thus, an insufficient consideration of time of day can contribute to additional variability in the findings. By accounting for age, time of day, and menstrual cycle phase, future studies should carefully consider the role of steroid hormones in deciphering the relationship between inflammation and MDD in both men and women.

Other studies in non-depressed healthy individuals have investigated sex differences in the depressogenic effects of an inflammatory challenge. In response to a bacterial endotoxin, healthy women show greater increases in depressed mood and feelings of social disconnectedness relative to men (Moieni et al., 2015). Further, feelings of social disconnectedness in response to the challenge were positively correlated with IL-6 and TNF- α levels in women but not in men (Moieni et al., 2015). Another study also found that women exhibited a larger inflammatory response to a bacterial endotoxin relative to men, but this effect was not associated with larger modifications in mood or state anxiety in women (Engler et al., 2016). Thus, while it appears that women mount a larger immune response to an acute inflammatory challenge (Moieni et al., 2015; Engler et al., 2016), whether this translates to greater alterations in mood is not clear.

Very few animal studies to date have systematically investigated whether biological sex or sex hormones influence the relationship between inflammation and depressive-like endophenotypes. These studies have generally investigated sex differences the inflammatory consequences of stress (Hudson et al., 2014; Bollinger et al., 2016), or sickness behaviour following an inflammatory challenge (Pitychoutis et al., 2009; Adzic et al., 2015; Brkic et al., 2016; Sens et al., 2017). A study in rats found that the effects of stress on microglial activation in the mPFC were sex-dependent (Bollinger et al., 2016). At baseline, females display more activated microglia than males, but following acute or chronic restraint stress, only female rats displayed reduced microglial activation (Bollinger et al., 2016). These findings are of interest considering other reports, predominantly in males, showing the opposite trend of increased microglial activation following stress exposure (Calcia et al., 2016). Because alterations in microglial activity have been shown to underlie stress-induced depressive-like behaviour (Kreisel et al., 2014), the findings of Bollinger and colleagues could have implications for sex differences in the behavioural consequences of stress exposure. Another study investigated sex difference in whether prior exposure to stress can prime the neuroinflammatory response to a subsequent stressor (Hudson et al., 2014). Specifically, adult mice were initially exposed to three days of repeated variable stress, then exposed to an acute restraint stressor 6 weeks later. Male mice that were stressed on both occasions displayed a sensitization of IL-1 β in the hippocampus and frontal cortex relative to male mice that were only exposed to acute restraint (Hudson et al., 2014). Interestingly, while baseline cytokine concentrations were higher in female mice, neither the initial exposure to variable stress, nor the re-exposure to an acute stressor had any significant effects on cytokine concentrations in the hippocampus or frontal cortex (Hudson et al., 2014). While keeping in mind that the literature directly examining sex as a factor in the neuroimmune

consequences of stress is extremely limited, it appears that males and females may show differential neuroinflammatory responses to stress, which could be linked to sex hormones and may contribute to sex differences in MDD.

Activation of the immune system using infectious or inflammatory agents induces a host of sickness behaviours that markedly overlap with a number of MDD symptoms, including reduced food intake, anhedonia, lethargy, and reduced activity (Maes et al., 2012). Thus, sickness behaviour is utilized as an immunological model of depression, and is generally achieved via the administration of bacterial endotoxins or inflammatory cytokines (reviewed in (Remus and Dantzer, 2016)). Sickness behaviour can be useful in modeling depression occurring in a subpopulation of individuals in which inflammatory processes are central to the disease mechanisms, but caution should be taken in generalizing findings to other subtypes of depression. Few studies to date have examined sex differences in sickness behaviour and its neurobiological correlates (Pitychoutis et al., 2009; Adzic et al., 2015; Brkic et al., 2016; Sens et al., 2017). Irrespective of sex, acute lipopolysaccharide (LPS) administration in mice resulted in increased depressive- and anhedonia-like behaviour (Sens et al., 2017). However, LPS resulted in longer lasting reductions in grooming behaviour and serotonergic turnover in females, and longer lasting reductions in overall food consumption in males (Sens et al., 2017), suggesting sex differences in the duration of behavioural and neurochemical alterations in response to LPS. In rats, another study reported a rather surprising finding of reduced immobility in the forced swim test following LPS treatment in females, but no significant behavioural alteration in males (Pitychoutis et al., 2009). This was coupled with a larger increase in serotonergic turnover in females relative to males (Pitychoutis et al., 2009). In another study, male and female rats showed similar behavioural alterations following seven days of LPS treatment, including

increased depressive-like behaviour, reduced sucrose consumption, and reduced activity (Adzic et al., 2015). Despite the similarities in behavioural outcomes, reduced hypothalamic brain BDNF was detected in females only, and increases in the inflammatory enzyme cyclooxygenase-2 (COX-2) were detected in males only (Adzic et al., 2015). Using a similar experimental design, the same group found evidence of nuclear translocation of the glucocorticoid receptor in the frontal cortex of male, but not female rats, in response to LPS treatment, despite similar behavioural outcomes (Brkic et al., 2016). Collectively, the data do not strongly support a higher susceptibility of either sex to the behavioural consequences of inflammatory activation. However, the neurobiological mechanisms driving the behavioural consequences of inflammatory agents may differ between males and females, therefore these models can be useful in identifying targets for sex-specific pharmacotherapies in inflammation-related MDD.

1.4 Neurobiology of Major Depressive Disorder

Individuals with depression present with anatomical, functional, neurochemical, cellular, and molecular abnormalities across several brain regions, but corticolimbic regions are particularly highlighted (Krishnan and Nestler, 2008; Koolschijn et al., 2009; Lorenzetti et al., 2009; Schmaal et al., 2016; Dean and Keshavan, 2017; Drysdale et al., 2017; Labonté et al., 2017). The sections below summarize the role of the hippocampus and frontal cortex in MDD and its models, as these regions were the focus of experiments described in Chapters 2-4.

1.4.1 The hippocampus in MDD and stress-based models

The hippocampus is a limbic region that has been extensively examined in the context of MDD. Several studies report reduced hippocampal volume in individuals with MDD relative to controls, an effect which is corroborated by meta-analyses (McKinnon et al., 2009; Schmaal et

al., 2016). Hippocampal volume reductions are primarily observed in individuals with MDD persisting longer than two years, and are generally limited to children and middle-aged to older adults (McKinnon et al., 2009), pointing to protective factors in younger adults. Interestingly, MDD-related volumetric reductions may be more apparent in men than in women (Frodl et al., 2002; Kronmüller et al., 2009), however this trend is not seen across all studies (MacQueen et al., 2003). These differences may be driven by age, as the mean age of participants was substantially younger in the study by MacQueen et al., suggesting that the sex differences volumetric reductions may be more robust later in adulthood. Further, one study found increased hippocampal volume in antidepressant-responding women relative to non-responding women, but this effect was not seen in men (Vakili et al., 2000). Taken together, pre-menopausal women may be more resistant to MDD-related hippocampal volume reductions, and treatment efficacy may be more tightly linked to hippocampal volume restoration in women. Intriguingly, one study found that prescribed antidepressants were associated with increased neurogenesis in the hippocampus of women, but not men (Epp et al., 2013), suggesting a possible mechanism of volumetric restoration.

Post-mortem studies indicate that the hippocampus is compromised in MDD beyond gross volumetric reductions. For example, MDD is associated with decreased expression of pre- and post-synaptic genes in the dentate gyrus and CA1 subfields of the hippocampus (Duric et al., 2013), indicating alterations in synaptic function or reductions in synapse number. Further, the packing density of glia, pyramidal, and granule cells is increased in all hippocampal subfields, suggesting a reduction in neuropil which could be linked to overall volumetric reductions (Stockmeier et al., 2004). The number of granule neurons is also reduced in the anterior, but not posterior, dentate gyrus of individuals with untreated MDD (Boldrini et al., 2013), perhaps

contributing to reports of overall reduced hippocampal volume (McKinnon et al., 2009). The restriction of this effect to the anterior region is also of importance, as that hippocampus shows functional heterogeneity across the anterior-posterior axis, such that the anterior hippocampus (ventral in rodents) plays a more prominent role in affective function and stress regulation, while the posterior hippocampus (dorsal in rodents) plays a more prominent role in cognitive function (Fanselow and Dong, 2010). However, it is important to note that the ventral hippocampus also plays important roles in cognition, including spatial working memory, contextual fear conditioning, olfactory working memory, and olfactory pattern separation (Ferbinteanu et al., 2003; Rudy and Matus-Amat, 2005; Kesner et al., 2011; Weeden et al., 2014). Recently, whole-transcriptome RNA-sequencing also showed significant alterations in inflammation-related genes in the hippocampus of individuals with MDD (Mahajan et al., 2018). Thus, collectively, the hippocampus appears to be compromised in MDD on a number of levels, ranging from gross morphology to cellular, synaptic, and molecular abnormalities.

The hippocampus highly expresses GRs (McEwen et al., 1968; Reul and De Kloet, 1985), and there is a wealth of evidence from rodent studies indicating that this region is particularly vulnerable to stress exposure. For example, repeated or chronic stress exposure reduces adult hippocampal neurogenesis (discussed below), results in dendritic atrophy in pyramidal neurons of the CA3 region, and in alterations in hippocampal gene expression (reviewed in (McEwen et al., 2016)). Importantly, stress exposure can result in sex-dependent effects on neuronal architecture in the hippocampus. For example, in CA3 pyramidal neurons, chronic restraint stress produced atrophy of the apical dendritic arbor in male rats, but the basal dendritic arbor in female rats (Galea et al., 1997; McLaughlin et al., 2010). Sex differences in the effects of stress on the hippocampus could be influenced by the sex hormones, as they modulate

many aspects of hippocampal plasticity. Ovarian hormones, for example, influence adult hippocampal neurogenesis (discussed ahead), the density of hippocampal synapses (Woolley and McEwen, 1992), neuronal excitability in CA1 and CA3 regions (Scharfman et al., 2003), and long-term potentiation (LTP) in the CA1 region (Warren et al., 1995). Thus, it is likely that interactions between stress and ovarian hormones can influence structural and functional plasticity in hippocampus.

1.4.2 Adult hippocampal neurogenesis: overview and functional significance

The hippocampus is a highly plastic structure that maintains the ability to produce new neurons throughout life in many mammalian species including humans (Eriksson et al., 1998; Gould et al., 1999; Boldrini et al., 2018). Adult-born granule neurons in the hippocampus arise from a population of neural stem cells (NSCs) and undergo several consecutive developmental stages before integrating into the existing hippocampal circuitry (Kempermann et al., 2015; Gonçalves et al., 2016). More specifically, type 1 radial glia-like cells (RGLs) reside in the subgranular zone (SGZ) of the hippocampal dentate gyrus and are thought to represent the pool of NSCs that give rise to adult-born granule cells. Type 1 cells can generate proliferating Type 2 intermediate progenitor cells (IPCs), which in turn give rise to Type 3 neuroblasts. Neuroblasts can subsequently differentiate and mature into granule neurons. A simplified illustration of the developmental stages of adult-born hippocampal neurons is depicted in Figure 1.4. For the purposes of this thesis, the process of adult hippocampal neurogenesis can be separated into four general stages: cell proliferation, migration, differentiation, and survival. Different experimental manipulations can affect one or more of these stages (Galea et al., 2013; Lucassen et al., 2015; Opendak and Gould, 2015), and this can be examined using various endogenous or exogenous

markers. For example, cell proliferation can be examined using the endogenous protein Ki67, which is expressed during all active phases of the cell cycle but not during G₀ phase (Scholzen and Gerdes, 2000). The polysialylated neuronal cell adhesion molecule (PSA-NCAM) is highly expressed in newly generated and migrating cells (Bonfanti, 2006), thus its expression can be used to examine cells undergoing migration into the granule cell layer (GCL). Immature neurons can be examined through the expression of doublecortin (DCX), a microtubule-associated protein that is expressed in immature neurons for 21 days after production in adult rats (Brown et al., 2003). Finally, adult-born cells can be identified through the administration of exogenous markers, including 5-bromo-2-deoxyuridine (BrdU). BrdU is a DNA synthesis marker that incorporates into the DNA of dividing cells by replacing thymidine nucleotides within a 2-hour window after administration (Miller and Nowakowski, 1988; Taupin, 2007). As such, BrdU allows for cell “birth-dating”, and its co-expression with the neuronal marker NeuN can be used to determine the proportion of BrdU-expressing cells that have differentiated into neurons.

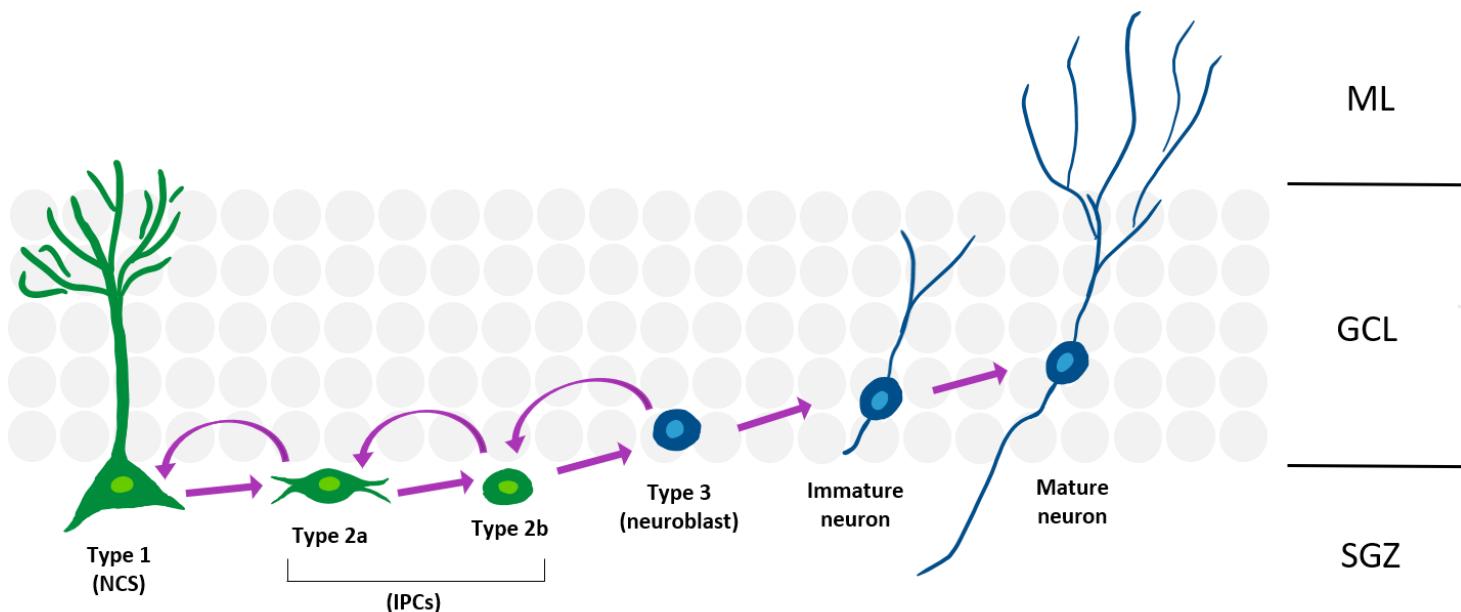


Figure 1.4 Stages of adult hippocampal neurogenesis. Radial glia-like neural progenitor cells give rise to mature granule neurons in the dentate gyrus.

Adult hippocampal neurogenesis is implicated in at least some functions of the hippocampal formation at large, including pattern separation, anxiety, and regulation of the neuroendocrine stress response (Christian et al., 2014; Anacker and Hen, 2017; Toda and Gage, 2018; Toda et al., 2019). Reduced hippocampal neurogenesis is observed in all animal models of depression including those that utilize exposure to stressors (Bessa et al., 2009; Brummelte and Galea, 2010; David et al., 2009; Gould et al., 1998; Green and Galea, 2008; Keilhoff et al., 2006). Further, factors that alleviate depression symptoms, including chronic antidepressant treatment and exercise, increase neurogenesis in the hippocampus (Bjørnebekk et al., 2006, 2005; Malberg et al., 2000). In males, adult hippocampal neurogenesis has been shown to be required for the efficacy of antidepressants in tests of anxiety (Santarelli et al., 2003) and to reverse HPA axis dysregulation (Surget et al., 2011). More recently, chemogenetic inhibition of neurogenesis specifically in the ventral dentate gyrus was shown to increase susceptibility to social defeat stress in male mice (Anacker et al., 2018), indicating that neurogenesis can confer stress resilience. In another recent study, chemogenetically suppressing the activity of new hippocampal neurons, without manipulating their number, blocked the antidepressant efficacy of fluoxetine in male and female mice (Tunc-Ozcan et al., 2019), although data was not separated by sex and the proportion of males versus females per group was not reported. The same study showed that activating new hippocampal neurons reversed the depressive-like effects of chronic stress exposure (Tunc-Ozcan et al., 2019). As mentioned previously, in humans, prescribed antidepressants were associated with increased neurogenesis in the hippocampus of females, but not males (Epp et al., 2013). Based on the collective evidence, a “neurogenic hypothesis” of depression has been put forth, theorizing that reductions in adult hippocampal neurogenesis are

implicated in MDD, and that treatment efficacy is related to a restoration of neurogenesis (Eliwa et al., 2017). It is important to note, however, that research surrounding the functional significance of neurogenesis has, to date, been almost exclusively performed in male subjects. Although the exact role of new neurons in the dentate gyrus remains somewhat elusive, especially in females, adult hippocampal neurogenesis is often utilized as a neuroplastic marker in animal models of depression.

The effects of stress on adult hippocampal neurogenesis can be sex-dependent. For example, exposure to acute predator odor or foot shock stress reduced cell proliferation in male rats, but no such effect was observed in female rats (Falconer and Galea, 2003; Shors et al., 2007). Sex-dependent alterations in hippocampal neurogenesis are also reported in animal models of chronic or repeated stress (Hillerer et al., 2013; Westenbroek et al., 2004). In individually housed rats, hippocampal cell proliferation was reduced after chronic foot-shock stress in males but increased in females (Westenbroek et al., 2004). Social isolation alone, which itself is a stressor, also resulted in differential effects on cell proliferation depending on sex, with decreased cell proliferation in females and no significant change in males (Westenbroek et al., 2004). Another study in rats examined sex differences in the effects of repeated restraint stress on hippocampal neurogenesis, and found reductions in cell proliferation only in males, and reductions in cell survival only in females, after a stress incubation period (Hillerer et al., 2013). Taken together, sex differences in the effects of stress can be observed across distinct stages of neurogenesis (i.e. proliferation and survival), and depend on the stress paradigm (acute, repeated, chronic). Because sex hormones are potent regulators of adult hippocampal neurogenesis (discussed below), sex differences in the consequences of stress on neurogenesis may be in part due to sex difference in the hormone milieu, however this has not been directly examined.

1.4.3 Adult hippocampal neurogenesis: regulation by ovarian hormones

There is convincing evidence that estrogens are important players in the regulation of adult hippocampal neurogenesis (Mahmoud et al., 2016b). This evidence is derived from studies that have investigated the influence of endogenous fluctuations and exogenous manipulations of estrogens. For example, in both rats and mice, higher levels of hippocampal cell proliferation are observed in proestrous females relative to estrous and diestrous females (Lagace et al., 2007; Rummel et al., 2010; Tanapat et al., 1999; Tzeng et al., 2014), reaching levels 50% higher than during diestrus (Tanapat et al., 1999). This evidence supports a pro-proliferative effect of estradiol, which peaks during proestrus (Butcher et al., 1974). In further support of this notion, in young-adult female rats, cell proliferation was reduced when examined one-week post-ovariectomy, and acute estradiol replacement restored cell proliferation to levels of sham controls, however this effect is dose-dependent and non-linear (Barha et al., 2009; Tanapat et al., 2005; Tanapat et al., 1999). Further, acute estradiol replacement fails to rescue cell proliferation levels when administered 4 weeks post-ovariectomy (Tanapat et al., 2005), indicating that progenitor cells in the hippocampus may become less sensitive to the effects of estradiol after a longer duration of ovarian hormone deprivation.

Relatively fewer studies have examined the effects of chronic estradiol replacement on the survival of new hippocampal neurons, but findings overall indicate that effects depend on the timing of estradiol exposure relative to that of cell proliferation. Specifically, the survival of new neurons is enhanced in cell populations that are produced after the initiation of estradiol treatment (McClure et al., 2013), but is suppressed in cell populations that are produced prior to the initiation of estradiol treatment (Barker and Galea, 2008; Chan et al., 2014). In other words,

the hormone-related environment during which the cells are generated may determines the outcome of chronic estradiol treatment on cell survival. Few studies have investigated the influence of progesterone on neurogenesis, whether in isolation or in unison with estrogens. One study found that a single dose of progesterone given 24 hours after estradiol administration decreased the estradiol-induced enhancement of cell proliferation (Tanapat et al., 2005). Another study found that chronic progesterone alone did not affect the survival of new neurons (labeled with BrdU 24 hours prior to 21 days of treatment), but partially restored an estradiol-benzoate reduction in survival (Chan et al., 2014). These studies therefore suggest that progesterone may antagonize some of the effects of estradiol on cell proliferation or survival.

Given the complexity of estrogen signaling, the mechanisms by which estrogens influence adult hippocampal neurogenesis have not been fully deciphered. However, the effects of estradiol on cell proliferation in the dentate gyrus appear to be at least partially mediated through ER α and ER β . In adult ovariectomized female rats, treatment with the ER α -selective agonist propylpyrazole-triol (PPT), or the ER β -selective agonist diarylpropionitrile (DPN), increased cell proliferation, suggesting that both ER subtypes participate in the pro-proliferative effects of estradiol. In addition, it is likely that estrogens can upregulate cell proliferation through direct interactions with ERs on progenitor cells in the SGZ, as both ERs were found to colocalize with progenitor cells, albeit at low levels (Mazzucco et al., 2006). Interestingly, treatment with the GPER agonist G1 decreased cell proliferation in adult ovariectomized rats (Duarte-Guterman et al., 2015), indicating an estradiol-independent role of GPER in hippocampal neurogenesis, or alternatively an antagonistic effect of GPER and ER α/β activation to maintain levels of neurogenesis. Lastly, unlike classical ERs, GPER did not co-localize with progenitor cells in the

SGZ (Duarte-Guterman et al., 2015), indicating that the effects of GPER activation on neurogenesis may be indirect.

1.4.4 The frontal cortex in MDD and stress-based models

The prefrontal cortex (PFC) is another brain region implicated in the pathogenesis of MDD. The PFC can be roughly separated into ventromedial (vm) and dorsolateral (dl) subregions on the basis of differential network connectivity, and differences in function and neuroanatomical features (Ongur, 2000; Kringelbach and Rolls, 2004). In general, dlPFC regions play a prominent role in cognitive and executive functions, whereas vmPFC regions are more linked to emotional and affective functions (Koenigs and Grafman, 2009). However it is important to note that this is a simplistic view of the human PFC, and that considerable debate remains surrounding how different subregions should be defined and labeled (Dixon et al., 2017). Nonetheless, functional imaging studies indicate that both regions display dysregulated activity patterns in MDD, albeit in opposite directions. Specifically, MDD is associated with a hyperactive vmPFC but a hypoactive dlPFC (Biver et al., 1994; Greicius et al., 2007; Drevets et al., 2008), and these alterations are putatively linked to mood and cognitive disturbances, respectively. More recently, imaging studies have explored MDD-related perturbations in functional connectivity between brain regions. For example, one study found that different patterns of dysfunctional connectivity may be linked to various subtypes or features of depression, and more specifically that dysregulated frontostriatal and orbitofrontal connectivity patterns were associated with anhedonia (Drysdale et al., 2017). Further, in a meta-analysis of resting state functional connectivity studies, MDD was found to be characterized by hypoconnectivity in frontoparietal regions important for regulation of attention and emotion (Kaiser et al., 2015). On a cellular and

molecular level, there are also reductions in the expression of synapse-related genes and an overall reduction in synapse number in the PFC of individuals with MDD (Feyissa et al., 2009; Kang et al., 2012). Further, in a recent meta-analysis of genome-wide association studies that included data from over 800,000 individuals, enrichment analyses highlight the importance of prefrontal brain regions in gene variants associated with MDD (Howard et al., 2019).

There is also considerable debate and controversy surrounding what (if anything) constitutes the rodent PFC and homologies to the human PFC (Laubach et al., 2018). However there is general agreement that what is considered to be the rodent PFC is homologous to regions in the human anterior cingulate cortex (ACC) (Laubach et al., 2018). It is also clear that there is substantial divergence between the rodent and human PFC, and that there are regions in the human dlPFC that do not exist in rodents (Petrides, 2005). While keeping this in mind, stress exposure in rodent models also results in molecular, architectural, and functional alterations in the frontal cortex (Arnsten, 2009; McEwen et al., 2016; Csabai et al., 2018). Interestingly, there are sex and hormone differences in the effects of stress exposure on neuronal architecture in this region. For example, the effects of repeated restraint stress on the apical dendritic arbor of pyramidal neurons in the anterior cingulate and prelimbic regions of the mPFC were in opposite directions in male versus female rats (Garrett and Wellman, 2009). Specifically, dendritic atrophy was observed in males, and dendritic expansion was observed in females, and this effect in females was dependent on estradiol (Garrett and Wellman, 2009). There are also sex differences in the effects of stress on neurons in the infralimbic region of the mPFC that project to the basolateral amygdala (BLA; Shansky et al., 2010, 2009), a circuit that is implicated in fear and anxiety. In male rats, these neurons are resistant to chronic restraint stress-induced dendritic atrophy (Shansky et al., 2009), but interestingly, the same population of neurons displays an

expansion of dendritic arbor in females rats treated with estradiol and exposed to the same stress paradigm (Shansky et al., 2010). In non-BLA projecting mPFC neurons, male rats show apical dendritic atrophy in response to chronic restraint stress (Shansky et al., 2009), but females are resistant to changes in dendritic arborization regardless of estradiol treatment (Shansky et al., 2010). Thus, stress exposure results in sex- and circuit- specific changes in dendritic arborization that are in some instances dependent on estradiol, and this may contribute to sex differences in MDD.

1.5 Behavioural and neuroendocrine tests

Across experiments presented in Chapters 2-4, we assessed animals on a battery of behavioural and neuroendocrine tests after stress exposure. These tests are briefly overviewed below.

1.5.1 Forced swim and tail suspension tests

The forced swim test (FST) is one of the most commonly used tools for antidepressant screening that is also employed as a behavioural readout in animal models of depression (Slattery and Cryan, 2012). The FST was developed using rats but has also been modified for use in mice (Porsolt et al., 1977, 1978). In brief, rodents are subjected to an inescapable swimming session, and the latency to assume an immobile posture or the total time spent immobile are scored. Immobility in the FST is reduced by a broad range of antidepressants, suggesting high predictive validity, and resulting its wide use as a screening tool in drug development (reviewed in Cryan and Slattery, 2012; Petit-Demouliere et al., 2005). The idea that increased immobility in the FST is indicative of depressive-like behaviour was therefore adopted retrospectively, based on the observations that pharmacological antidepressants or electroconvulsive treatment can reduce immobility in the FST (Katz, 1981; Willner, 1997). In addition, antidepressant that enhance

serotonergic tone selectively increase swimming, whereas those that enhance catecholaminergic tone selectively increase struggling (Detke et al., 1995). Importantly, the rat FST generally requires two swimming sessions, which are thought to be required for eliciting “learned helplessness” in the second session (Slattery and Cryan, 2012). In mice, for reasons that remain unknown, the effects of antidepressant agents or manipulation to induce depressive-like phenotypes can be observed with only one swimming session (Slattery and Cryan, 2012). Further, compared to rats, mice typically show higher baseline levels of immobility in the FST (Can et al., 2012).

The Tail Suspension Test (TST) is an additional putative test for depressive-like behaviour and a commonly used tool for antidepressant screening (Steru et al., 1985; Cryan et al., 2005). Although the TST is used in mice (Can et al., 2011), it is conceptually very similar to the FST. The TST is based on the observation that when subjected to an acute tail suspension stressor, mice will display an immobile posture after initially engaging in escape-oriented movements (Steru et al., 1985). Similar to the FST, immobility in the TST can be reduced by antidepressant agents and increased by manipulations such as chronic stress exposure (reviewed in Cryan et al., 2005).

1.5.2 Sucrose preference and consumption tests

Anhedonia refers to the reduced ability to experience interest or pleasure, and is one of the two core symptom of MDD, the other being depressed mood (American Psychiatric Association, 2013). The consumption of, or the preference for, a palatable sucrose solution, are two putative measures of anhedonia-like behaviour that are commonly used in rats and mice (Willner, 2017; Liu et al., 2018). These tests are typically performed in the home cage, where rodents are given a

choice between a palatable sucrose solution and plain water. Sucrose consumption is measured relative to overall body mass, whereas sucrose preference is calculated as the percentage of sucrose consumed over total amount of liquid consumed. A decrease in sucrose consumption or preference is presumed to represent a reduction in the hedonic value of sucrose, or a reduction in the motivation to consume sucrose (Scheggi et al., 2018). Sucrose consumption or preference are reduced in stress-based animal models of depression, and are sensitive to chronic antidepressant treatment (Willner, 2017). Importantly, as with most other behavioural tests, the sucrose preference and consumption tests were developed and have been primarily validated using male subjects.

1.5.3 Novelty suppressed feeding test

Comorbid anxiety disorders are common in MDD, especially in women (Marcus et al., 2008). The novelty suppressed feeding test (NSF) is used as a measure of anxiety-like behaviour in rats and mice, based on the phenomenon of hyponeophagia (Dulawa and Hen, 2005). After a period of food deprivation, rodents are individually placed in a novel open arena containing food in the center, and the latency to begin feeding is used as an index of anxiety-like behaviour. NSF is thought to be a conflict-based test, in which the animal faces a choice between entering the center to consume the food or avoiding entry into the potentially dangerous arena (Dulawa and Hen, 2005). Adding to the predictive validity of this test, latency to feed is sensitive to chronic, but not acute, administration of classical antidepressants (Bodnoff et al., 1988; Santarelli et al., 2003; Dulawa and Hen, 2005). This mirrors the actions of these drugs in humans, in which alleviation of MDD symptoms typically requires weeks of treatment (Blier, 2003). Interestingly,

the ability of antidepressants to reduce latency to feed in NSF has been shown to require adult hippocampal neurogenesis in male mice (Santarelli et al., 2003).

1.5.4 Dexamethasone suppression test

Some individuals with MDD have impaired HPA axis negative feedback inhibition, which can be revealed using the dexamethasone (DEX) suppression test (Ising et al., 2007; Stetler and Miller, 2011). DEX is a potent synthetic glucocorticoid that selectively binds to GRs, and as DEX does not readily access the brain, its effects are thought to be primarily mediated via actions at the level of the pituitary (Miller et al., 1992; Cole et al., 2000). DEX fails to effectively suppress cortisol in some individuals with MDD (Ising et al., 2007; Stetler and Miller, 2011), indicating impairments in HPA axis negative feedback inhibition. DEX can be also be used to assess the glucocorticoid-mediated negative feedback function of the HPA axis in rodents (Gobinath et al., 2016). The ability of DEX to suppress corticosterone can be tested in response to an acute stressor (Gobinath et al., 2016), but few studies have used the DEX suppression test within valid animal models of MDD (Nollet et al., 2012).

1.6 Thesis Overview and Objectives

Given the complex roles of ovarian hormones in MDD, this thesis sought to clarify the role of ovarian hormones by examining their influence on the outcomes of stress exposure in female rats and mice. More specifically, the experiments in this thesis explored whether and how ovarian hormones can influence depressive-like behaviour, antidepressant efficacy, and neuroplastic and neuroimmune correlates both under non-stress conditions and in response to stress exposure. To this end, we systematically examined the effects of ovarian hormone deprivation (Chapters 2-4), and estradiol and ER agonist replacement (Chapter 4), on the abovementioned outcomes using

female mice and rats. Below are the specific objectives and hypotheses of the experiments described in Chapters 2-4:

Chapter 2 investigated the role of ovarian hormones in depressive-like endophenotypes and antidepressant efficacy within a chronic unpredictable stress model in female rats. We hypothesized that long-term ovarian hormone deprivation would increase the expression of depressive-like phenotypes under chronic stress conditions and reduce antidepressant efficacy and associated neuroplasticity.

Chapter 3 explored how ovarian hormones can influence the behavioural and neuroinflammatory consequences of sub-chronic stress exposure in female mice. We hypothesized that ovarian status would dictate the behavioural and neuroinflammatory response to stress.

Chapter 4 aimed to dissect the contribution of ER α and ER β to the effects of 17 β -estradiol under chronic stress conditions in female mice, with a focus on behavioural, neuroplastic, and neuroimmune outcomes. We hypothesized that 17 β -estradiol would protect against the development of depressive-like phenotypes and that its actions would be differentially mediated by ER α and ER β .

Chapter 2: Ovarian hormones, but not fluoxetine, impart resilience within a chronic unpredictable stress model in middle-aged female rats

2.1 Introduction

Women are more than twice as likely as men to develop depression, a disparity that is particularly striking during the reproductive years of women (Gutiérrez-Lobos et al., 2002). Notably, periods that involve dramatic fluctuations and/or reductions in ovarian hormones, such as the postpartum and perimenopause, carry an increased risk of developing depression in women (Hendrick et al., 1998; Cohen et al., 2006; Soares, 2014). Together, these data provide compelling evidence for a role of ovarian hormones in the pathoetiology of depression.

Indeed, in healthy women, pharmacologically-induced reductions in ovarian hormones triggered sub-clinical depression scores, and estradiol concentrations were negatively associated with depressive symptoms (Frokjaer et al., 2015). In addition, withdrawal from a hormone simulated pregnancy increased depressive symptoms in women with a history of postpartum depression (Bloch et al., 2000). Thus, ovarian hormone fluctuations contribute to the development of depressive symptoms in women. Similarly in rats, estradiol withdrawal after hormone simulated pregnancy leads to depressive-like phenotypes (Galea et al., 2001; Green and Galea, 2008; Green et al., 2009). Importantly, in this model, treatment with estradiol or an estrogen receptor (ER) β agonist prevented the development of depressive-like behaviors (Galea et al., 2001; Green et al., 2009). Ovariectomy itself can increase depressive- and anxiety-like behaviors while treatment with estradiol or selective estrogen receptor modulators (SERMs) can restore this effect (Okada et al., 1997; Walf et al., 2004; Bekku and Yoshimura, 2005; Li et al., 2014). Taken together, these findings corroborate the notion that reductions in ovarian hormones

may increase the risk for developing depressive-like phenotypes. However, most of these studies did not utilize a model of depression, and thus the face and construct validity of such studies is uncertain. The present study aimed to fill this gap by investigating the role of ovarian hormones in the development of depressive-like phenotypes within a valid animal model of depression.

Ovarian hormones are also implicated in antidepressant efficacy (Thase et al., 2005). For example, in postmenopausal women with depression, antidepressant treatment was more successful at alleviating symptoms when prescribed alongside hormone therapy than when given alone (Thase et al., 2005), suggesting that ovarian hormones may enhance antidepressant efficacy. Furthermore, in women with depression, estradiol has been given as an adjunct therapy or a standalone antidepressant, with some success (Ahokas et al., 2001; Rasgon et al., 2007; Moses-Kolko et al., 2009). Similarly, 17 β -estradiol treatment in ovariectomized rats augmented the effects of sertraline, a selective serotonin reuptake inhibitor (SSRI), to reduce immobility in the forced swim test (Sell et al., 2008). However, this latter study was not conducted in conjunction with an animal model of depression, and thus it is unclear whether similar findings would be obtained from animals with depressive-like phenotypes. Our current study aimed to determine whether ovarian hormones influence antidepressant efficacy in an animal model of depression in middle-aged rats.

The hippocampus exhibits compromised structural plasticity in depressed patients, as a meta-analysis showed that untreated depression is associated with reduced hippocampal volume, which is seen two years after diagnosis (McKinnon et al., 2009). Decreased hippocampal neurogenesis is observed in all animal models of depression (Green and Galea, 2008; Bessa et al., 2009; Wainwright et al., 2011), and reductions in neural progenitor cells (NPCs) are detected

in the hippocampus of individuals with depression (Boldrini et al., 2012). Conversely, antidepressant treatment restores the reductions in neurogenesis and NPCs in animal models and individuals with depression, respectively (Green and Galea, 2008; Bessa et al., 2009; Boldrini et al., 2012). Notably, antidepressant use results in a greater increase in hippocampal volume in depressed women than depressed men (Vakili et al., 2000), and the pro-neurogenic effects of antidepressants are seen in post-mortem tissue of women, but not men (Epp et al., 2013). Thus, ovarian hormones may modulate the effects of antidepressants on neurogenesis in the hippocampus.

There is also a growing recognition for the role of inflammation in depression and chronic stress (Miller et al., 2009). Remarkably, microglial activation is increased in the prefrontal cortex, insula, and anterior cingulate cortex by approximately 30% in depressed patients relative to controls (Setiawan et al., 2015). Increased inflammation and microglial activation in the hippocampus are also evident in animal models of chronic stress (Frank et al., 2007; Kreisel et al., 2014). Despite the well-established anti-inflammatory effects of ovarian hormones in a variety of disease models (Habib and Beyer, 2015), to our knowledge, the influence of ovarian hormones on microglia in relation to chronic stress or SSRI exposure has not been investigated, and thus the current study aimed to fill this gap.

Disturbances in the hypothalamic-pituitary-adrenal (HPA) axis are arguably the best characterized endocrine markers of depression. Specifically, a meta-analysis showed that depressed individuals display elevated levels of serum cortisol (Stetler and Miller, 2011), and at least a subset of depressed individuals show impairments in the HPA negative feedback system (Ising et al., 2007; Stetler and Miller, 2011). Antidepressant efficacy is more tightly linked to the

restoration of HPA function in women compared to men (Binder et al., 2009), and hypogonadal women have impaired HPA negative feedback function (Maes et al., 1992; Young et al., 1993). Thus, similar to the effects of ovarian hormones on the behavioral efficacy of antidepressants, ovarian hormones may potentiate the efficacy of antidepressants to restore HPA axis dysfunction.

In this study, we investigated whether ovarian hormones impart resilience against the behavioral and endocrine outcomes of chronic unpredictable stress in middle-aged female rats. We further investigated whether ovarian hormones influence the efficacy fluoxetine, an SSRI, under conditions of chronic stress. We examined depressive- and anxiety-like behaviors (forced swim test, novelty suppressed feeding test, and sucrose preference test), endocrine measures (corticosterone concentrations and HPA axis negative feedback function), and neuroplastic and neuroimmune markers (neurogenesis, PSA-NCAM, and microglia number and morphology). We hypothesized that ovarian hormones would be associated with resilience against the expression of depressive-like phenotypes under chronic stress conditions, and that antidepressant efficacy would be modulated by ovarian status in middle-aged female rats.

2.2 Methods

Animals

Forty female Sprague-Dawley rats (Charles River Laboratories, Montreal, Canada), weighing 200–250g, were used in this study. Animals were pair-housed in a female-only colony room and given *ad-libitum* access to food (Purina rat chow) and water. Colony rooms were temperature and humidity controlled ($21 \pm 1^\circ\text{C}$; $50 \pm 10\%$, respectively), and maintained on a 12-hour light/dark cycle (lights on at 07:00). All procedures were approved by the Animal Care

Committee at the University of British Columbia and performed in agreement with ethical guidelines set by the Canadian Council on Animal Care. All efforts were made to reduce the number of animals used and to minimize their suffering.

Surgery

Animals were randomly assigned to receive bilateral ovariectomy (OVX, n=20) or sham surgery (Sham, n=20), which were performed at approximately five months of age, as we have done previously (Barha et al., 2010). Surgeries were performed under isoflurane anesthesia, with ketamine (30mg/kg, Bimeda-MTC, Cambridge, ON), xylazine (2mg/kg, Bayer HealthCare, Toronto, ON) and bupivacaine (applied locally; 4mg/kg, Hospira Healthcare Corporation, Montreal, QC). Following recovery, animals were handled only during cage cleaning procedures, and left otherwise undisturbed until the beginning of the chronic unpredictable stress (CUS) procedure at approximately 9 months of age.

BrdU administration

To label dividing progenitor cells in the dentate gyrus and their progeny, all rats received two intraperitoneal injections of 5-bromo-2'-deoxyuridine (BrdU; 200mg/kg; Sigma–Aldrich, St. Louis, MO, USA), given 8 hours apart, one day prior to the initiation of CUS. Two BrdU injections were chosen due to the lower rates of cell proliferation in the hippocampus of middle-aged rodents (Kuhn et al., 1996; Rao et al., 2005). Further, the timing of BrdU administration was chosen to delineate the effects of CUS (and subsequently antidepressant treatment) on the survival of newly produced cells, independent of effects on cell proliferation.

Chronic Unpredictable Stress (CUS)

A CUS paradigm was used as we have done previously (Wainwright et al., 2011), with some modifications. Briefly, all animals were exposed to a variety of stressors, applied twice daily in a semi-random order at unpredictable times for a duration of 6 weeks. We allowed for at least 2 hours between stressors. The stressors and their descriptions are listed in Table 2.1.

Stressor	Duration	Description
Wet bedding	2 hours	200 ml of tap water in home cage, rats moved to a dry and clean cage afterwards
Food and water deprivation	4 hours	Rats deprived of food and water, always prior to the sucrose preference test
Tail pinch	5 mins	Plastic clothespin placed at base of tail
Cage tilt	2 hours	Home cage tilted at 45°
Elevated platform	5 mins	Rats placed on a plexiglass platform (20x20 cm) at 90 cm above ground.
Continuous lighting	36 hours	Colony lights on for 36 hours
Restraint	1 hours	Rats restrained in well ventilated plexiglass tubes
Social isolation	18 hours	Rats individually housed from 15:00-10:00
Soiled cage	2 hours	Rat pairs placed in cage soiled by other rats. Rats moved to a clean cage afterwards
White noise	1 hour	Rats exposed to 80 dB of white noise
Strobing light	1 hour	Strobing light at 1 flash/second in an otherwise dark room, during light cycle
Tail bleed	2 mins	Blood sample taken via tail vein nick
Food deprivation	16 hours	Rats deprived of food but not water

Table 2.1 Stressors included in the Chronic Unpredictable Stress paradigm. Two stressors were presented daily, in a pseudo-random order, for a duration of 6 weeks.

Drug preparation and treatment

The first 3 weeks of CUS were utilized to induce depressive-like phenotypes. During the last 3 weeks of CUS (timeline depicted in Figure 2.1), rats received daily subcutaneous injections of fluoxetine hydrochloride (Sequoia Research Products Ltd, Pangbourne, UK) or vehicle (Veh). Fluoxetine was dissolved in 5% DMSO (Sigma–Aldrich, St. Louis, MO, USA) in 0.9% saline, and given at a dose of 5mg/kg. Vehicle treatment consisted of 5% DMSO in 0.9% saline. Thus, we obtained four treatment groups (n=10 each), with drug and ovarian status as the between-subject variables: (i) vehicle-treated sham-operated rats (Veh-Sham); (ii) fluoxetine-treated sham-operated rats (FLX-Sham); (iii) vehicle-treated ovariectomized rats (Veh-OVX); and (iv) fluoxetine-treated ovariectomized rats (FLX-OVX). An intermediate dose of fluoxetine at 5mg/kg was chosen to in order to avoid a possible ceiling effect, which may mask potential differences between ovarian status groups in the response to treatment. This rationale was based on prior work in ovariectomized middle-aged female rats showing that while 10mg/kg FLX decreased immobility in the forced swim test without hormone replacement, a low dose of 1.5mg/kg FLX decreased immobility only when administered in conjunction with estradiol replacement (Récamier-Carballo et al., 2012).

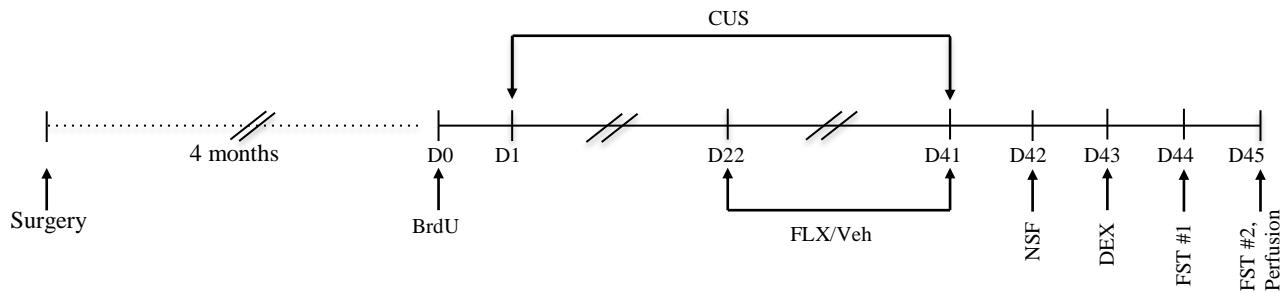


Figure 2.1 Timeline depicting the sequence of experimental events. Sham surgery (Sham) or bilateral ovariectomy (OVX) were performed at 5 months of age. Following a four month delay, rats were subjected to 6 weeks of chronic unpredictable stress (CUS; 24 hours after bromodeoxyuridine (BrdU) administration). Fluoxetine (FLX) or vehicle (Veh) treatment were administered daily for the final 3 weeks of CUS. Behavioral and neuroendocrine testing were initiated 24 hours after the final day of CUS, in the order shown (one test/day), with the exception of the sucrose preference test, which was administered weekly throughout CUS (not shown). Ninety minutes after the second session of FST, the animals were perfused and tissue was collected. D indicates day of experiment, beginning at BrdU administration on D0. NSF = novelty suppressed feeding, DEX = dexamethasone suppression test, FST = forced swim test.

Behavioral testing

Behavioral testing occurred following the 6 weeks of CUS, with the exception of the sucrose preference test, which was administered weekly (see Figure 2.1).

Sucrose preference test. Rats were acclimatized to the 1% sucrose solution and the two-bottle procedure by introducing two identical bottles to their home cage for 24 hours, one containing 1% sucrose and the other tap water. After acclimatization, the sucrose preference test was administered once prior to CUS to obtain a baseline measure of sucrose preference, and weekly thereafter until the termination of CUS (last test was on the last day of CUS). Briefly, rats were single-housed and simultaneously deprived of food and water for 4 hours, then presented with two bottles, one containing 1% sucrose and the other tap water. The test lasted 1 hour beginning at the start of the dark-phase (19:00-20:00hrs), after which the rats were re-paired with cage

mates. All bottles were weighed before and after the test, and sucrose preference was calculated using the formula: sucrose preference = (sucrose consumed/(sucrose + water consumed)) × 100. The right-left placement of the sucrose and water bottles were counter-balanced for all animals between test days.

Novelty-Suppressed Feeding Test (NSF). The apparatus consisted of an open arena (60 × 60 × 50 cm) in which three pellets of a palatable food (Kellogg's Froot Loops) were placed in the center. Prior to the test, and to avoid neophobic reactions, the rats were acclimatized to the food by introducing four Froot Loops to the home cage for three consecutive days. On test day, the food-deprived rats (18 hours) were individually placed in the arena, at a consistent corner of the chamber, and the latency to start feeding was measured. The rats were then immediately returned to the home cage, and the amount of Purina rat chow (in grams) consumed within the first hour was measured. The NSF arena was thoroughly cleaned with 70% EtOH between animals.

Forced Swim Test (FST). The FST was conducted as we previously described (Wainwright et al., 2011). Briefly, each rat was subjected to two FST sessions in a vertical glass cylinder filled with clean water (25 ± 0.5 °C) to a depth of 30 cm. Rats were individually placed in the cylinder for 15 minutes in the first session, and 5 minutes in the second session, which occurred 24 hours later. The test was video-taped, and behavior was subsequently scored using the BEST Collection Software (Educational Consulting, Hobe Sound, FL, USA), by an observer blinded to experimental condition. Time spent in each of three distinct behaviors was scored: (1) Immobility = floating with only those movements necessary to maintain the head above water; (2) Swimming = active paddling movements of forelimbs and/or hindlimbs; and (3) Struggling = vigorous climbing-like movements, with forelimbs surfacing above the water.

Blood sampling for basal corticosterone quantification

To assess changes in basal corticosterone levels throughout CUS, blood samples were collected every 10 days via the tail-vein, wherein the first sample was collected prior to the first stressor on day 1 of CUS. The procedure was consistently performed between 07:00-07:45hrs and completed within 2 minutes of touching the home cage to avoid acute stress-induced increases in corticosterone. Blood samples were allowed to clot over night at 4°C, then centrifuged at 1000 x g for 15 minutes and serum aliquots were stored at -20°C until processing.

Dexamethasone Suppression Test

To evaluate the integrity of the glucocorticoid-dependent negative-feedback function of the HPA axis, the Dexamethasone suppression test (DEX) was administered 48 hours after the termination of CUS (see Figure 2.1). DEX was administered between 8:00-10:30hrs to all rats in order to avoid major circadian fluctuations in corticosterone. In short, 3 hours following the administration of Dexamethasone (in propylene glycol; i.p.; 100 ug/kg; Sigma), blood was collected via the tail vein immediately before and after 30 minutes of restraint stress, and again 30 minutes after the termination of restraint (60 minutes after the first collection). Rats were left undisturbed in their home cages during recovery from restraint. Blood samples were allowed to clot over night at 4°C, then centrifuged at 1000 x g for 15 minutes and serum aliquots were stored at -20°C until processing.

Estrous cycle phase determination

In order to account for potential effects of estrous cycle phase on behavior and cell proliferation, vaginal cells were collected by lavage on experimental days 42-45 (Figure 2.1), estrous cycle

phase was determined as previously described (Brummelte and Galea, 2010a). Given that the animals were in middle age at the beginning of CUS, the analysis of vaginal cells was also intended to determine if any rats in the sham group had reached persistent anestrous, however all rats were found to be cycling.

Tissue collection

Ninety minutes following the second FST session, animals were deeply anesthetized with sodium pentobarbital and blood was collected via cardiac puncture. Animals were then transcardially perfused with cold 0.9% saline followed by 4% paraformaldehyde (PFA, Sigma-Aldrich) in 0.1M phosphate buffer (PB). Brains were extracted immediately and post-fixed in 4% PFA for 24 hours, then transferred to 30% sucrose solution in 0.1M PB (pH 7.4) and stored at 4 °C until sectioning. Brains were sliced in 40µm coronal sections using a Leica SM2000R microtome (Richmond Hill, ON, Canada), and were stored at -20 °C in a cryoprotective medium (0.1M PBS, 30% ethylene glycol and 20% glycerol; Sigma) until processing. Adrenals, thymus and ovaries (in sham-operated rats) were also extracted and weighed. Blood samples were allowed to clot over night at 4°C, then centrifuged at 1000 x g for 15 minutes and serum aliquots were stored at -20°C until processing.

Immunohistochemistry

All staining was performed on free-floating brain sections, and unless otherwise specified, incubation periods were conducted at room temperature and on a rotator.

BrdU. Sections were rinsed 3x10 minutes with 0.1M Tris-phosphate buffer (TBS; pH 7.4) between each of the following steps. After a 30 min incubation in 0.6% hydrogen peroxide

(H₂O₂), sections were transferred to 2N HCL for 30 min at 37 °C to denature DNA. Sections were then incubated in 0.1M Borate buffer for 10 min then blocked in TBS+ (containing 0.1% Triton X and 3% normal donkey serum (NDS)) for 30 min. Sections were then transferred to the primary antibody solution (1:200 mouse monoclonal antibody against BrdU, Roche Diagnostics, Laval, Quebec, Canada) in TBS+ at 4 °C for 20 h, and then the secondary antibody solution for 4 h (1:200 anti-mouse IgG biotinylated, Vector Laboratories, Burlington, Ontario, Canada) in TBS+. Next, tissue was incubated in avidin-biotin complex for 1.5 h (ABC; Elite kit; 1:50; Vector Laboratories). Immunoreactants were visualized by a peroxidase-diaminobenzidine (DAB) reaction (Vector Laboratories) for 7 min. Finally, sections were mounted on glass slides and allowed to dry, then counterstained with cresyl violet, dehydrated in increasing graded ethanol, defatted with xylenes, and cover-slipped with Permount.

Ki67. Sections were rinsed 3x10 min with 0.1M phosphate-buffered saline (PBS; pH 7.4) between each of the following steps. After a 25 min incubation in 0.3% H₂O₂, sections were transferred to a primary antibody solution for 16 h, containing 1:1000 Rabbit anti-Ki67 (Vector Laboratories) monoclonal antibody solution in 0.1M PBS, 1% Normal Goat Serum (NGS, Vector Laboratories), and 0.5% Triton X. Afterwards, sections were incubated in a secondary antibody solution for 1 h, containing 1:200 biotinylated Goat anti-rabbit IgG (Vector Laboratories, in 0.1M PBS), then in ABC (Vector Laboratories) for 40 min. Finally, immunoreactivity was visualized with a 5 min DAB reaction (Vector Laboratories) and sections were mounted on glass slides and allowed to dry. Tissue was dehydrated in increasing graded ethanol, defatted with xylenes, and cover-slipped with Permount.

BrdU/NeuN. Sections were rinsed 3x10 min with 0.1M PBS (pH 7.4) between each of the following steps. Sections were incubated for 24 h at 4 °C in a primary antibody solution containing 1:250 mouse anti-NeuN (Chemicon/Millipore), 3% Triton-X + 3% Normal donkey Serum (NDS) in 0.1M PBS. Tissue was then transferred to a secondary antibody solution for 18 h at 4°C containing 1:200 donkey anti-mouse Alexa 488 (Invitrogen, Eugene, OR) in 0.1M PBS. Next, sections were incubated in 4% PFA for 10 min, then washed 2x10 min in 0.9% NaCl. To denature DNA, sections were subsequently incubated in 2N HCl at 37 °C for 30 min. Sections were then transferred to a primary antibody solution for 24 h at 4°C, containing 1:500 Rat anti-BrdU (AbD Serotech, Oxford, UK) in 0.1M PBS + 3%NDS + 3% Triton-x. Next, sections were incubated in 1:500 Donkey anti-rat Cy3 (Jackson ImmunoResearch, Westgrove, PA) in 0.1M PBS for 24 h at 4°C. Sections were mounted on glass slides and cover-slipped with PVA-DABCO.

PSA-NCAM. Sections were rinsed 5x10 min with 0.1M TBS (pH 7.4) between each of the following steps. Sections were incubated in 0.3% H₂O₂ for 20 min, then transferred to a primary antibody solution at 4°C for 48 h, containing 1:1000 mouse anti-PSA-NCAM (chemicon/Millipore) in 0.1M PBS + 0.3% Triton-X + 3% Normal horse Serum (NHS). Next, sections were incubated in a secondary antibody solution containing 1:250 horse anti-mouse IgG biotinylated (Vector) in 0.1M PBS for 24 h at 4°C. Sections were then incubated in ABC for 2 h, and immunoreactivity was visualized with a 5 min DAB reaction (Vector Laboratories). Sections were finally mounted on glass slides and allowed to dry, then dehydrated in increasing graded ethanol, defatted with xylenes, and cover-slipped with Permount.

Iba-1. Sections were rinsed 3x10 min with 0.1M PBS (pH 7.4) between each of the following steps. Following a 25 min incubation in 0.3% H₂O₂, sections were blocked for 1 h with 10% normal goat serum (NGS) in 0.5% Triton X in 0.1 M PBS. Next, sections were incubated in a primary antibody solution for 18 h, containing 1:1000 anti-Iba-1 (Wako, Osaka, Japan) in 10% NGS and 0.4% Triton X in 0.1M PBS. Afterwards, sections were transferred to a secondary antibody solution of 1:500 biotinylated anti-rabbit (Vector Laboratories) in 0.4% Triton-X and 2.5% NGS in PBS. Then, sections were incubated in ABC (Vector Laboratories) in 0.4% Triton-X in PBS for 1 h, and immunoreactants were visualized by a Ni-DAB reaction (Vector Laboratories) for 7 min. Finally, sections were mounted on glass slides and allowed to dry, then counterstained with cresyl violet, dehydrated in increasing graded ethanol, defatted with xylenes, and cover-slipped with Permount.

Microscopy and cell counting

An experimenter blinded to treatment condition completed all immunohistochemical quantification under a Nikon E600 microscope equipped with epifluorescence. Representative photomicrographs are depicted in Figure 2.2. BrdU- and Ki67-immunoreactive (ir) cells were counted under 100 × magnification in every 10th section of the hippocampus along the rostral-caudal axis. To obtain an estimate of total immunoreactive cells in the dentate gyrus, raw numbers were multiplied by 10 (Hamson et al., 2013). BrdU- and Ki67-ir cells in the hilus were counted separately from those in the granule cell layer (GCL) and subgranular zone (SGZ, band approximately 50µm between the GCL and hilus). Counts were segregated in this way as cells in the hilus are considered ectopic and give rise to a distinct population of cells (Cameron et al., 1993). Furthermore, cells were quantified separately in the dorsal and ventral regions of the

hippocampus, according to previously established coordinates (Banasr et al., 2004). The dorsal hippocampus is thought to be functionally distinct from the ventral hippocampus with the former being more important for spatial learning, and the latter being more important for stress and affect (Fanselow and Dong, 2010). To determine the proportion of BrdU-ir cells of neuronal phenotype, BrdU-ir cells in every 10th section of the hippocampus were examined for co-expression of NeuN under $60 \times$ magnification. An exhaustive count of PSA-NCAM and Iba-1-ir cells was completed in four hippocampal sections per animal, two sections each from the dorsal and ventral regions of the hippocampus (Kondratiuk et al., 2015), with approximately the following respective coordinates: (Bregma -3.12, -3.48, 6.00, and -6.36). PSA-NCAM-ir cells were counted in the GCL only, whereas Iba-1-ir cells were counted in the GCL+SGZ and within an approximately $50\mu\text{m}$ band of the molecular layer (ML). Dentate gyrus areas (GCL and Hilus) were measured using digitized images of the sections and the software ImageJ (NIH).

Morphological analysis of Iba-1 immunoreactive cells

An experimenter blinded to treatment condition performed morphological analysis of Iba-1 immunoreactive cells in the GCL+SGZ. Specifically, using the measure feature of the NIS-Elements Basic Research software (Nikon) and under a Nikon E600 microscope, the number and length of processes were measured in 20 randomly selected cells from each animal, including 10 cells each in the dorsal and ventral regions of the hippocampus. The average length of processes was calculated for each cell, using the total length and number of processes for that cell, and subsequently an average value was taken for each animal, following previously established methods (Nemeth et al., 2014).

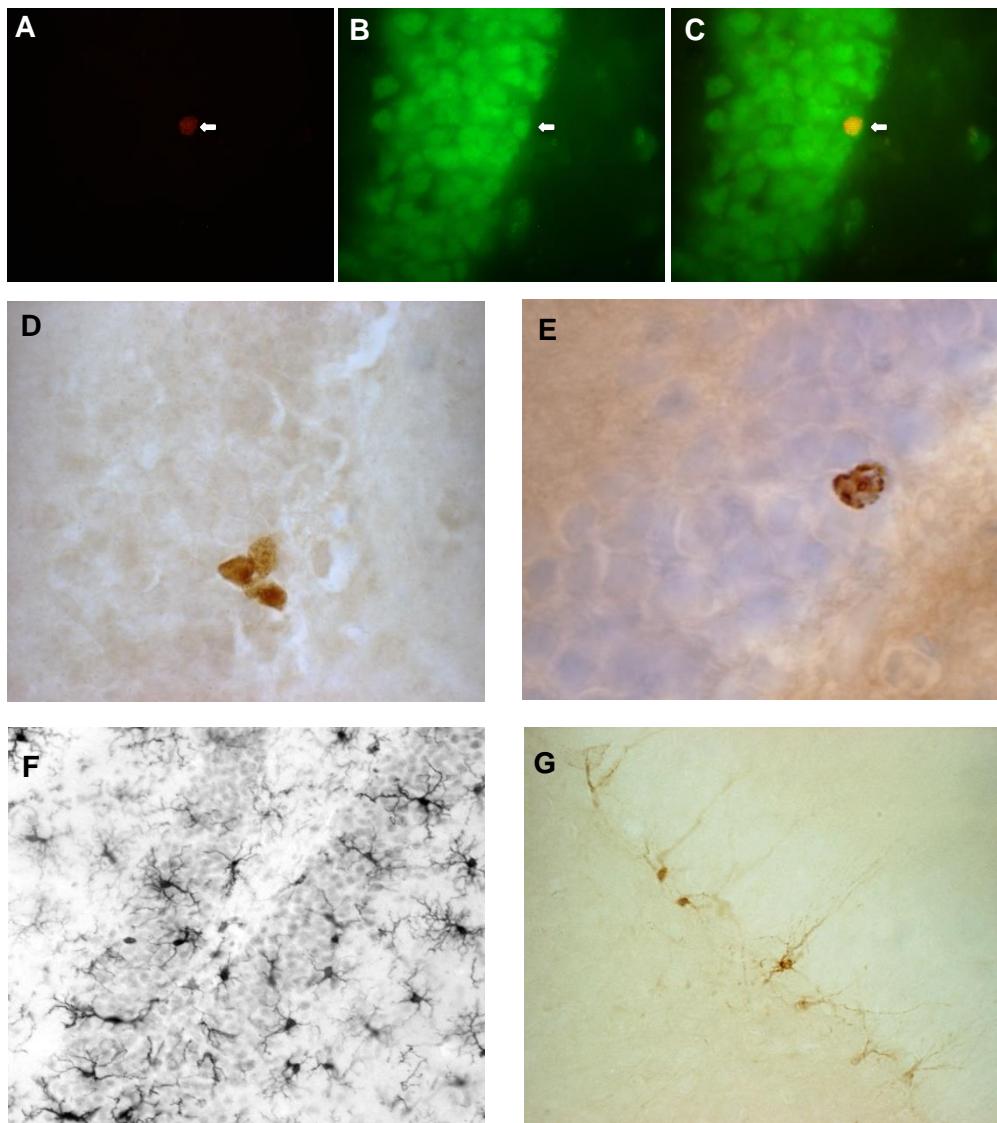


Figure 2.2 Representative photomicrographs of the granule cell layer in the dentate gyrus. (A) BrdU-immunoreactive (ir; red), (B) NeuN-ir (green) (C) BrdU/NeuN-ir (merged image), (D) Ki67-ir, (E) BrdU-ir. (F) Iba-1-ir and (G) PSA-NCAM-ir cells. Images A-C, F, and G viewed at 40 \times magnification, D and E at 100 \times magnification.

Serum hormone quantification

Radioimmunoassay kits were used according to the manufacturers' instructions to quantify serum corticosterone (Corticosterone, double-antibody RIA, MP Biomedicals, Solon, OH) and

17β -estradiol concentrations (Estradiol, Ultra-sensitive RIA, Beckman Coulter, Mississauga, Ontario). Samples were run in duplicates, and inter- and intra-assay coefficients were < 10%.

Statistical analyses

All statistical tests were performed using Statistica software (Tulsa, OK). Behavioral measures (Forced swim test, Novelty Suppressed Feeding, Sucrose Preference Test), serum 17β -estradiol concentrations and organ mass were each analyzed using factorial analysis of variance (ANOVA) with ovarian status (Sham, OVX) and drug treatment (Veh, FLX) as the between-subject factors. Percent sucrose preference and percent change in body mass were analyzed using repeated-measures ANOVA with ovarian status (Sham, OVX) and drug treatment (Veh, FLX) as the between-subjects variables, and week as the within-subject variable. Serum corticosterone (CORT) concentrations (basal or after DEX challenge) were each analyzed using a repeated-measures ANOVA with day (1, 10, 20, 30, 40) or time (0, 30, 60 min) as the within-subject variable, and ovarian status (Sham, OVX) and drug treatment (Veh, FLX) as the between-subjects variables. Ki67-ir, BrdU-ir, Iba-1-ir and PSA-NCAM-ir cells, and the percentage of BrdU/NeuN colabeled cells were each analyzed using a repeated-measures ANOVA with ovarian status (Sham, OVX) and drug treatment (Veh, FLX) as the between-subjects variables, and hippocampal region (dorsal, ventral) and dentate gyrus area (GCL+SGZ, hilus, or GCL+SGZ, ML) as the within-subject variables. A priori we expected differences between ovarian status groups on antidepressant efficacy. Post-hoc tests utilized Newman-Keul's comparisons and any *a priori* comparisons were subjected to a Bonferroni correction ($\alpha=0.05$). Unless otherwise stated adding estrous cycle phase as a covariate did not significantly alter the results.

2.3 Results

Ovariectomy increased immobility and decreased swimming and struggling in the forced swim test

As predicted, OVX increased immobility in the FST (Figure 2.3A; $F(1, 36) = 15.99$, $p = 0.0003$, main effect of ovarian status). Interestingly, fluoxetine treatment did not significantly alter immobility in either group ($p= 0.20$), and there was no treatment by ovarian status interaction ($p=0.83$). Similarly, OVX decreased swimming and struggling in the FST (Figure 2.3B and C; $F(1, 36) = 10.40$, $p < 0.003$, $F(1, 36)=4.68$, $p=0.037$, respectively; main effect of ovarian status), but there were no other significant effects (all p 's > 0.2). When analyzing with estrous phase as a covariate, there was a significant behaviour by ovarian status interaction ($F(2, 70)=3.71$, $p=0.029$), in which OVX increased immobility ($p<0.0002$), decreased swimming ($p<0.003$), but had no significant effect on struggling ($p>0.3$).

Ovariectomy increased anxiety-like behavior in the novelty suppressed feeding test

As expected, OVX increased latency to feed in the NSF (Figure 2.3D; $F(1, 36)= 18.50$, $p<0.0001$, main effect of ovarian status), but there were no other significant effects or interactions (all p 's >0.2). A priori comparisons revealed only a trend for fluoxetine to increase latency to feed in Sham rats ($p=0.09$). Importantly, the groups did not differ on home cage food consumption in the hour following NSF (all p 's > 0.1).

Ovariectomy increased anhedonia-like behavior in the sucrose preference test

Because drug treatment was initiated after 3 weeks of CUS, we ran a repeated measures ANOVA on sucrose preference with only weeks 4-6 of CUS as the within-subject variable. This revealed a significant CUS week by ovarian status interaction ($F(2, 72)=5.1$, $p=0.009$), in which, as

expected, OVX rats had significantly lower sucrose preference on week 4 ($p<0.0004$; Figure 2.3E). Further, there was a significant CUS week by drug treatment interaction ($F(2, 72)=3.39$, $p=0.039$), such that fluoxetine treatment significantly increased sucrose preference on week 6 of CUS ($p < 0.025$) but not any other week (all p 's > 0.4), regardless of ovarian status (Figure 2.3F). There were also significant main effects (ovarian status, and CUS week; all p 's < 0.008), but no other significant interactions (all p 's > 0.08). Importantly, the groups did not differ significantly on baseline sucrose preference prior to CUS (all p 's > 0.05).

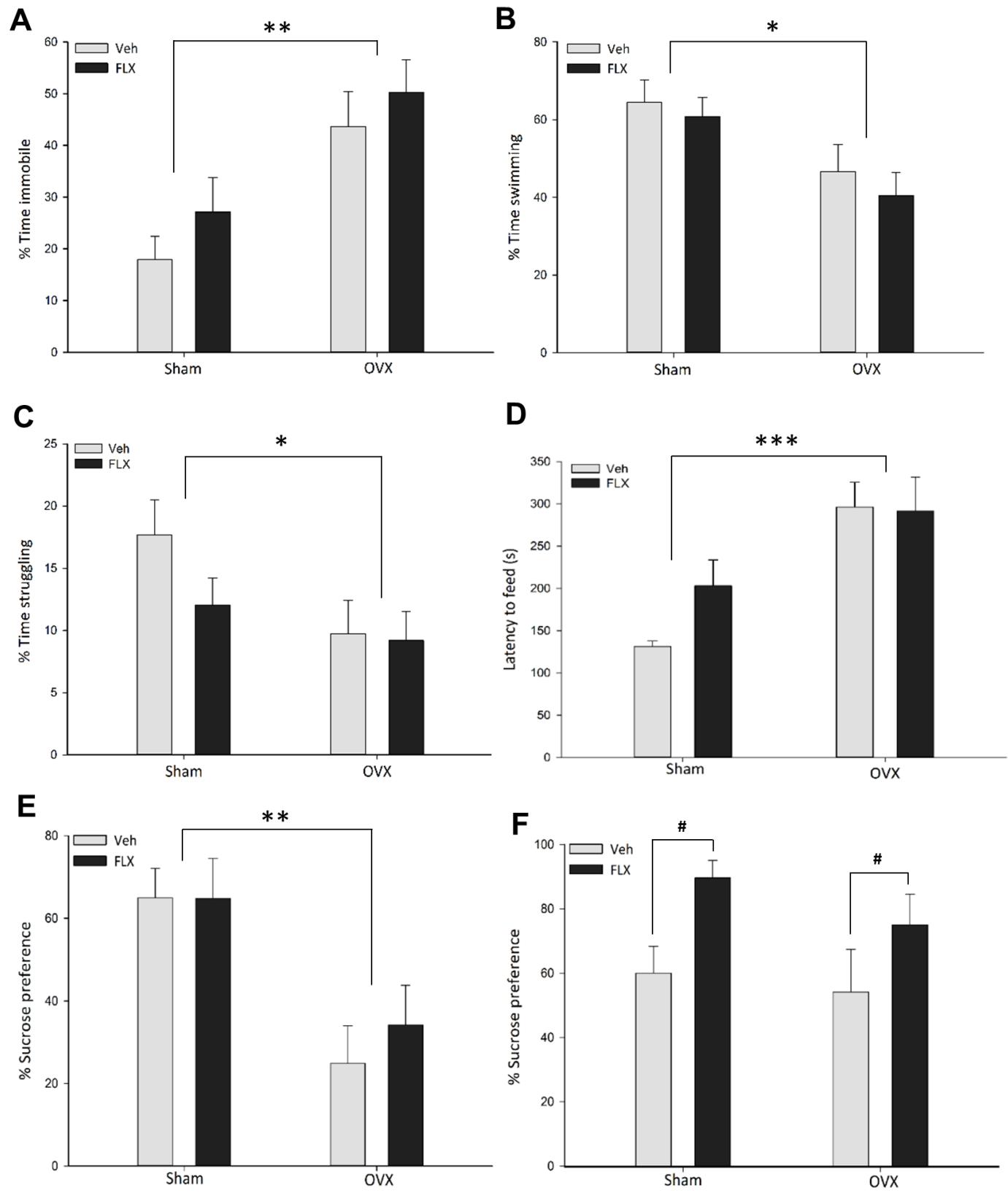


Figure 2.3 Effects of ovariectomy (OVX) and/or fluoxetine (FLX) treatment on behavior in the forced swim (A-C), novelty suppressed feeding (D), and sucrose preference (E-F) tests. In comparison to Sham controls, OVX significantly increased immobility (A), and reduced swimming (B) and struggling (C) in the forced swim test, but fluoxetine treatment did not have significant effects (A-C). (D) OVX significantly increased latency to feed in the novelty suppressed feeding test, but there was no significant effect of fluoxetine treatment ($p=0.09$ for FLX to increase latency to feed in Sham rats). OVX significantly decreased percent sucrose preference on week 4 of CUS, in comparison to Sham controls (E). Fluoxetine significantly increased percent sucrose preference on week 6 of CUS, relative to vehicle treatment (F). * Indicates $p < 0.05$, ** indicates $p < 0.0004$, and *** indicates $p < 0.0001$ in relation to Sham controls. # Indicates $p < 0.025$ in comparison to vehicle treatment. Veh = vehicle, FLX = fluoxetine. Data is represented in mean values + standard error of the mean (SEM).

Fluoxetine treatment significantly decreased body mass in OVX groups

Fluoxetine treatment significantly reduced the percent change in body mass in OVX but not sham rats from week 4 of CUS onwards, when compared to weeks 1-3 (all p 's < 0.03 ; week by drug treatment by ovarian status interaction: $F(5, 180)=2.72$, $p=0.021$; Figure 2.4A).

Additionally, on week 6, the percent change in body mass was significantly greater in fluoxetine-treated OVX rats when compared to vehicle-treated OVX rats ($p=0.047$). There were also significant main effects (ovarian status and CUS week, all p 's < 0.01), and a CUS week by drug treatment interaction ($p>0.00001$), but no other significant main effects or interactions (all p 's > 0.15).

Ovariectomy impaired glucocorticoid-dependent negative feedback on the HPA axis

OVX rats had significantly higher post-stress (DEX 30) CORT concentrations compared to Sham rats ($p < 0.0001$; time by ovarian status interaction; Figure 2.4B, $F(2, 62)=7.63$, $p=0.0001$). There were no significant differences in serum CORT concentrations at baseline (DEX 0, $p=0.74$) or after recovery from stress (DEX 60, $p=0.94$). Fluoxetine significantly reduced post-stress (DEX 30) CORT concentrations in Sham ($p=0.023$) but not OVX ($p=0.33$) rats. There was

also a significant main effect of ovarian status ($p=0.005$), and a trend toward a drug treatment effect with fluoxetine attenuating CORT concentrations ($p=0.097$).

Sham rats showed an initial increase in percent change in basal CORT that was reduced by ovariectomy

OVX rats showed no significant percent change in baseline CORT across all days of CUS examined, but the percent change was significantly lower than Sham groups across the first twenty days (Figure 2.4C, $F(3, 87)=3.83$, $p=0.013$, time by ovarian status interaction). In Sham rats, the percent change in CORT was significantly reduced by day 40 in comparison to days 10 and 20 (p 's <0.05). There were also significant main effects of time ($p=0.017$) and ovarian status ($p=0.0006$), but no other significant effects.

Ovariectomy reduced relative adrenal mass and modulated the effect of fluoxetine treatment on relative adrenal mass.

OVX significantly reduced relative adrenal mass (Figure 2.4D, $F(1,35)=5.90$, $p<0.02$, ovarian status by drug treatment interaction). Interestingly, post-hoc tests revealed only weak trends for fluoxetine to decrease relative adrenal mass in Sham rats ($p=0.096$) and to increase relative adrenal mass in OVX rats ($p=0.093$). There was also a significant main effect of ovarian status ($F(1,35)=61.53$, $p<0.0001$) but no other significant effects. Additionally, the groups did not differ on relative thymus mass (all p 's > 0.15).

Ovariectomy decreased serum 17β -estradiol concentrations

As expected, OVX significantly decreased serum 17β -estradiol concentrations in samples collected prior to perfusion (Figure 2.4E, $F(1,36)=18.88$, $p=0.0001$, main effect of ovarian status). There were no other significant effects or interactions (all p 's >0.4).

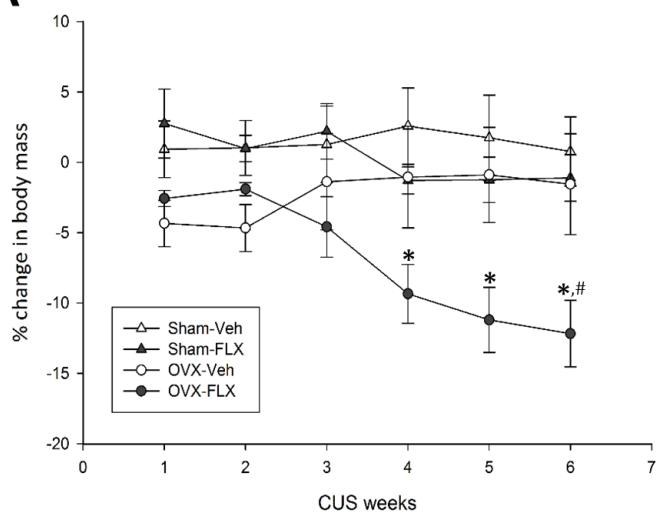
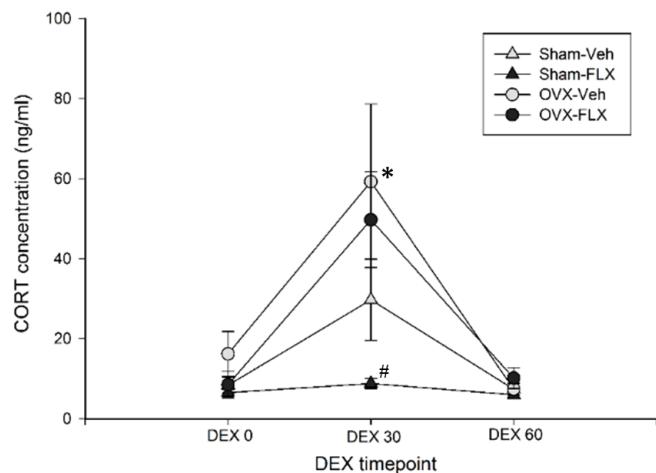
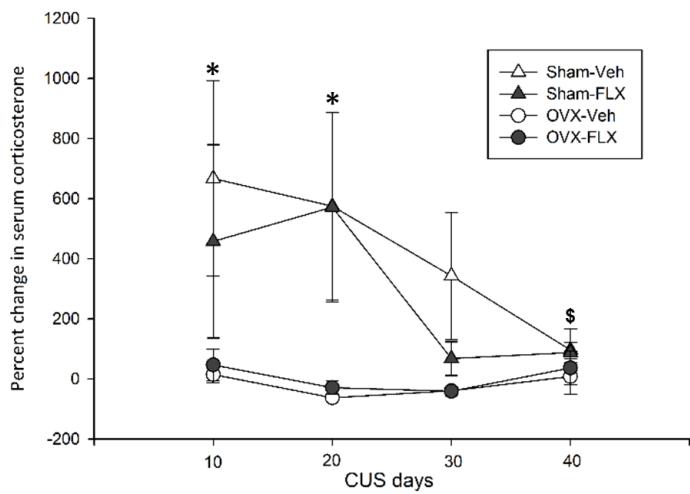
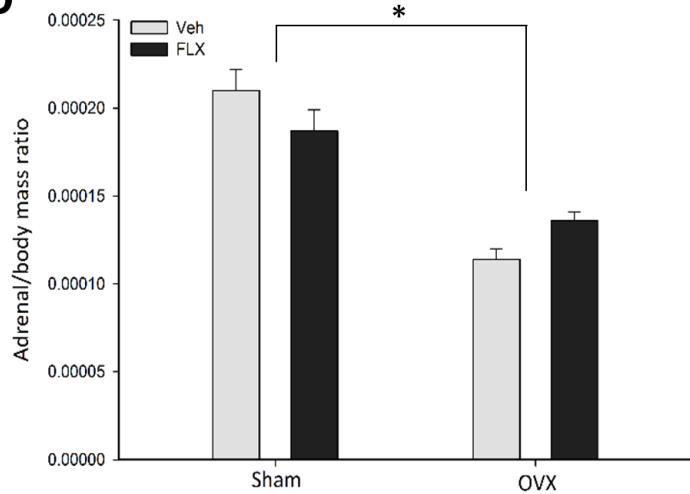
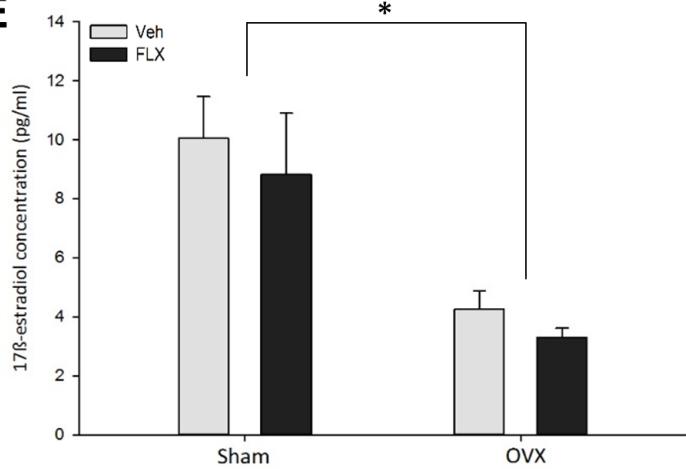
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Figure 2.4 Percent change in body mass, adrenal mass, and corticosterone and 17 β -estradiol concentrations. (A) Percent change in body mass across weeks of chronic unpredictable stress (CUS). Fluoxetine treatment significantly reduced body mass in ovariectomized (OVX) but not sham rats from week 4-6 of CUS when compared to weeks 1-3; * indicates p<0.03. Fluoxetine treatment significantly decreased body mass in OVX rats on week 6, relative to vehicle treatment; # indicates p<0.05. (B) Corticosterone (CORT) concentrations in the dexamethasone (DEX) suppression test. OVX rats had significantly higher post-stress CORT concentration relative to Sham rats (DEX 30); * indicates p=0.0001. Fluoxetine significantly reduced post-stress CORT in sham rats only, relative to vehicle treatment; # indicates p=0.023. (C) Percent change in basal serum CORT was significantly higher in Sham relative to in OVX groups across the first twenty days; * indicates p=0.013. In Sham rats, the percent change in CORT was significantly reduced by day 40 in comparison to days 10 and 20; \$ indicates p's <0.05. (D) OVX significantly reduced adrenal/body mass ratio; * indicates p<0.02. (E) 17 β -estradiol concentrations from serum taken on perfusion day. OVX significantly decreased serum 17 β -estradiol relative to Sham; * denotes p=0.0001. OVX = ovariotomy, Veh = vehicle, FLX = fluoxetine. Data is represented in mean values \pm SEM.

Ovarian status and fluoxetine treatment did not significantly affect dentate gyrus volume

Apart from the expected volume differences between the GCL and hilus ($F(3, 102)=323.84$, $p<0.00001$, main effect of region), there were no significant differences between ovarian status or drug treatment groups (all p's >0.3). Thus, the number of immunoreactive cells, instead of density, was used in all analyses.

Fluoxetine treatment increased the number of Ki67-ir cells in the GCL: dependent on hippocampal region and ovarian status

Fluoxetine treatment significantly increased the number of Ki67-ir cells in both the dorsal and ventral GCL+SGZ (p's < 0.008) but not in the hilus (p's >0.94; Figure 2.5A, $F(1, 36)=9.00$, $p<0.005$, region by area by treatment interaction). Furthermore, when compared to Sham groups, OVX groups had a significantly higher number of Ki67-ir cells in the GCL+SGZ ($p<0.006$) but not in the hilus ($p=0.96$; area by ovarian status interaction $F(1, 36)=4.261$, $p=0.046$). A priori, we expected differences in the effects of fluoxetine treatment based on ovarian status, and

comparisons revealed that in relation to vehicle, fluoxetine treatment significantly increased the number of Ki67-ir cells in both the dorsal and ventral GCL+SGZ in OVX rats (p 's <0.0002), but only in the ventral GCL+SGZ in Sham rats (ventral p =0.00017, dorsal p =0.96). In order to account for the effects of estrous cycle phase on cell proliferation (Tanapat et al., 1999), the number of Ki67-ir cells were analyzed with estrous phase as a covariate, but this did not change the main findings, and there were no significant effects of the covariate (all p 's>0.78).

Fluoxetine increased the number of PSA-NCAM-ir cells in the ventral GCL in an ovarian status-dependent manner

There was a strong trend for fluoxetine treatment to increase the number of PSA-NCAM-ir cells in the ventral but not dorsal GCL+SGZ ($F(1, 33)$ =3.884, p =0.057, region by treatment interaction; Figure 2.5B). A priori comparisons indicated that fluoxetine significantly increased the number of PSA-NCAM-ir cells in the ventral GCL+SGZ of OVX animals (p =0.0025), but not in any other group or region (all p 's>0.1). There was a significant main effect of region ($F(3,108)$ =101.16, p =0.0000), a trend for a main effect of fluoxetine treatment (p =0.084) but no other significant effects (all p 's>0.3).

Fluoxetine treatment increased the number of BrdU-ir cells in the dorsal GCL in an ovarian status-dependent manner

Interestingly, fluoxetine treatment significantly increased the number of BrdU-ir cells in the dorsal GCL of OVX rats (Figure 2.5C; $F(1, 34)$ =7.42, p =0.01; region by ovarian status by treatment interaction). As expected, there was a main effect of region and of area on BrdU-ir (all p 's<0.0001). There were no other significant main effects or interactions (all p 's > 0.1). Additionally, there were no group differences in the percentage of BrdU/NeuN colabeled cells,

such that the average percentage of colabeled cells was above 75% for all groups (Table 2.2; all p's> 0.75).

Ovarian status	Drug treatment	% of BrdU/NeuN colabeled cells
Sham	Veh	77.50 ± 6.77
	FLX	75.85 ± 4.90
OVX	Veh	75.35 ± 7.08
	FLX	77.54 ± 4.65

Table 2.2 Percentage of BrdU/NeuN colabeled cells in the granule cell layer of the dentate gyrus. The groups did not differ significantly (all p's>0.75). Data in means ± standard error of the mean. Veh= vehicle, FLX=fluoxetine, OVX=ovariectomy.

Fluoxetine treatment decreased the number of Iba-1-immunoreactive cells in the ventral GCL, but had no effect on the average length of microglial processes

Fluoxetine treatment significantly decreased the number of Iba-1-ir cells in the GCL+SGZ ($p=0.0011$; Figure 2.5D) but not the ML ($p=0.55$; $F(1, 35)=4.56$, $p=0.04$ area by treatment interaction), and there was a trend towards a main effect of drug treatment ($p<0.06$). Planned comparisons revealed that fluoxetine decreased the number of Iba-1-ir cells in the ventral ($p=0.000008$, $p=0.008$ in Sham and OVX, respectively) but not dorsal ($p=0.016$, $p= 0.39$, in Sham and OVX, respectively) GCL+SGZ. There were main effects of region and of area (all p's<0.0001), but no other significant effects (all p's>0.1). The average length of processes was significantly higher in the ventral relative to the dorsal GCL+SGZ, regardless of ovarian status (Table 2.3; $F(1, 35)=6.75$, $p=0.014$, main effect of region). There were no other significant main effects or interactions (all p's>0.19).

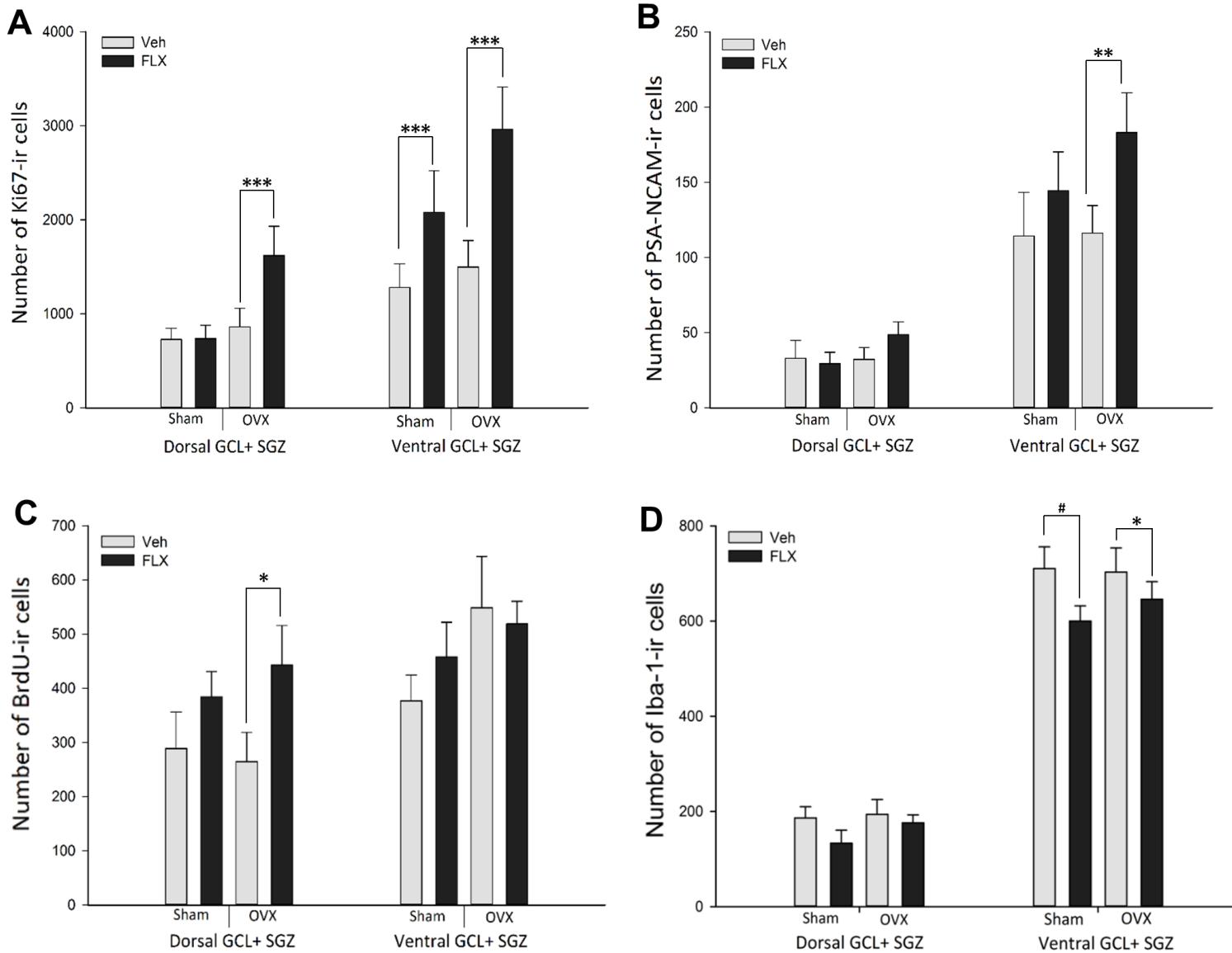


Figure 2.5 Number of Ki67, PSA-NCAM, BrdU, and Iba-1 immunoreactive cells in the dentate gyrus. (A) Fluoxetine increased Ki67-immunoreactive (ir) cells in the dorsal and ventral granule cell layer (GCL) and subgranular zone (SGZ) in ovariectomized (OVX) rats, and in the ventral GCL+SGZ in Sham rats, relative to vehicle treatment. (B) Fluoxetine increased PSA-NCAM-ir cells in the ventral GCL+SGZ in OVX rats, compared to vehicle treatment. (C) Fluoxetine increased BrdU-ir cells in the dorsal GCL+SGZ in OVX rats, compared to vehicle treatment. (D) Fluoxetine treatment significantly decreased Iba-1-ir cells in the ventral GCL, relative to vehicle treatment. Data is represented in mean values + SEM. Veh = vehicle, FLX = fluoxetine, OVX = ovariectomy. * indicates $p < 0.01$, ** indicates $p < 0.003$, *** indicates $p < 0.0002$, and # indicates $p < 0.000001$, in relation to vehicle treatment.

Ovarian status	Drug treatment	Average length of processes	
		dorsal dentate gyrus	ventral dentate gyrus
Sham	Veh	149.22 ± 4.76	158.73 ± 4.33
	FLX	152.06 ± 5.05	157.63 ± 4.91
OVX	Veh	154.15 ± 4.11	162.01 ± 6.18
	FLX	159.40 ± 2.65	162.59 ± 4.01

Table 2.3 Average length of processes of Iba-1-immunoreactive cells in the granule cell layer of the dentate gyrus. The average length of processes was significantly higher in the ventral dentate gyrus relative to the dorsal dentate gyrus ($p=0.014$), but there were no other significant main effects or interactions (all p 's > 0.19). Data in means ± standard error of the mean. Veh = vehicle, FLX = fluoxetine, OVX = ovariectomy.

Higher levels of estradiol were associated with reduced immobility in the forced swim test

Interestingly, serum estradiol concentrations were negatively correlated with percent time spent immobile in FST in fluoxetine ($r = -0.65$, $p = 0.043$; Figure 2.6A) but not vehicle treated rats ($r = 0.098$, $p = 0.79$; Figure 2.6B).

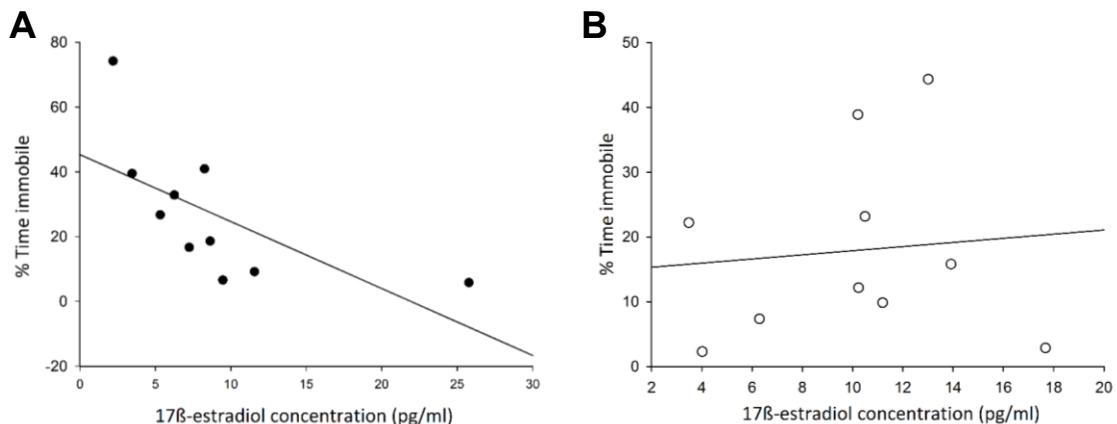


Figure 2.6 Correlations between serum estradiol concentrations and percent time spent immobile in the forced swim test

in Sham rats. Serum estradiol concentrations were negatively correlated with percent immobility in the forced swim test in fluoxetine-treated rats ((A) $r = -0.65$, $p = 0.043$) but not vehicle-treated rats ((B) $r = 0.098$, $p = 0.79$). If the outlier (serum estradiol = 25.8 pg/ml) is removed from the analysis in (A), the correlation between serum estradiol concentrations and percent time spent immobile is strengthened ($r = -0.81$, $p = 0.007$).

2.4 Discussion

Here, we report that under conditions of chronic stress, long-term ovarian hormone deprivation increased the vulnerability of middle-aged female rats to develop depressive-like behavioral and endocrine phenotypes. Interestingly, we found that fluoxetine treatment showed weak behavioral and endocrine efficacy; fluoxetine treatment increased sucrose preference only in the last week of CUS and improved HPA negative feedback in Sham but not ovariectomized rats. Fluoxetine treatment was also associated with significant changes in neurogenesis, PSA-NCAM and microglia expression, in a region-specific and ovarian status-dependent manner. To our knowledge, this is the first report to integrate the behavioral, neurogenic, and neuroendocrine consequences of long-term ovarian hormone deprivation in tandem with chronic stress and antidepressant treatment. A schematic summary of findings is included in Figure 2.7.

A	Behavior			Endocrine	Neural					
	FST Immobility	NSF Latency	SPT preference	DEX suppression	Ki67	BrdU	PSA-NCAM	Iba-1 number	Iba-1 length of processes	
OVX (Relative to sham)	↑	↑	↓*	↓	=	=	=	=	=	
B	Behavior			Endocrine	Neural					
	FST Immobility	NSF Latency	SPT Sucrose preference	DEX suppression	Ki67	BrdU	PSA-NCAM	Iba-1 number	Iba-1 length of processes	
Sham (FLX relative to Veh)	=	=	↑**	↑	=	↑	=	=	=	
OVX (FLX relative to Veh)	=	=	↑**	=	↑	↑	↑	=	=	

Figure 2.7 Schematic summary of behavioral, endocrine, and neural findings. (A) The effects of long-term ovariectomy in comparison to Sham controls. (B) The effects of fluoxetine compared to vehicle treatment, separated by ovarian status. OVX: ovariectomy, FLX: fluoxetine, Veh: vehicle, FST: Forced Swim Test, NSF: Novelty Suppresses Feeding, SPT: Sucrose Preference Test, DEX: dexamethasone. ↑ indicates significant increase, ↓ indicates significant decrease, = indicates no significant change. * indicates significance only in week 4 of chronic unpredictable stress, ** indicates significance only in week 6 of chronic unpredictable stress.

Ovarian hormones imparted resilience against the development of anxiety- and depressive-like behaviors under chronic stress conditions

Long-term ovarian hormone deprivation markedly increased the percent time spent immobile and decreased the percent time spent swimming in the FST, compared to Sham controls. This indicates that under conditions of chronic stress, ovarian hormones may afford resilience against the development of depressive-like behavior. Our findings are in partial agreement with a study

indicating that long-term, but not short-term, ovarian hormone deprivation increased immobility in the FST in mice subjected to CUS (Lagunas et al., 2010). However, much of the previous literature on the role of ovarian hormones in depressive-like behavior is comprised of studies in which animals are not subjected to any prior stress manipulations or model of depression (Rachman et al., 1998; Récamier-Carballo et al., 2012; Li et al., 2014). While these studies demonstrate that short-term ovariectomy alone (1-5 weeks) increased immobility in the FST, long-term ovariectomy (3 - 15 months) did not alter immobility in the FST (de Chaves et al., 2009; Estrada-Camarena et al., 2011). Therefore, these past findings suggest that the increase in depressive-like behavior as a result of ovariectomy alone may be transient. Taken together with our current data, this indicates that long-term ovarian hormone deprivation may result in a state of enhanced vulnerability to depressive-like phenotypes in the face of chronic stress.

In a similar manner, in the present study we found that long-term ovarian hormone deprivation increased anxiety-like behavior in the novelty suppressed feeding test, in comparison with Sham controls. This agrees with previously reported anxiolytic effects of estradiol and estrogen receptor agonists (Lund et al., 2005; Walf and Frye, 2005), and with studies indicating increased anxiety-like behavior with longer periods of ovarian hormone depletion (Picazo et al., 2006; Lagunas et al., 2010). Here, we observe a trend for fluoxetine to increase latency to feed in Sham, but not ovariectomized rats. This is not completely surprising given previous reports suggesting anxiogenic effects of fluoxetine treatment (Silva et al., 1999; Belzung et al., 2001; Leuner et al., 2004). Interestingly, one study found that chronic fluoxetine treatment increased anxiety-like behavior in female but not male rats (Leuner et al., 2004). This sex difference coupled with our current data suggests that ovarian hormones may be implicated in the anxiogenic effects of fluoxetine, however this requires further investigation.

In line with our other behavioral findings, sucrose preference was also significantly reduced by long-term ovariectomy. Because there were no differences between ovarian status groups in baseline sucrose preference prior to CUS, it appears that long-term ovariectomy does not itself produce anhedonia-like behavior, but rather increases the susceptibility to anhedonia-like behavior under chronic stress conditions. Importantly, this effect was not seen at all time points, however this may not be surprising given that sucrose anhedonia is less robust in female compared to male rodents (Dalla et al., 2005, 2008; Grippo et al., 2005; Kamper et al., 2009). Regardless of ovarian status, fluoxetine treatment resulted in a significant increase in sucrose preference after the last week of CUS, but had no significant effect on behavior in any other test (NSF and FST). This indicates that in middle-aged female rats, a 5mg/kg dose of fluoxetine may be efficacious in alleviating some but not all depressive-like behavioral phenotypes. Our findings are somewhat inconsistent with another study showing that non-efficacious doses of fluoxetine and estradiol, when administered in concert, act synergistically to reduce immobility in the FST in ovariectomized middle-aged female rats (Récamier-Carballo et al., 2012). However, important differences between the study by Recamier-Carballo et al. and ours may explain the inconsistencies in findings. These differences include the length of ovarian hormone deprivation (4 months in the current study versus 3 weeks in Récamier-Carballo et al., 2012), treatment regimen (3 weeks of fluoxetine treatment in our study versus 3 injections within 24 hours in Récamier-Carballo et al., 2012), and condition (rats were exposed to chronic stress in our study but were stress-naive in Recamier-Carballo et al., 2012). Our data are, however, consistent with previous studies in which estradiol was shown to increase resilience. For example, estradiol replacement in ovariectomized rats reduced the incidence of helplessness after inescapable shock (Bredemann and McMahon, 2014). In addition, estradiol afforded resilience to cognitive deficits

in the face of repeated stress (Wei et al., 2014). Collectively, findings from this study and others indicate that ovarian hormone deprivation may result in a state of enhanced vulnerability to the deleterious effects of chronic stress.

It is important to note that it is not possible to ascertain the reason behind the lack of behavioural efficacy of fluoxetine treatment in the FST or NSF from the current data. In order to address this caveat, future work in middle-aged female rats should compare the response to additional doses of fluoxetine, and/or other SSRIs. However, it is also important to note that although SSRIs are the first-line pharmacological treatment for major depression, only a third of patients will respond to these drugs following initial treatment (Trivedi et al., 2006; Kornstein et al., 2013). Even in patients that are initially responsive to treatment, up to 57% will have depressive symptoms return due to a loss of drug efficacy. Thus, our findings are at least somewhat consistent with the clinical literature, and perhaps it is not surprising that we see limited behavioral efficacy with SSRIs in this report.

17 β -estradiol was associated with a facilitated antidepressant-like effect of fluoxetine to decrease immobility in the forced swim test

Higher levels of serum 17 β -estradiol were associated with reduced immobility in the FST only in the fluoxetine-treated Sham group (fluoxetine-treated ovariectomized group had undetectable estradiol levels). Because the same relationship was not seen in the vehicle treated Sham group, this suggests that in chronically stressed female rats, higher levels of endogenous 17 β -estradiol may facilitate the effect of fluoxetine to reduce immobility in the FST. Our findings are in line with a previous report demonstrating that in ovariectomized rats, sub-optimal doses of 17 β -estradiol act synergistically with fluoxetine to reduce immobility in the FST (Récamier-Carballo

et al., 2012). Our findings also parallel the human literature, which indicates that in post-menopausal women with depression, SSRIs are more efficacious in alleviating symptoms when prescribed in conjunction with hormone therapy (Thase 2005), however other studies have failed to show this effect (Kornstein et al., 2013). The mechanisms that underlie the ovarian hormone-mediated enhancement of antidepressant efficacy are not clearly delineated. However, estradiol increases serotonergic tone via enhanced serotonin synthesis (Bethea et al., 1999), reduced degradation (Gundlah et al., 2002), decreased autoreceptor-mediated inhibition (Lu and Bethea, 2002), and possibly reduced serotonin transporter levels (Frokjaer et al., 2015). Thus, such mechanisms may be at play in the facilitation of antidepressant effects.

Ovarian hormones imparted resilience against glucocorticoid-dependent negative feedback impairment

Coinciding with our behavioral results, long-term OVX rats that had undergone CUS exhibited impaired HPA negative feedback compared to Sham rats in the dexamethasone suppression test. This finding signifies that ovarian hormones may protect against HPA negative feedback impairment in the context of chronic stress. In general, ovarian hormones potentiate acute stress-induced activation of the HPA axis (Burgess and Handa, 1992) and impair HPA negative feedback inhibition (Weiser and Handa, 2009). These findings may initially seem contradictory to our current data in which DEX suppression of CORT release was reduced in OVX rats. However, a critical consideration is that in the aforementioned studies, the subjects were not previously exposed to chronic stress. Thus, these findings coupled with our own suggest that the role of ovarian hormones to regulate HPA axis negative feedback may be drastically affected by chronic stress. Intriguingly, our findings translate to a study of pre- and post- menopausal

women, all of whom were diagnosed with depression, in which resistance to DEX suppression was found in a significantly higher proportion of postmenopausal women as compared to premenopausal women (Young et al., 1993). Furthermore, our data are also consistent with the finding that estradiol improved HPA negative feedback function in the DEX suppression test in women made hypogonadal (Lee et al., 2012). Lastly, we found that fluoxetine improved HPA negative feedback function in Sham, but not ovariectomized rats. To our knowledge, there are no studies examining antidepressant efficacy to normalize HPA negative feedback function in depressed women with differing estradiol levels. Taken together, this suggests that ovarian hormones may protect against HPA axis negative feedback dysregulation in women with depression, and similarly as a result of chronic stress exposure in female rodents.

In the present study, basal levels of serum CORT were reduced in OVX relative to Sham rats, but the percent change in basal CORT did not change throughout CUS, while it was increased in Sham controls. This finding is in agreement with prior research indicating that ovarian hormones exert stimulatory effects on basal HPA axis activity (see Goel et al., 2014 for review).

Fluoxetine treatment increased neurogenesis and PSA-NCAM in a region-specific and ovarian status-dependent manner

In the present study, we found no significant differences in levels of cell proliferation (Ki67 expression), neurogenesis (BrdU/NeuN), or PSA-NCAM expression in the dentate gyrus of vehicle treated Sham and long-term OVX middle-aged rats. These findings are in accordance with previous literature showing that unlike short-term ovariectomy (1 week), long-term ovariectomy (3-4 weeks) does not produce significant changes in neurogenesis (Tanapat et al.,

1999) or PSA-NCAM expression (Banasr et al., 2001). The normalization of neurogenesis levels following longer periods of ovariectomy is unlikely related to *de novo* synthesis of estrogens in the brain, as after three weeks of ovariectomy, levels of estradiol in the hippocampus of adult female rats are not detectable (Barker and Galea, 2009), and hippocampal estradiol correlates with plasma estradiol (Kato et al., 2013). Thus, alternative mechanisms may be at play in the return of neurogenesis levels after long-term ovariectomy, including ovariectomy-induced reductions in basal corticosterone (Goel et al., 2014). Together, our results indicate that in middle-aged rats exposed to chronic stress, long-term deprivation of ovarian hormones does not alter neurogenesis or PSA-NCAM expression in the dentate gyrus.

Fluoxetine treatment increased cell proliferation, neurogenesis, and the expression of PSA-NCAM within the dentate gyrus. These findings are in line with prior reports indicating that chronic treatment with fluoxetine upregulates the same markers (Malberg et al., 2000; Guirado et al., 2012). Although in older age fluoxetine did not increase neurogenesis in the dentate gyrus (Couillard-Despres et al., 2009), our findings are consistent with others showing that fluoxetine upregulated PSA-NCAM in middle-aged male rats (Guirado et al., 2012). Interestingly, our current data show that fluoxetine treatment, when initiated after BrdU administration, increased adult hippocampal neurogenesis, unlike what is seen in stress-naïve, young adult male rats (Malberg et al., 2000). Indeed, our finding is consistent with another study using young adult female rats (Vega-Rivera et al., 2015), perhaps indicating a sex difference in the effect of fluoxetine administration on the survival of new neurons. Interestingly, the fluoxetine-mediated upregulation of cell proliferation, neurogenesis, and PSA-NCAM was seen despite its lack of behavioral efficacy in the majority of tests, suggesting that these neural changes are not

necessarily synonymous with behavioral efficacy, partially consistent with the existing literature in young adult male rats and mice (Bessa et al., 2009; Santarelli, 2003).

Somewhat surprisingly, the effect of fluoxetine treatment to enhance cell proliferation (Ki67-ir cells) was seen in both the dorsal and ventral hippocampus of OVX rats, but only in the ventral hippocampus of Sham rats. Similarly, fluoxetine-mediated enhancement of neurogenesis and PSA-NCAM was exclusive to OVX rats, in the dorsal and ventral hippocampus, respectively. Thus, contrary to our initial expectations, the potential for plasticity in response to fluoxetine treatment is not reduced with long-term ovarian hormone deprivation, but rather appears to be enhanced. The mechanism underlying this unexpected finding is not clear, however, because ovarian hormone generally have stimulatory effects on the HPA axis (Goel et al., 2014)), it is possible that higher levels of basal corticosterone in Sham rats may interact with fluoxetine to prevent the upregulation of cell proliferation observed in OVX rats. Considering that in the present study there were no significant differences in the effects of fluoxetine on behavior between OVX and Sham groups, the functional consequences of this differential upregulation of neuroplasticity based on the ovarian hormone milieu remain elusive.

Interestingly, fluoxetine treatment improved HPA negative feedback function in Sham rats, but had more profound pro-neurogenic effects in OVX rats. This is somewhat inconsistent with previous research in young adult male rodents, in which newly generated hippocampal neurons were shown to be required for antidepressants to improve HPA axis negative feedback function (Surget et al., 2011), and data indicating that hippocampal neurogenesis may be important for HPA negative feedback regulation (Snyder et al., 2011). This discrepancy is interesting, as it suggests that the relationship between hippocampal neurogenesis and HPA axis

function may differ between males and females. It is important to note that it is not without precedent that neurogenesis may have different functions or links to behavior/stress in males versus females, as sex differences in neurogenesis have been noted in response to spatial learning (Chow et al., 2013; Yagi et al., 2016), trace eyeblink conditioning (Dalla et al., 2009), acute and chronic stress (Falconer and Galea, 2003; Westenbroek et al., 2004), adolescent stress exposure (Barha et al., 2011), and chronic estradiol treatment (Barker and Galea, 2008).

Fluoxetine treatment reduced microglial number in the ventral dentate gyrus but had no significant effect on microglial morphology

Regardless of ovarian status, fluoxetine treatment significantly reduced the number of Iba-1-ir microglia in the ventral dentate gyrus, partially consistent with previous findings showing imipramine treatment reverses the initial increase and subsequent decline in microglial number as a result of chronic unpredictable stress (Kreisel et al., 2014). However, our data provide no evidence for alterations in microglial activation with fluoxetine treatment, as there was no effect of fluoxetine on the number or length of processes of Iba-1-ir cells. Thus, our findings suggest that SSRI treatment may be associated with a reduction in microglia-mediated neuroinflammation, however this requires further investigation as microglial function depends on its activational state (Kettenmann et al., 2011) and levels of pro- versus anti-inflammatory cytokines. Importantly, our findings are in partial agreement with human literature suggesting that antidepressant treatment is associated with a reduction of peripheral inflammation, as measured by reduced pro-inflammatory cytokines such as IL-6 (Basterzi et al., 2005) and TNF- α (Tuglu et al., 2003), findings that were confirmed by a meta-analysis (Eller et al., 2008). Taken together with our present findings, this suggests that antidepressants may exert their effects, at

least in part, through the amelioration of microglia-mediated neuroinflammation in the hippocampus.

Our findings reveal no baseline differences in the number or morphology of Iba-1-ir microglial cells between OVX and Sham groups. This is somewhat surprising considering that ovarian hormones inhibit microglial proliferation *in vivo* (Ganter et al., 1992), and can directly alter microglial function and morphology both *in vivo* and *in vitro* (Habib and Beyer, 2015). Our findings are also in contrast with a study in middle-aged female rats, in which Iba-1 mRNA expression in the hippocampus was significantly increased when measured approximately 40 days after ovariectomy (Sárvári et al., 2014). However, in our present study, the period of ovarian hormone deprivation was substantially longer (4 months), and thus compensatory mechanisms may be at play to return baseline levels of Iba-1 expression.

Ovarian status-dependent effects of fluoxetine on body mass

Fluoxetine treatment reduces body mass or attenuates weight gain in rodents (McGuirk et al., 1992). In the present study, fluoxetine reduced body mass in OVX rats only. Although the functional outcome of fluoxetine-induced body mass reduction is unknown, ovarian hormones appear to protect against this effect.

2.5 Conclusions

In summary, ovarian hormones afforded resilience against the development of depressive-like phenotypes in middle-aged female rats exposed to chronic unpredictable stress. Long-term ovariectomy increased depressive- and anxiety-like behaviors in the forced swim, novelty suppressed feeding, and sucrose preference tests after exposure to chronic unpredictable stress. Further, long-term ovariectomy resulted in significant impairments in HPA axis negative

feedback inhibition, as revealed by the dexamethasone suppression test. Thus, our behavioral and endocrine findings converge to suggest that ovarian hormones may be key determinants of stress resilience. With the exception of the sucrose preference and dexamethasone suppression tests, chronic fluoxetine treatment at 5mg/kg did not produce antidepressant-like effects, regardless of ovarian status. However, higher levels of endogenous 17 β -estradiol were associated with a facilitation of fluoxetine to decrease immobility in the forced swim test. Despite its limited efficacy, fluoxetine treatment was associated with reduced microglial number in the ventral dentate gyrus, and increased cell proliferation, neurogenesis, and PSA-NCAM expression, effects that varied by ovarian status and region. Our findings underscore the need for considering the role of ovarian hormones in stress-resilience, depression, antidepressant efficacy, and the neural correlates of antidepressant use in females.

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Chapter 3: Ovarian status dictates the neuroinflammatory and behavioral consequences of sub-chronic stress exposure in middle-aged female mice

3.1 Introduction

Major Depressive Disorder (MDD), a leading cause of disability and ill-health worldwide (Friedrich, 2017), is approximately twice more prevalent in women than men (Kessler and Bromet, 2013; Salk et al., 2017). In addition to this robust difference in prevalence rates, disease presentation and pathophysiology differ significantly between men and women (reviewed in Eid et al., 2019). Although there is an encouraging increase in studies investigating sex differences in depression and in animal models of stress exposure, studies that target female-specific factors that may contribute to risk or resilience to depression remain scarce. Ovarian hormones play a role in mood regulation and influence depression in women (Brummelte and Galea, 2010b; Soares, 2013; Hantsoo and Epperson, 2015) and therefore represent one such target. For example, there is an increased susceptibility to develop depression during the reproductive years in women compared to men (Gutiérrez-Lobos et al., 2002). These data provide the basis for the hypothesis that ovarian hormones may increase risk for depression in women. Other lines of evidence, however, support the contrasting hypothesis that ovarian hormones may afford resilience against depression. Specifically, events that are characterized by large reductions in ovarian hormones, including the postpartum and perimenopause, are associated with an increased risk for depression in women (Cohen et al., 2006; Freeman et al., 2004; Hendrick et al., 1998; Soares, 2014). In rodent studies, ovariectomy alone increases depressive-like behavior in the short term and increases susceptibility to the development of depressive-like behavior in response to chronic stress exposure in the long term (Lagunas et al., 2010; Li et al., 2014;

Mahmoud et al., 2016a). Taken together, this literature highlights the complex and seemingly paradoxical roles of ovarian hormones in mood regulation and depression. Importantly, ovarian hormones regulate a wide range of physiological processes and systems that are compromised in depression, including stress response and immune systems (Goel et al., 2014; Klein and Flanagan, 2016). Therefore, examining the interplay between endocrine and immune systems in the context of stress exposure may clarify the role of ovarian hormones in depression.

There is mounting evidence of immune dysregulation in depression (Hodes et al., 2015; Miller and Raison, 2016). At least a subpopulation of individuals with MDD present with increased markers of inflammation, supported by several meta-analyses which highlight that IL-6 and TNF- α are increased in the blood and cerebral spinal fluid (Dowlati et al., 2010; Liu et al., 2012; Haapakoski et al., 2015; Wang and Miller, 2018). Further, poor antidepressant efficacy has been linked to an inability to normalize dysregulated inflammatory processes (Syed et al., 2018). Stress exposure, a major risk factor for depression (Kendler et al., 1999), can also activate inflammatory responses in the brain and periphery (Gouin et al., 2012; Felger et al., 2015). Importantly, sex differences exist across many immune processes, mediated in part by sex hormones (Klein and Flanagan, 2016). Several reports document the anti-inflammatory actions of estrogens and progesterone in the brain; in response to acute inflammatory challenges *in vitro* and *in vivo*, estradiol, selective estrogen receptor modulators (SERMs), and progesterone have all been shown to ameliorate microglia inflammatory activity and the secretion of proinflammatory cytokines including IL-1 β , TNF- α , and IL-6 (Vegeto et al., 2001; Suuronen et al., 2005; Tapia-Gonzalez et al., 2008; Smith et al., 2011; Lei et al., 2014). Further, in models of ischemic stroke and brain injury, both estrogens and progesterone have well-documented neuroprotective and anti-inflammatory properties (Sayeed and Stein, 2009; Villa et al., 2016).

However, the immunomodulatory actions of estrogens and progesterone are complex, as they can produce pro-inflammatory and neurotoxic effects depending on many factors, including age and sex (Hsieh et al., 2016; Selvamani and Sohrabji, 2010a; Straub, 2007). Despite the recognition that ovarian hormones influence immune processes, the role of ovarian hormones at the intersection of depression and immune dysregulation is not well known. This is particularly important during the transition to menopause, a time of heightened risk for MDD (Freeman et al., 2004; Cohen et al., 2006) that is also marked by substantial changes in ovarian and immune function (Burger et al., 2002; Giebing-Kröll et al., 2015). The current study targets this gap in the literature by examining how the ovarian hormone milieu can influence the depressogenic and inflammatory consequences of stress exposure in middle-aged mice.

A better understanding of endocrine-immune interactions in the pathogenesis of depression may be achieved by investigating downstream intracellular signaling pathways that are elicited by stress exposure. Mitogen-activated protein kinase (MAPK) pathways represent a logical target, as they regulate a wide range of cellular processes in response to diverse stimuli, including hormones and cytokines (Cargnello and Roux, 2011). Further, studies in humans and animal models of depression indicate that MAPK signaling pathways are compromised in the disorder (Miller and Raison, 2006; Hollos et al., 2018; Wang and Mao, 2019). The extracellular signal-regulated kinase (ERK) subfamily of MAPKs has received most attention in the context of depression (Wang and Mao, 2019), however the stress-activated protein kinases, c-Jun NH₂-terminal kinase (JNK) and p38 MAPK, may also be implicated (Miller and Raison, 2006; Hollos et al., 2018). Here, we quantified phosphorylated MAPKs to explore cellular mechanisms that may underly the role of ovarian hormones in risk or resilience in the face of stress exposure.

Disruptions in synaptic function and number are evidenced following stress and thought to be key factors in the pathogenesis of stress-related disorders such as depression (Duman et al., 2016). The prefrontal cortex (PFC) may be particularly vulnerable; in addition to reports of reduced PFC volume in depression (Drevets, 2000; Drevets et al., 2008), post-mortem studies find reductions in the number of synapses and in the expression of synapse-related genes in the PFC (Feyissa et al., 2009; Kang et al., 2012). In rodent models, chronic stress exposure results in dendritic remodeling in the frontal cortex and hippocampus (reviewed in McEwen et al., 2016), in addition to alterations in spines and synaptic markers (Orlowski et al., 2012; Workman et al., 2013). Importantly, neuronal remodeling in the frontal cortex and hippocampus in response to stress is influenced by sex and sex hormones (Galea et al., 1997; Shansky et al., 2009, 2010). Further, inflammatory processes could be implicated in synaptic alterations in stress and depression, as synaptic pruning is mediated by microglia during development and possibly in disease (Paolicelli et al., 2011; Hong et al., 2016). Therefore, examining synaptic markers may also clarify endocrine-immune interactions in stress and depression.

In this study, we used female mice to examine the effects of long-term ovariectomy on the behavioral and inflammatory consequences of sub-chronic stress exposure in middle age. We chose middle age because few studies have examined this important time point in females, and because perimenopause represents a time of increased vulnerability to depression in women. We measured cytokine concentrations in the periphery, and in the hippocampus and frontal cortex, areas known to be susceptible to stress exposure and compromised in depression (McKinnon et al., 2009; Howard et al., 2019). We also quantified phosphorylated MAPKs and postsynaptic density protein 95 (PSD-95) in the frontal cortex. We hypothesized that ovarian hormone

deprivation will dictate behavioral outcomes and modulate cytokines, cell signaling proteins, and PSD-95 expression in response to stress exposure.

3.2 Methods

Animals and surgery

Young-adult female C57BL/6N mice were purchased from Charles River Laboratories (Quebec, Canada). Mice received bilateral ovariectomy (OVX; n = 14) or sham surgery (Sham; n = 14) at Charles River Laboratories in young adulthood at 8 weeks of age, and arrived at our facility at 9 weeks of age. Mice were group housed (2-3/cage) in a temperature- and humidity- controlled colony room ($21 \pm 1^\circ\text{C}$; $50 \pm 10\%$ humidity), maintained on a 12-hour light/dark cycle (lights on at 07:00 h), and provided *ad libitum* access to food and water. Mice were left undisturbed, apart from weekly cage changing, until the beginning of further experimental manipulations in middle age, at 11-months-old. The reason mice were tested at middle age was two fold: 1) this is a time of heightened vulnerability to depression in women (Freeman et al., 2004; Cohen et al., 2006) that few animal studies target, and 2) previous rodent studies suggest that differences in susceptibility to stress exposure may emerge only after longer periods of ovarian hormone deprivation (Lagunas et al., 2010). Although our ovariectomized groups may not closely model the menopausal transition as surgery was performed in young adulthood, our sham-operated groups were tested during the transition to reproductive senescence, and as such are an appropriate model for the menopausal transition (Koebele and Bimonte-Nelson, 2016). All procedures were approved by the Animal Care Committee at the University of British Columbia and were performed in accordance with the ethical guidelines set by the Canadian Council on Animal Care.

Sub-chronic variable stress exposure

In each ovarian status condition (OVX, Sham), half the mice were exposed to 6 days of sub-chronic variable stress, adapted from previous methods (Hodes et al., 2015a; Labonté et al., 2017). We chose a 6-day paradigm to examine group differences in early indicators of immune dysregulation in response to stress. Mice in the stress condition were transferred to a separate colony room and allowed to acclimatize for two weeks prior to stress exposure. Stress-exposed mice were housed separately to avoid the transmission of stress-induced olfactory cues to non-stressed mice (Brechbuhl et al., 2013). The protocol consisted of three stressors, applied once per day on days 1-3, and repeated on days 4-6 (see timeline in Figure 3.1). Mice were exposed to foot shock stress on days 1 and 4 (100 shocks at 0.45mA, randomly distributed across 1 hour), tail suspension stress on days 2 and 5 (1 hour, suspended using laboratory tape, mid-way across the tail), and restraint stress on days 3 and 6 (1 hour, in a well-ventilated 50ml falcon tube, in the home cage). Mice in non-stress groups were handled daily for 5 minutes across days 1-6, but otherwise left undisturbed. Body weight was monitored daily across the stress exposure period and in non-stressed controls.

Behavioral testing

A battery of behavioral tests was conducted between experimental days 7 and 9 (Figure 3.1). All mice underwent behavioral testing, and except for the sucrose preference test, all tests were conducted under red light conditions and in designated testing rooms. Animals were acclimatized to the rooms for 1 hour prior to testing.

Splash test (ST). Self-grooming behavior in the ST was used as an index of self-care-like and motivational behavior, adapted from previous methods (Hodes et al., 2015a; Isingrini et al.,

2010). Mice were sprayed three times on the back with a 10% sucrose solution (in tap water), then individually placed in an empty clean cage for 5 mins. The test was filmed, and time spent grooming was scored by an experimenter blinded to condition. Immediately after the self-grooming test, mice were individually housed in clean cages, deprived of food in preparation for the novelty suppressed feeding test, and returned to their colony room. Mice remained individually housed for the remainder of the experiment as per Hodes et al., 2015a.

Novelty Suppressed Feeding (NSF). The NSF test was used to assess anxiety-like behavior, and testing was performed according to published protocols (Hodes et al., 2015a; Santarelli et al., 2003). Mice were food-deprived overnight, then individually placed in an arena (50x50x20cm) covered with clean bedding and a single pellet of regular chow in the centre. Latency to start feeding was measured as an index of anxiety-like behavior. Mice that did not feed within 10 minutes were removed from the arena and assigned a latency of 600 seconds in the analysis. Bedding was replaced between mice. To account for potential appetite differences between groups, home cage latency to feed and amount of food consumed within 5 minutes were measured.

Sucrose Consumption Test (SCT). The SCT was used to assess anhedonia-like behavior and performed according to previous protocols (Hodes et al., 2015a). Mice were first acclimatized to two bottles in the home cage, both filled with tap water. After 24 hours and following the NSF test, the bottles were replaced with two new bottles, one containing tap water and the other 1% sucrose (in tap water). The right-left position was counterbalanced between animals in each condition. The bottles were removed 24 hours later, weighed, then returned to the cage for an additional 24 hours with the right-left position switched for each mouse. Importantly, to avoid

hunger-related changes in sucrose consumption, sucrose was presented after food was returned to the home cage following NSF.

Forced Swim Test (FST). The FST was used to assess stress-coping behavior and was conducted according to standard protocols (Can et al., 2012). Mice were individually placed in a 4L glass beaker filled with clean water at $25 \pm 0.5^{\circ}\text{C}$ to a depth of 15cm for a duration of 6 minutes. The test was filmed, and an observer blinded to experimental condition scored two aspects of passive-coping behavior: 1) latency to first bout of immobility and 2) amount of time spent immobile across the duration of the test. Passive-coping behavior was defined as immobility with only movements necessary to remain floating or to keep the head above water.

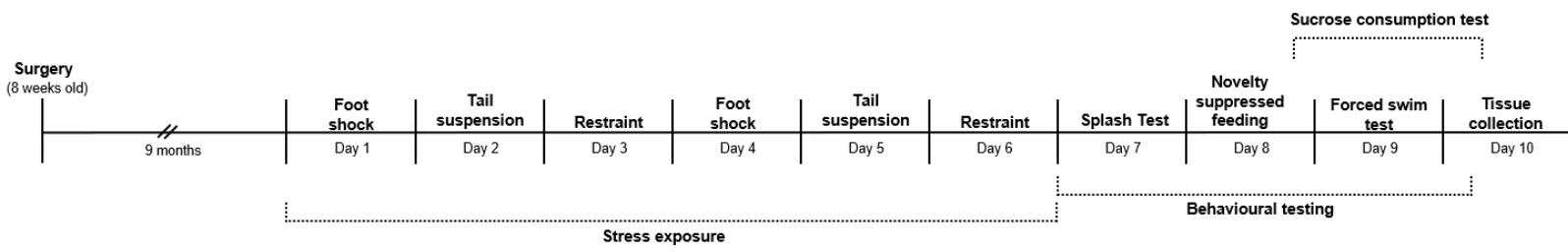


Figure 3.1 Experimental timeline. Mice received bilateral ovariectomy or sham surgery at 8 weeks of age. At 11 months of age, mice in the stress condition were subjected to foot shock stress on days 1 and 3, tail suspension stress on days 2 and 4, and restraint stress on days 3 and 6. All mice were tested on the splash test on day 7, the novelty suppressed feeding test on day 8, the forced swim test on day 9, and the sucrose consumption test on days 8-10 (starting after NSF on day 8). Tissue was collected on day 10.

Tissue collection and processing

Mice were euthanized via rapid decapitation, and trunk blood was collected into EDTA-coated tubes then centrifuged at 4°C and $1,000 \times g$. for 15 minutes and plasma was stored at -80°C . Immediately following decapitation, the frontal cortex and hippocampus were micro-dissected on a cold surface, flash-frozen on dry ice, then stored at -80°C . Frontal cortex was collected

anterior to the genu of the corpus colossum, and the entire rostral-caudal extent of the hippocampus was collected. Adrenal glands were also collected and weighed immediately.

Electrochemiluminescence immunoassay kits from Meso Scale Discovery (MSD; Rockville, MD) were used according to manufacturer instructions for cytokine, PSD-95, and cell signaling protein measurements. A Sector Imager 2400 (MSD) was used to read the plates, and data was analyzed using the Discovery Workbench 4.0 software from MSD. Frontal cortices and hippocampi from all mice were homogenized individually using an Omni Bead Ruptor (Omni international, Kennesaw, GA) with 200 μ l and 150 μ L of cold lysis buffer, respectively. Homogenates were centrifuged at 4°C and 1,000 \times g. for 15 minutes, and stored at -80°C. For all assays (cytokine, PSD-95, cell signaling phosphoproteins), frontal cortex and hippocampus values were normalized to total protein concentrations, which were quantified using the Pierce Micro BCA Protein Assay Kit (ThermoFisher Scientific) used according to manufacturer instructions, with samples run in triplicates. Cell signaling proteins and PSD-95 were only quantified in the frontal cortex, as hippocampal tissue remaining after cytokine quantification was insufficient for these assays.

Cytokine quantification in brain and plasma

V-PLEX proinflammatory Panel 1 kits (Mouse) from MSD (Rockville, MD) were used to measure cytokine concentrations in plasma, frontal cortex, and hippocampus. Plates arrived pre-coated with primary antibodies for the simultaneous quantification of Interferon- γ (IFN- γ), Interleukin-1 β (IL-1 β), Interleukin-2 (IL-2), Interleukin-4 (IL-4), Interleukin-5 (IL-5), Interleukin-6 (IL-6), chemokine (C-X-C motif) ligand 1 (CXCL1), Interleukin-10 (IL-10), Interleukin-12p70 (IL-12p70), and tumor necrosis factor- α (TNF- α). This panel was chosen

because it allows for the quantification of several pro- and anti-inflammatory cytokines as well as the chemokine CXCL1, thus provides a comprehensive view of the cytokine milieu. All samples were run in duplicates, and sample and secondary antibody incubations were performed according to manufacturer instructions. Lower limits of detection (LLOD), which differed between analytes and plates (2 plates total), were as follows: IFN- γ : 0.033-0.076 pg/ml; IL-1 β : 0.048-0.069 pg/ml; IL-2: 0.32-0.79 pg/ml; IL-4: 0.077-0.091 pg/ml; IL-5: 0.097-0.10 pg/ml; IL-6: 0.83-0.85 pg/ml; CXCL1: 0.15-0.17 pg/ml; IL-10: 0.18-0.62 pg/ml; IL-12p70: 11.8-13.1 pg/ml; TNF- α : 0.082-0.096 pg/ml. In plasma, IL-12p70 was not detectable in 75% of samples, and IL-4 was not detectable in 79% of samples, and thus were not included in analyses.

Cell signaling phosphoprotein and PSD-95 quantification in the frontal cortex

Cell signaling phosphoproteins (phospho-ERK1/2 (pERK1/2), pMEK1/2, pJNK, pp33, and pSTAT3) and PSD-95 were measured in frontal cortex samples using kits from MSD (Rockville, MD). Primary antibody pre-coated plates were used for sample and secondary antibody incubations according to manufacturer's instructions, with samples run in duplicates, and results reported after normalization to total protein concentrations in each sample.

Radioimmunoassays for hormone quantification in plasma

Plasma corticosterone concentrations from samples collected at euthanasia were quantified using a commercially available radioimmunoassay kit used in accordance with manufacturers' instructions (corticosterone double-antibody radioimmunoassay kit, MP biomedicals, Solon, OH). To avoid handling-induced elevations in circulating glucocorticoids, trunk blood samples were collected within 2 minutes of the experimenter entering the colony room. The inter- and intra-assay coefficients of variation were below 10%.

An Ultra-Sensitive Estradiol Radioimmunoassay kit (Beckman Coulter, Prague, Czech Republic) was used according to manufacturers' instructions to measure 17 β -estradiol concentrations in plasma samples collected at euthanasia. Assay sensitivity is 2.2pg/ml, and average inter- and intra-assay coefficients of variation were below 10%.

Vaginal cytology for estrous cycle assessment

Estrous cycle stage can affect behavior, measures of neural plasticity, and immune function (Woolley et al., 1990; Beagley and Gockel, 2003; Meziane et al., 2007), thus vaginal lavage samples were obtained on behavioral testing days, and estrous stage was determined according to previous methods (Cora et al., 2015). Plasma was collected on the last day for estradiol quantification. We expected irregular estrous cycling in at least a proportion of sham-operated mice as they were middle-aged at testing. Mice in persistent diestrus, persistent estrus, or that displayed abnormalities in the length or order of estrous cycle stages were all classified as irregularly cycling, as we have done previously (Galea et al., 2018). Ovariectomized mice were also lavaged to control for potential effects of the procedure.

Statistical analyses

All statistical analyses were performed using Statistica software (Tulsa, OK). Behavioral measures, cytokine concentrations, IL-6:IL-10 ratio, corticosterone concentrations, PSD-95, and cell signaling protein levels were each analyzed using factorial analysis of variance (ANOVA), with ovarian status (OVX, Sham) and stress condition (stress, non-stress) as the between-subject factors. Body mass was used as a covariate when analyzing sucrose consumption, immobility in the forced swim test, self-grooming behavior in the splash test, and adrenal mass. Estrous cycle stage (proestrus, non-proestrus) was used as a covariate in behavioral analyses. 17 β -estradiol

concentrations were used as a covariate in corticosterone, cytokine, cell signaling, and PSD-965 analyses. Covariate effects are only mentioned when significant. Post-hoc analyses utilized Newman-Keul's comparisons and any *a priori* comparisons were subjected to a Bonferroni correction. A Chi-square test was used to compare the frequency of mice that had regular estrous cycles between sham-operated groups. To assess whether cycle regularity affected outcomes in sham-operated mice, we analyzed data from sham-operated mice separately using ANOVAs with cycle regularity (regular, irregular) and stress condition (stress, non-stress) as the between-subject factors. Specifically, we analyzed all behavioral outcomes and key cytokines and cell signaling phosphoproteins that were found to be significantly affected in initial ANOVAs. However, we did not find any significant effects of cycle regularity nor of the interaction between stress condition and cycle regularity (all p's >0.2), therefore these analyses are not further reported in the results section. Pearson's correlations were performed between variables of interest. Outliers that fell more than 2.5 standard deviations away from the mean were removed from analyses. Principal Component Analyses (PCA) were used to reduce the cytokine data into a smaller number of uncorrelated variables and to obtain information about the amount of variance accounted for by potential cytokine networks within the data. PCAs were followed by ANOVAs on individual principal component scores as we have done previously (Eid et al., 2019a), to assess the effects of ovarian status and stress exposure on cytokine networks.

3.3 Results

Body mass was significantly reduced across days of stress exposure regardless of ovarian status

Long-term ovariectomy significantly increased body mass ($F(1, 24)=22.72, p<0.0001$), therefore to investigate the effects of sub-chronic stress exposure we calculated body mass as a percentage of mass on experimental day 1. Body mass percentage decreased significantly across the 6 days of stress exposure ($F(4, 96)=32.967, p<0.0001$; stress condition by day interaction; Figure 3.2). Specifically, in stress-exposed groups, there was a significant decline on day 3 relative to day 2, and on every day thereafter (p 's <0.006). Body mass percentage was also significantly different between stress and non-stress groups on days 3-6 (p 's <0.007). There were also significant main effects of stress condition and day (p 's <0.0001), a trend toward a significant day by ovarian status interaction ($p=0.064$), but no other significant main effects or interactions (p 's >0.095).

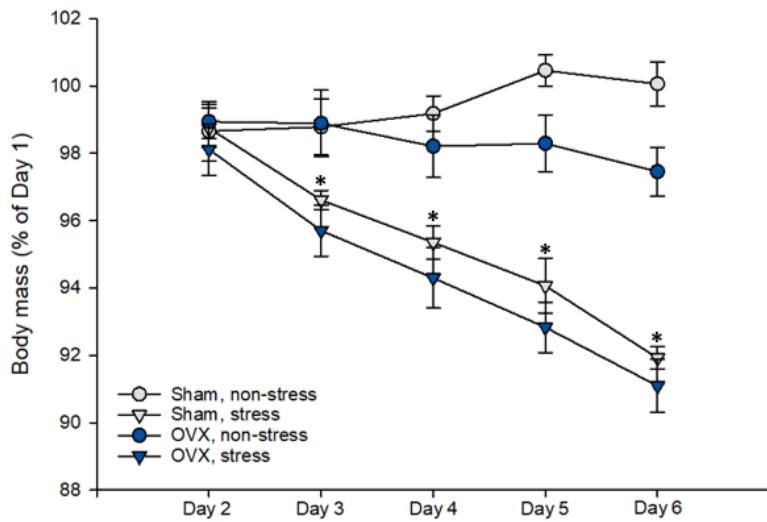


Figure 3.2 Body mass across days, shown as a percentage of body mass on experimental day 1. Regardless of ovarian status, body mass decreased significantly across days with sub-chronic stress exposure; * indicates $p<0.007$, significantly different from day-matched non-stress groups, and stress groups on prior day/s of stress exposure. Data in means \pm standard error of the mean. OVX, ovariectomized

Long-term ovariectomy reduced circulating 17 β -estradiol concentrations but stress exposure did not affect 17 β -estradiol concentrations or estrous cycling

Plasma 17 β -estradiol concentrations were significantly reduced by long-term ovariectomy ($F(1, 18)=9.20$, $p=0.007$; main effect of ovarian status). Stress condition did not affect 17 β -estradiol concentrations, nor did the interaction of stress condition and ovarian status (all p 's >0.2 ; Table 3.1). These results do not change when analyzing estrous cycle regularity as a covariate: a significant main effect of ovarian status remains ($p = 0.018$), but there was no significant main effect of the covariate, and no significant effect of stress condition nor a stress condition by ovarian status interactions (p 's >0.2). Within sham-operated groups, there was no significant difference in the frequency of mice that had regular estrous cycles ($\chi^2(1) = 0.31$, $p = 0.58$; Table 3.1). Further, there were no significant differences in 17 β -estradiol concentrations between regularly vs. irregularly cycling mice, regardless of stress condition (all p 's >0.4). It should be noted that 17 β -estradiol concentrations in sham-operated groups were considerably lower than what would be expected in young-adult intact mice (~20-60pg/ml across estrous cycle (Walmer et al., 1992)), indicating our that sham-operated groups were in a transitional state to reproductive senescence.

Group	17 β -estradiol (pg/ml)	% of mice regularly cycling
Sham, non-stress	10.34. \pm 1.85	42.86
Sham, stress	10.32 \pm 2.20	28.57
OVX, non-stress	4.30 \pm 0.96	N/A
OVX, stress	7.52 \pm 0.71	N/A

Table 3.1 Plasma 17 β -estradiol concentrations from trunk blood at decapitation and percentage of sham-operated mice with regular estrous cycles. Data in means \pm standard error of the mean. OVX, ovariectomized.

Basal plasma corticosterone concentrations and adrenal mass did not differ significantly between groups

Basal plasma corticosterone concentrations did not differ significantly between groups (all p's >0.3; Table 3.2), but there was a significant covariate effect of plasma 17 β -estradiol concentrations ($p = 0.027$). Similarly, adrenal mass was not significantly affected by ovarian status, stress exposure, nor their interaction (p's >0.3; Table 3.2).

Group	CORT (pg/ml)	Adrenal mass (mg)
Sham, non-stress	42.48 ± 8.99	6.99 ± 0.66
Sham, stress	33.79 ± 6.70	8.06 ± 0.53
OVX, non-stress	31.92 ± 6.88	8.50 ± 0.88
OVX, stress	39.58 ± 8.62	8.16 ± 0.69

Table 3.2 Basal plasma corticosterone concentrations and adrenal mass. Data in means ± standard error of the mean. OVX, ovariectomized; CORT, corticosterone.

Ovariectomy reduced latency to immobility under non-stress conditions, and sub-chronic stress exposure reduced latency to immobility in sham-operated mice only

Under non-stress conditions, ovariectomy significantly decreased latency to immobility in the FST ($p=0.01$; *a priori* comparisons; ovarian status by stress condition interaction: $F(1, 18)=1.8372$, $p=0.19$). Further, exposure to sub-chronic stress significantly decreased the latency to immobility in sham-operated ($p=0.012$; Figure 3.3A) but not in ovariectomized mice ($p=0.54$). There was also a significant main effect of stress condition ($p=0.025$) but not of ovarian status ($p=0.22$). The percentage of time spent immobile in FST did not significantly differ between groups (all p's >0.09; Table 3.3)

Sub-chronic stress exposure decreased sucrose consumption regardless of ovarian status

Stress exposure significantly reduced sucrose consumption ($F(1, 23)=4.87, p=0.038$; main effect of stress condition; Figure 3.3B). There was also a trend for ovariectomy to reduce sucrose consumption ($F(1, 23)=3.98, p=0.067$), but no other significant effects (p 's > 0.4). In comparison, water consumption was not significantly affected by body mass, ovarian status, stress condition, nor their interaction (all p 's > 0.4; Table 3.3).

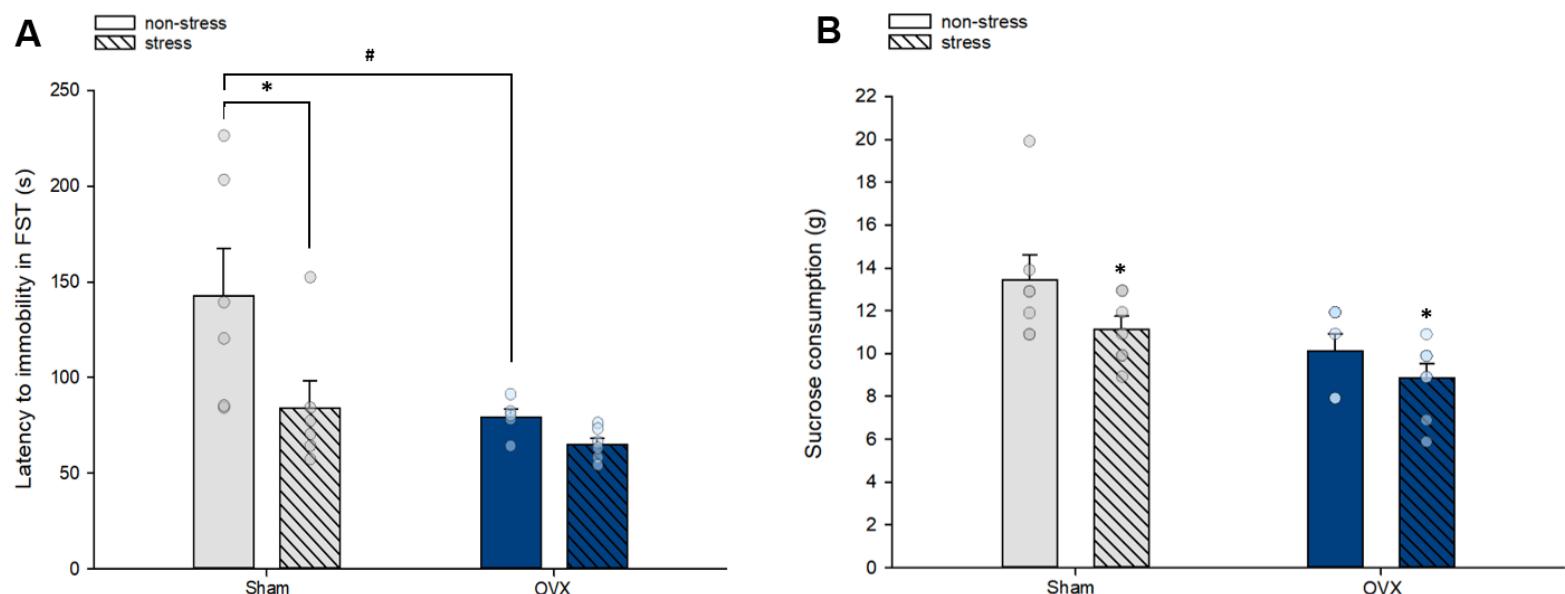


Figure 3.3 Latency to first bout of immobility in the Forced Swim Test and sucrose consumption in the Sucrose

Consumption Test. (A) Ovariectomy significantly reduced latency to immobility under non-stress conditions, and sub-chronic stress exposure reduced latency to immobility in sham-operated mice only; # indicates $p=0.01$ and *indicates $p=0.012$. (B) Stress exposure decreased sucrose consumption, regardless of ovarian status, and there was a trend for ovariectomy to reduce sucrose consumption; * indicates $p=0.038$, main effect of stress condition. FST = forced swim test; OVX = ovariectomy. Data in means + standard error of the mean.

Sub-chronic stress exposure and ovarian status did not significantly affect behavior in the splash and novelty suppressed feeding tests

Anxiety-like behavior as measured in the novelty suppressed feeding test and self-grooming behavior in the splash test were not significantly altered by ovariectomy, sub-chronic stress exposure, nor their interaction (all p's >0.1; Table 3.3).

Group	% time spent immobile in FST	Latency to feed in NSF (s)	% time spent grooming in ST	H ₂ O consumption (g)
Sham, non-stress	64.96 ± 5.37	542.83 ± 36.35	14.35 ± 3.04	8.29 ± 0.81
Sham, stress	58.79 ± 2.94	521.29 ± 40.01	19.98 ± 1.53	7.00 ± 0.44
OVX, non-stress	73.62 ± 2.05	507.86 ± 59.72	14.71 ± 4.31	8.00 ± 0.79
OVX, stress	65.88 ± 3.55	600.00 ± 0.00	18.32 ± 2.78	9.14 ± 1.26

Table 3.3 Behavioral data. Data in means ± standard error of the mean. OVX, ovariectomized; FST, forced swim test; NSF, novelty suppressed feeding; ST, splash test.

Principal component (PC) analyses in cytokine data:

Frontal cortex: PC1 scores were reduced by long-term ovariectomy, and PC2 scores were increased by stress exposure in sham-operated mice only

The model generated 3 principal components, accounting for 81.8% of the variance within the frontal cortex cytokine dataset. Variance explained by principal component 1 (PC1) = 52.8%, PC2 = 17.1 %, and PC3 = 11.9%. Factor loadings are shown in Table 3.4. ANOVA results reveal that ovariectomy significantly reduced PC1 scores ($F(1, 24)=5.23$, $p=0.031$; main effect of ovarian status; Figure 3.4A), but there was no significant main effect of stress condition, nor an ovarian status by stress condition interaction (p's >0.6). As such we will refer to PC1 as “Ovarian hormone sensitive” cytokines. Stress significantly increased PC2 scores in sham ($p = 0.008$), but not ovariectomized mice ($p = 0.51$; *a priori* comparisons, ovarian status by stress condition

interaction: $F(1, 24)=2.526$, $p<0.13$; Figure 3.4B). There was also a significant main effect of stress condition ($p = 0.019$), but not of ovarian status ($p = 0.55$). For PC3 scores, there were no significant main effects of ovarian status or stress condition, nor a significant interaction (p 's >0.19). Thus, in the frontal cortex we identify 8 cytokines that were proportionally more sensitive to ovarian hormone status based on component loadings(IL-2, IL-4 IL5, IL-6, IL-10, TNF- α , IL-12p70 and the chemokine CXCL1) and 3 cytokines sensitive to both stress and ovarian hormone status (IL-1 β , TNF- α and IL-4).

Hippocampus: Stress exposure increased PC2 scores regardless of ovarian status, and there were trends for long-term ovariectomy to increase PC1 and decrease PC2 scores

The model generated 3 principal components, accounting for 81.4% of the variance within the hippocampus cytokine dataset. Variance explained by principal component 1 (PC1) = 58.0%, PC2 = 14.8%, and PC3 = 8.6%. Factor loadings are shown in Table 3.4. ANOVAs reveal a weak trend for ovariectomy to increase PC1 scores ($F(1, 23)=3.02$, $p=0.096$; main effect of ovarian status; Figure 3.4C), and no significant main effect of stress condition, nor an ovarian status by stress condition interaction (p 's >0.3). Stress significantly increased PC2 scores regardless of ovarian status ($F(1, 23)=4.40$, $p=0.047$; Figure 3.4D), and there was a trend toward significance for ovariectomy to decrease PC2 scores ($F(1, 23)=3.67$, $p=0.068$), but no significant stress condition by ovarian status interaction ($p > 0.6$). For PC3 scores, there were no significant main effects of ovarian status or stress condition, nor a significant interaction (p 's >0.3). Thus, these analyses in the hippocampus identify 8 cytokines that were sensitive to ovarian hormone status based on component loadings (IL-2, IL-4 IL5, IL-6, IL-10, TNF- α , IL-12p70, IFN- γ) and 3 cytokines sensitive to stress (IL-1 β , TNF- α and the chemokine CXCL1).

Plasma: PC scores did not differ significantly between groups

The model generated 2 principal components, accounting for 48.2% of the variance within the plasma cytokine dataset. Variance explained by principal component 1 (PC1) = 29.0%, and PC2 = 19.2%. Factor loadings are shown in Table 3.4. ANOVAs reveal no significant main effects of stress condition or ovarian status, nor a significant interaction for PC1 or PC2 scores (p's > 0.06; Figure 3.4E-F).

Cytokine	Frontal cortex			Hippocampus			Plasma	
	PC1	PC2	PC3	PC1	PC2	PC3	PC1	PC2
IFN- γ	0.22	-0.30	0.91	0.85	-0.03	-0.24	0.59	0.17
IL-10	0.93	-0.21	0.25	0.90	-0.19	0.09	-0.25	0.25
IL-1 β	0.22	0.85	0.36	0.45	0.58	-0.35	0.59	-0.67
IL-2	0.90	0.00	-0.08	0.89	-0.19	-0.17	0.73	-0.25
IL-5	0.87	-0.23	0.08	0.65	-0.30	0.71	0.24	0.65
IL-6	0.77	0.10	-0.09	0.88	-0.007	0.19	0.67	0.24
CXCL1	0.73	0.35	-0.30	0.17	1.13	0.44	0.65	0.03
TNF- α	0.75	0.47	0.18	0.64	0.54	-0.31	0.26	0.67
IL-12p70	0.90	-0.11	-0.17	0.92	-0.16	0.064	N/A	N/A
IL-4	0.49	-0.66	0.04	0.89	-0.11	0.09	N/A	N/A

Table 3.4 Principal component analyses loading table. High loadings indicated in bold.

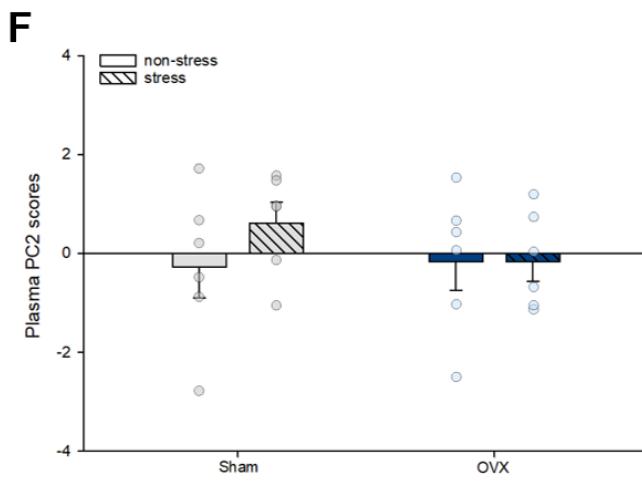
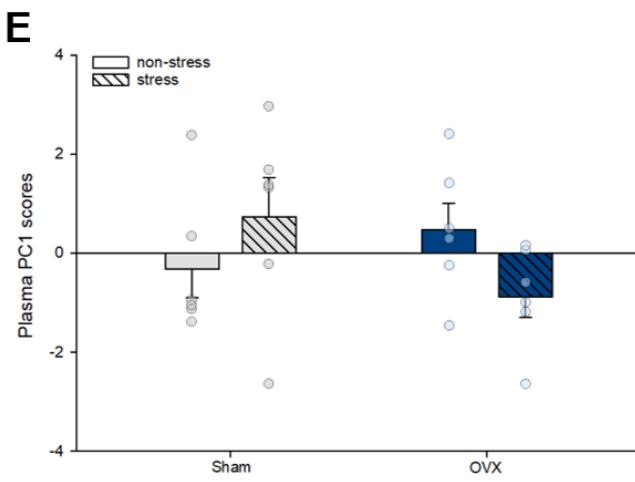
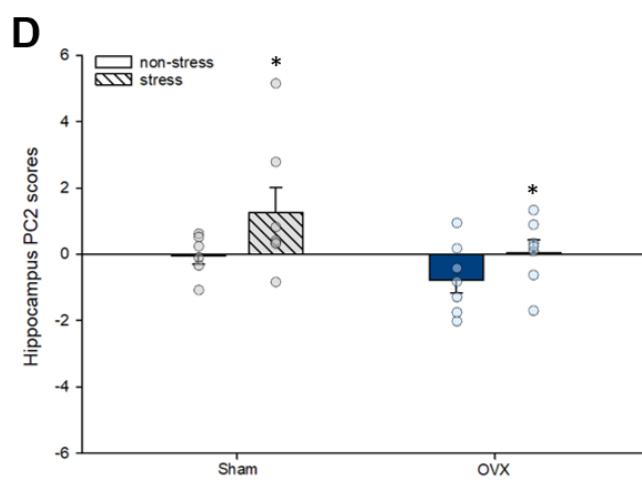
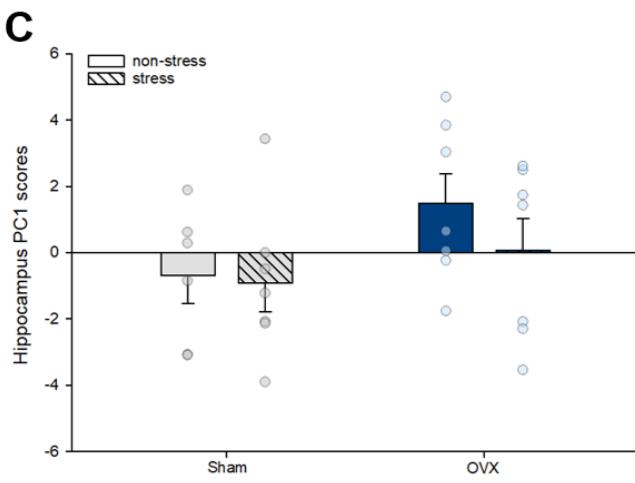
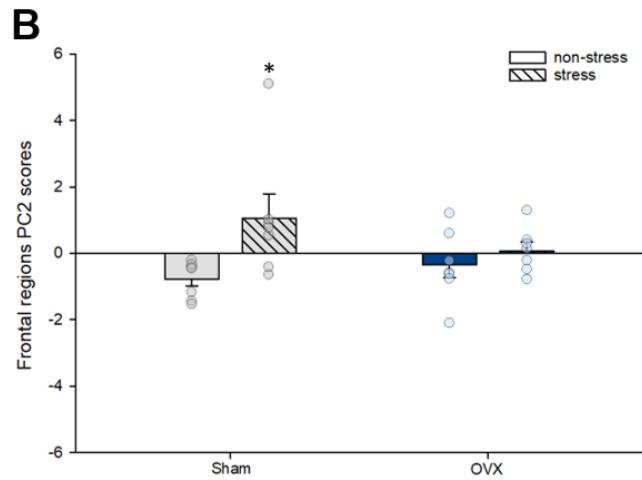
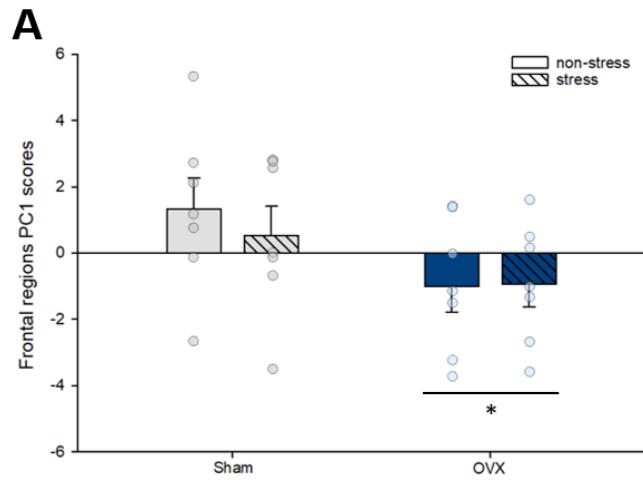


Figure 3.4 Principal component analyses scores in cytokine data. (A) Long-term ovariectomy significantly decreased Principal Component (PC) 1 scores in frontal cortex; * indicates $p=0.031$, main effect of ovarian status. (B) Exposure to sub-chronic stress increased PC2 scores in the frontal cortex in sham-operated mice only; * indicates $p=0.008$, significantly higher than non-stress sham-operated group. (C) There was a weak trend ($p=0.096$) for ovariectomy to increase PC1 scores in the hippocampus. (D) Sub-chronic stress exposure increased PC2 scores in the hippocampus, regardless of ovarian status, and there was a trend for ovariectomy ($p=0.064$) to reduce PC2 scores; * indicates $p=0.047$, main effects of stress condition. There were no significant group differences in PC1 or PC2 scores in plasma (E-F). PC = principal component; OVX = ovariectomy. Data in means + standard error of the mean.

Individual cytokine ANOVAs:

Long-term ovariectomy reduced the concentrations of several cytokines in the frontal cortex

Long-term ovariectomy significantly reduced the concentrations of the following cytokines/chemokine in the frontal cortex: TNF- α ($F(1, 24)=5.41$, $p=0.029$; Figure 3.5A); CXCL1 ($F(1, 24)=4.44$, $p=0.046$; Figure 3.5B); IL-10 ($F(1, 22)=9.80$, $p=0.0049$; Figure 3.5C); IL-12p70 ($F(1, 24)=6.40$, $p=0.018$; Figure 3.5D), IFN- γ ($F(1, 23)=5.89$, $p=0.023$; Figure 3.5E), and IL-6 ($F(1, 24)=4.16$, $p=0.05$; Figure 3.5F). For each of these cytokines, there was no significant main effect of stress condition, and no stress condition by ovarian status interaction (p 's > 0.2).

Sub-chronic stress exposure significantly increased IL-1 β in the frontal cortex of sham-operated mice only

Exposure to stress increased IL-1 β concentrations in the frontal cortex of sham-operated ($p=0.018$, a priori comparison) but not ovariectomized mice ($p=0.98$; $F(1, 24)=3.1706$, $p=0.088$; stress condition by ovarian status interaction; Figure 3.5G). There was also a trend toward a

significant main effect of stress exposure to increase IL-1 β ($p= 0.082$), but no significant main effect of ovarian status ($p = 0.32$).

Sub-chronic stress exposure significantly decreased IL-4 in the frontal cortex

Stress exposure significantly reduced IL-4 concentrations in the frontal cortex ($F(1, 24)=5.28$, $p=0.031$; main effect of condition; Figure 3.5H). This effect was driven by a larger decrease in sham-operated mice, as *a priori* comparisons reveal a trend toward a significant difference between sham-operated groups ($p = 0.041$) but not between ovariectomized groups ($p = 0.28$). There was also a trend toward significance for ovariectomy to reduce IL-4 ($p = 0.085$), but no significant stress condition by ovarian status interaction ($p = 0.46$). There were no significant effects of stress condition or ovarian status, nor an interaction to affect IL-2 (P 's > 0.25 ; Figure 3.5I) or IL-5 (p 's > 0.25 ; Figure 3.5J) concentrations in the frontal cortex.

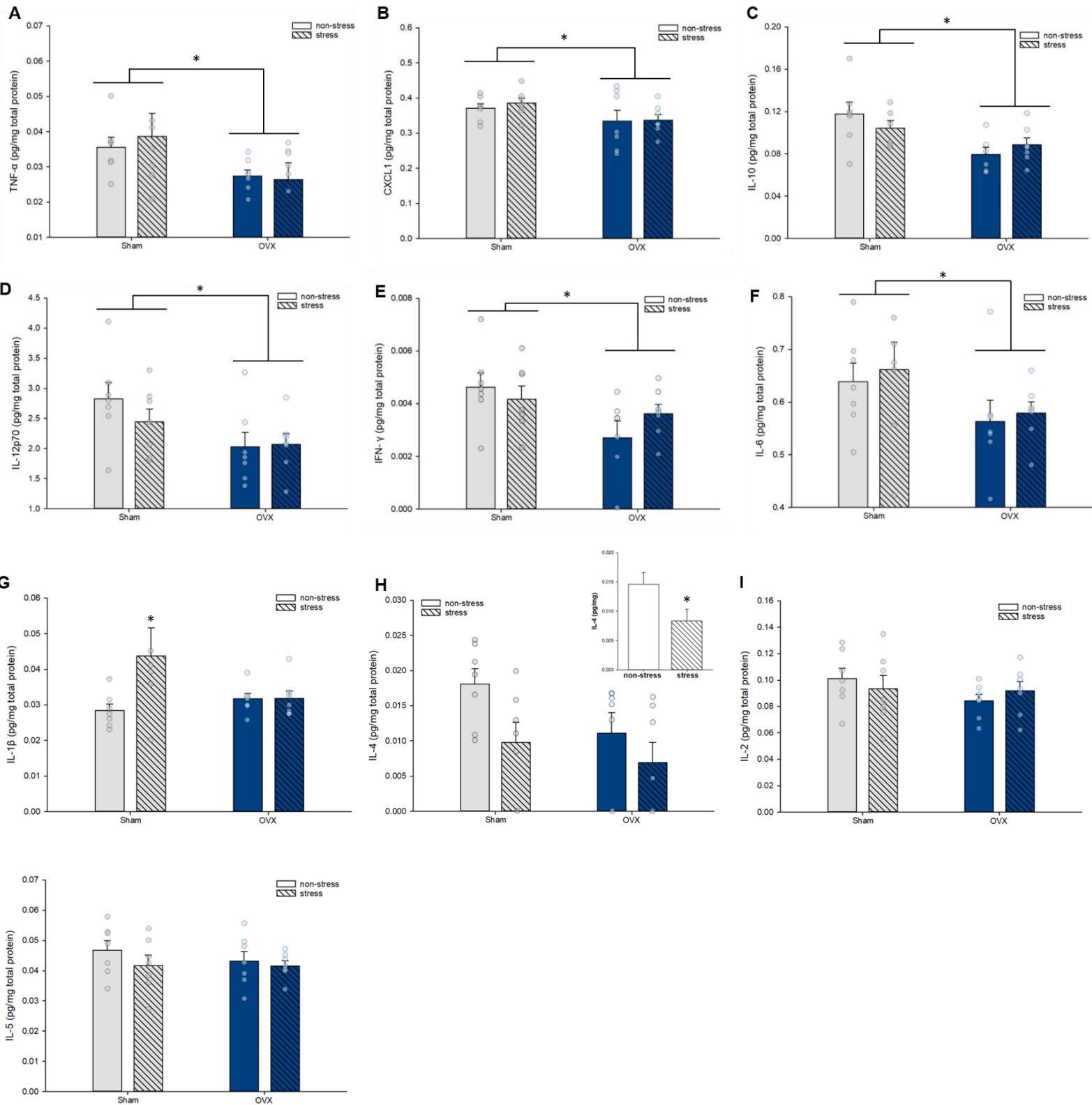


Figure 3.5 Cytokine concentrations in the frontal cortex, normalized by total protein concentrations. Long-term ovariectomy reduced the concentrations of TNF- α (A) CXCL1 (B), IL-10 (C), IL-12p70 (D), IFN- γ (E), and IL-6 (F) regardless of stress exposure; * indicates significant main effects of ovarian status (p 's <0.05). (G) Stress exposure increased IL-1 β in the frontal cortex of sham-operated mice only; * indicates p = 0.018, significantly higher than non-stress sham group. (H) Stress exposure significantly reduced IL-4; inset graph depicts main effect of stress condition, * indicates p = 0.031, significantly lower than non-stress. Concentrations of IL-2 (I) and IL-5 (J) did not differ significantly between groups. OVX, ovariectomized; IL, interleukin; CXCL1, chemokine (C-X-C motif) ligand 1; TNF- α , tumor necrosis factor- α ; IFN- γ , Interferon- γ . Data in means + standard error of the mean.

Long-term ovariectomy increased IL-2, IL-5, and IL-6 concentrations in the hippocampus

Long-term ovariectomy significantly increased IL-2 ($F(1, 22)=8.92$, $p<0.007$; Figure 3.6A), IL-5 ($F(1, 22)=8.37$, $p=0.008$; Figure 3.6B), and IL-6 ($F(1, 23)=5.80$, $p=0.024$; Figure 3.6C) concentrations in the hippocampus. For each of these cytokines, there was no significant main effect of stress condition, nor a stress condition by ovarian status interaction (all p 's >0.18).

Sub-chronic stress exposure increased IL-1 β in the hippocampus of sham-operated mice only, and decreased IL-12p70 concentrations regardless of ovarian status

Sub-chronic stress exposure increased IL-1 β concentrations in the hippocampus of sham-operated ($p=0.04$) but not ovariectomized mice ($p=0.78$; $F(1, 23)=1.82$, $p=0.19$; *a priori* comparison; stress condition by ovarian status interaction; Figure 3.6D). There were no significant main effects of stress condition ($p=0.09$) or ovarian status ($p>0.5$). Regardless of ovarian status, exposure to sub-chronic stress significantly decreased hippocampal IL-12p70 concentrations ($F(1, 23)=4.38$, $p=0.048$; Figure 3.6E). There was no significant main effect of ovarian status nor an ovarian status by stress condition interaction (p 's >0.1). There was also a weak trend for stress exposure to reduce IL-10 ($p=0.082$; Figure 3.6F), but no significant group differences for hippocampal IL-4 (p 's > 0.2; Figure 3.6G), IFN- γ (p 's > 0.29; Figure 3.6H), TNF- α (p 's > 0.27; Figure 3.6I), or CXCL1 (p 's > 0.3; Figure 3.6J).

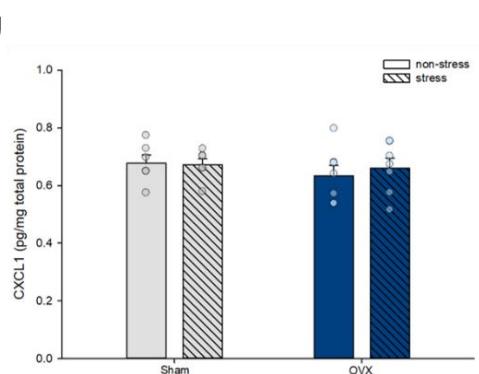
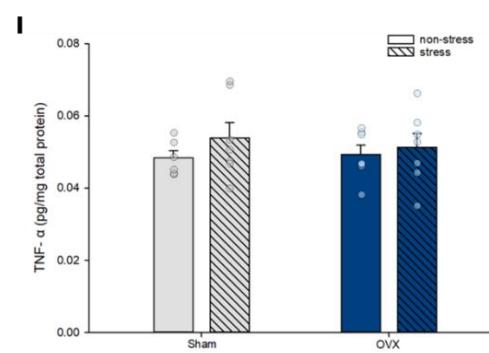
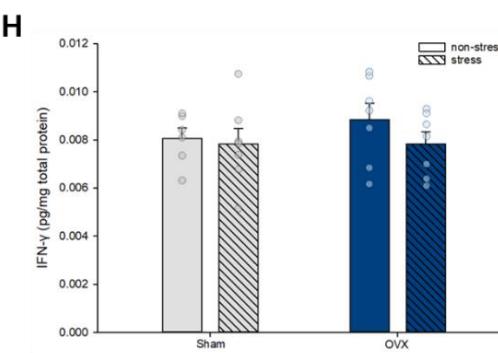
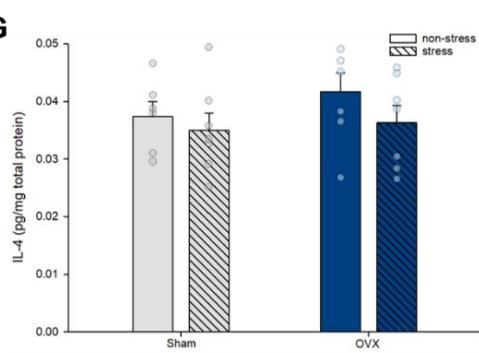
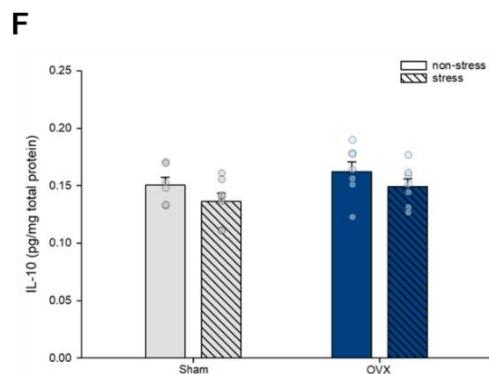
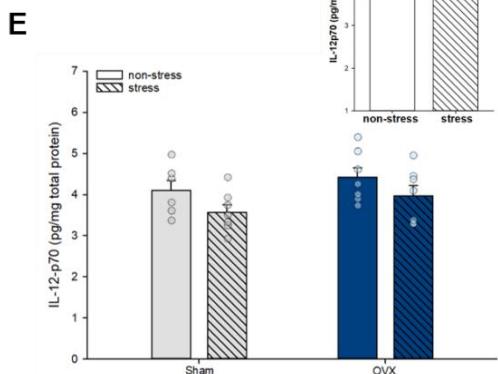
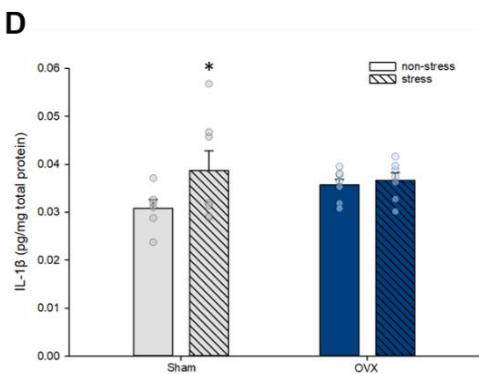
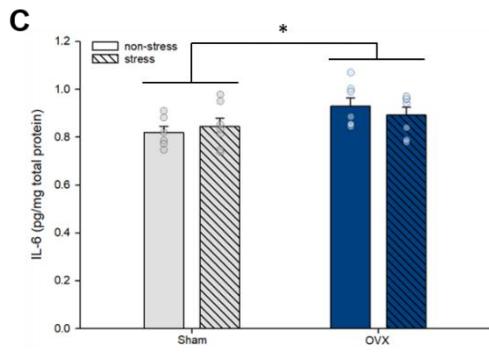
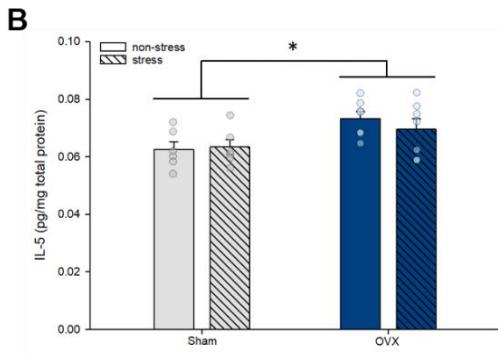
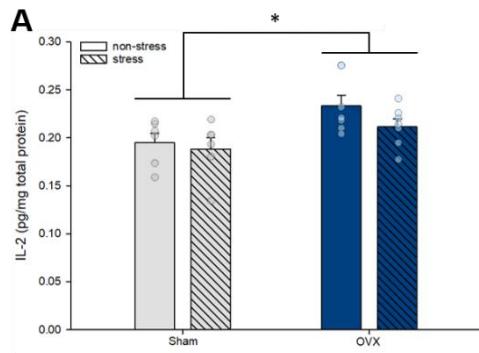


Figure 3.6 Cytokine concentrations in the hippocampus, normalized by total protein concentrations. Long-term ovariectomy significantly increased concentrations of IL-2 (A), IL-5 (B), and IL-6 (C) in the hippocampus; * indicates $p < 0.03$, main effect of ovarian status. (D) Stress exposure increased IL-1 β in the hippocampus of sham-operated mice only; * indicates $p = 0.04$, significantly higher than non-stress sham group. (E) Sub-chronic stress exposure significantly decreased IL-12p70 concentrations, regardless of ovarian status; inset graph depicts main effect of stress condition, * indicates $p < 0.05$, significantly lower than non-stress. Hippocampal concentrations of IL-10 (F), IL-4 (G), IFN- γ (H), TNF- α (I), and CXCL1 (J) did not significantly differ between groups. OVX, ovariectomized; IL, interleukin; CXCL1, chemokine (C-X-C motif) ligand 1; TNF- α , tumor necrosis factor- α ; IFN- γ , Interferon- γ . Data in means + standard error of the mean.

Ovariectomy significantly increased plasma CXCL1 concentrations

Ovariectomy significantly increased CXCL1 concentrations in plasma ($F(1,20)=6.62$, $p=0.018$; main effect of ovarian status; Figure 3.7A), but there was no significant main effect of stress condition ($p=0.23$) nor an ovarian status by stress condition interaction ($p = 0.53$). There were trends toward significance for an ovarian status by stress condition interaction to affect plasma IL-10 ($F(1, 20)=3.85$, $p=0.064$; Figure 3.7B) and IL-6 ($F(1, 19)=3.2520$, $p=0.087$; Figure 3.7C) concentrations, but no significant main effects of ovarian status nor stress condition in both cases (p 's > 0.19). There were no significant main effects of stress condition or ovarian status nor a significant interaction for plasma IL-5 (all p 's > 0.29 ; Figure 3.7D), TNF- α (p 's > 0.14 ; Figure 3.7E), IFN- γ (p 's > 0.4 ; Figure 3.7F), IL-2 (p 's > 0.6 ; Figure 3.7G), or IL-1 β (p 's > 0.2 ; Figure 3.7H).

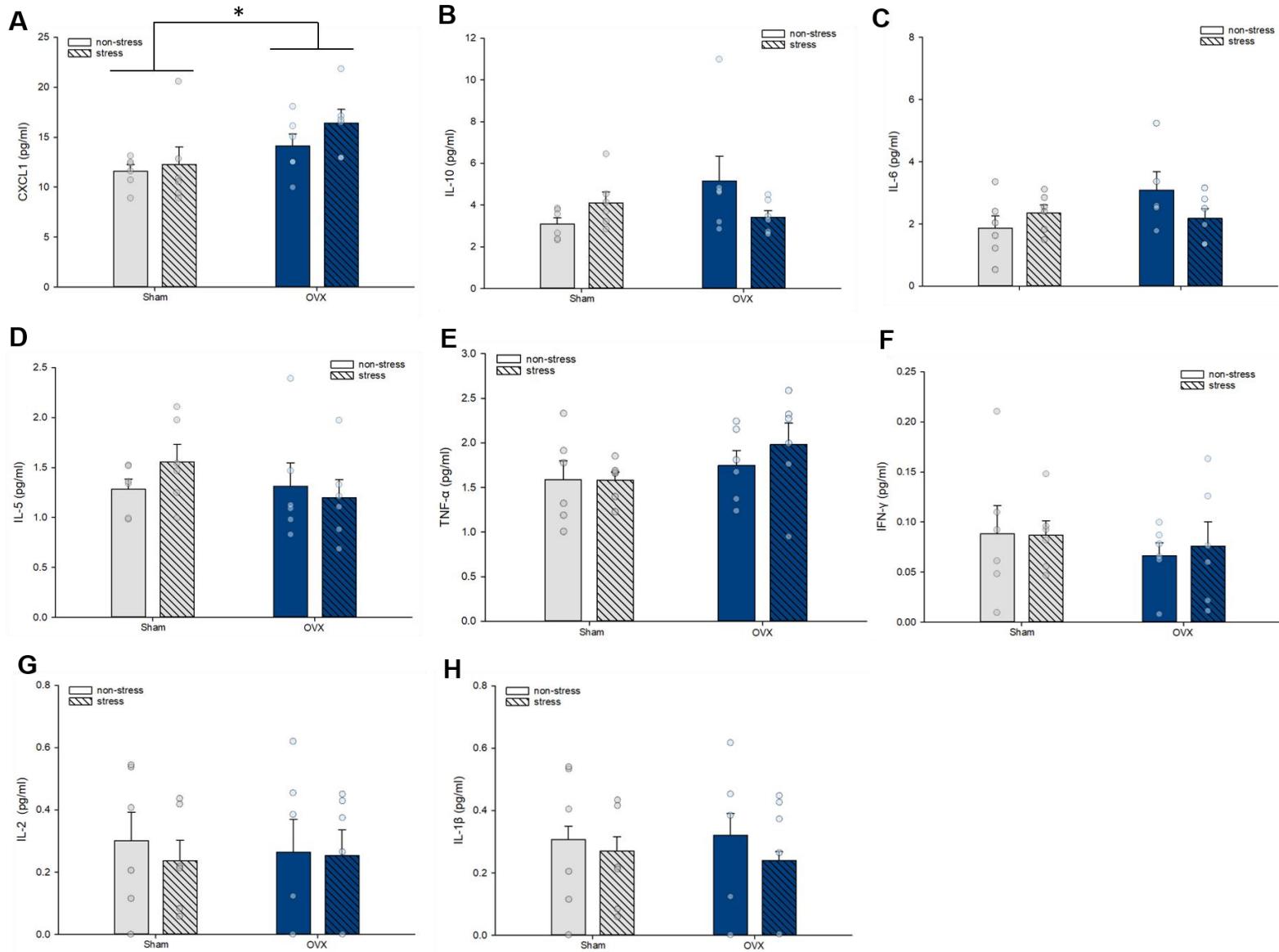


Figure 3.7 Cytokine concentrations in plasma. (A) Ovariectomy significantly increased CXCL1 concentrations in plasma; * indicates p= 0.018, main effect of ovarian status. There were no significant group differences in plasma concentrations of IL-10 (B), IL-6 (C), IL-5 (D), TNF- α (E), IFN- γ (F), IL-2 (G), or IL-1 β (H). OVX, ovariectomized; IL, interleukin; CXCL1, chemokine (C-X-C motif) ligand 1; TNF- α , tumor necrosis factor- α ; IFN- γ , Interferon- γ . Data in means + standard error of the mean.

Sub-chronic stress exposure increased IL-6:IL-10 ratio in the frontal cortex and hippocampus in sham-operated mice only

IL-6:IL-10 ratios were analyzed as an indicator of pro- to anti-inflammatory cytokine balance as previously described (Wood et al., 2015; de Brito et al., 2016). In both brain regions, sub-chronic stress exposure significantly increased IL-6:IL-10 ratio in sham-operated mice only (frontal cortex: $p = 0.033$; ovarian status by stress condition interaction: $F(1, 22)=4.72$, $p=0.041$, Figure 3.8A; hippocampus: $p = 0.015$; *a priori* comparisons; stress condition by ovarian status interaction: $F(1, 23)=1.9553$, $p=0.17$, Figure 3.8B). In the hippocampus, there was also a significant main effect of condition, with stress increasing the ratio ($p=0.025$), but not of ovarian status ($p=0.87$), and there were no significant main effects in the frontal cortex (p 's >0.12). Finally, under non-stress conditions, ovariectomy significantly increased IL-6:IL-10 ratio in the frontal cortex only ($p = 0.014$; Figure 3.8A). For plasma IL-6:IL-10 ratios, there were no significant main effects, nor a significant interaction (all p 's >0.3 ; Figure 3.8C).

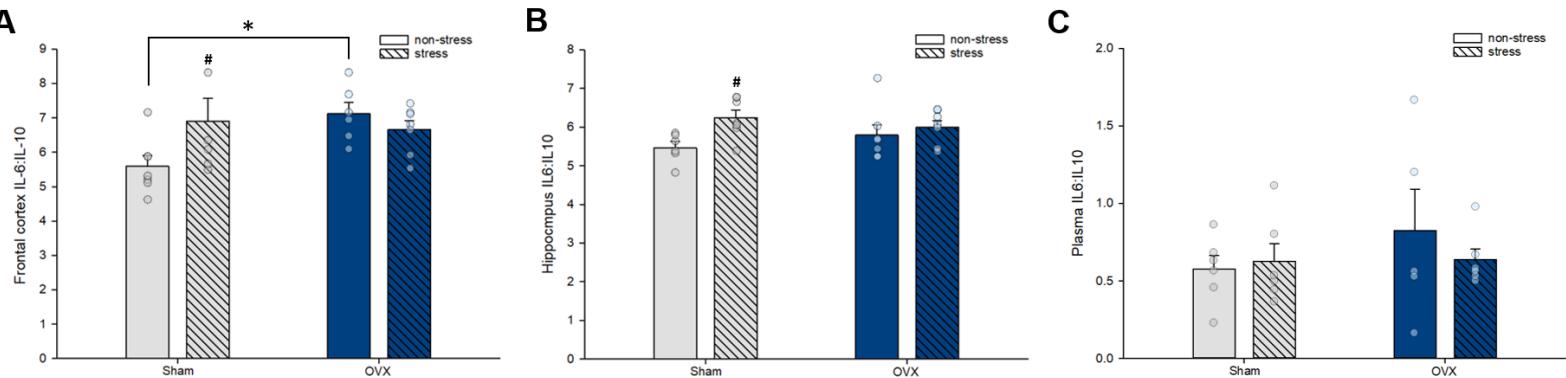


Figure 3.8 Interleukin-6 to interleukin-10 ratio in the frontal cortex (A), hippocampus (B), and plasma (C). (A)

Ovariectomy significantly increased IL-6:IL-10 ratio in the frontal cortex, and sub-chronic stress exposure increased IL-6:IL-10 ratio in sham-operated mice only. * indicates $p=0.014$ and # indicates $p=0.033$, significantly higher than non-stress sham group. (B) sub-chronic stress exposure increased IL-6:IL-10 ratio in the hippocampus in sham-operated mice only. # indicates $p=0.015$, significantly higher than non-stress sham group. (C) There were no significant group differences in plasma IL-6:IL-10 ratio.

OVX, ovariectomized; IL, interleukin. Data in means + standard error of the mean.

pERK1/2 and pMEK1/2 were significantly reduced by long-term ovariectomy and the effects of stress were dependent on ovarian status

Ovariectomy significantly decreased pERK1/2 and pMEK1/2 expression in the frontal cortex under non-stress conditions, relative to sham-operated mice (both p 's = 0.032). Sub-chronic stress exposure significantly decreased pERK1/2 expression in sham-operated mice ($p= 0.023$), but not in ovariectomized mice ($p=0.35$; ovarian status by stress condition interaction: $F(1, 24)=5.68$, $p=0.025$, Figure 3.9A). Conversely, sub-chronic stress exposure significantly increased pMEK1/2 expression in ovariectomized mice ($p= 0.042$), but not in sham-operated mice ($p = 0.31$; significant ovarian status by stress condition interaction $F(1, 24)=5.065$, $p=0.034$, Figure 3.9B). A similar, albeit non-significant trend was observed for pp38 expression (ovarian

status by stress condition interaction ($F(1, 24)=3.844$, $p=0.062$; Figure 3.9C), and there were no significant main or interaction effects for pJNK or pSTAT3 expression (all p 's >0.14 ; Figure 3.9D-E).

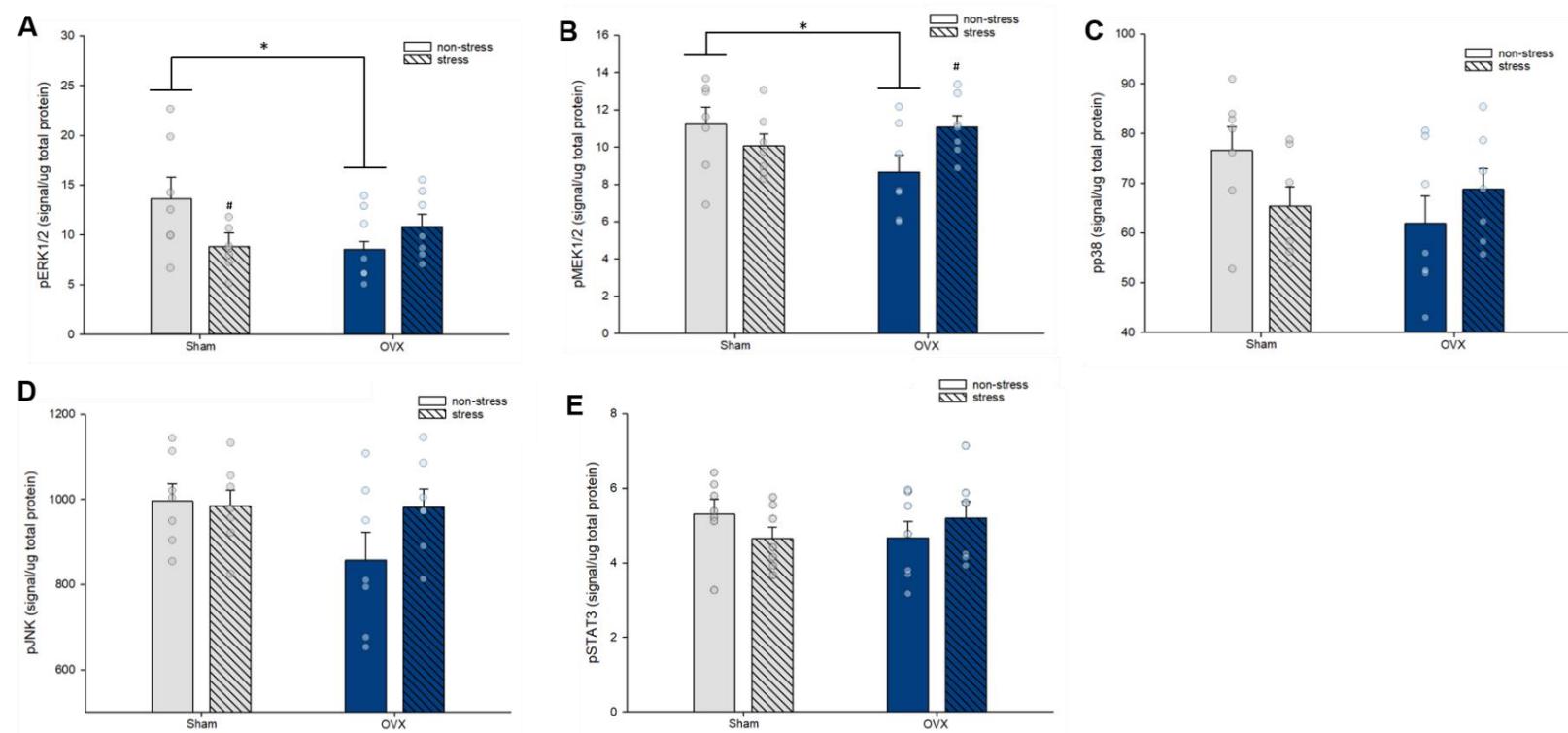


Figure 3.9 Cell signaling phosphoprotein expression in the frontal cortex, normalized by total protein levels. (A) Under non-stress conditions, ovariectomy decreased pERK1/2 expression, and stress exposure decreased pERK1/2 expression in sham-operated mice only; * indicates $p = 0.032$, significant difference between non-stress groups; # indicates $p = 0.023$, relative to non-stress sham-operated mice. (B) Under non-stress conditions, ovariectomy decreased pMEK1/2 expression, and stress exposure increased pMEK1/2 expression in ovariectomized mice only; * indicates $p = 0.032$, significant difference between non-stress groups; # indicates $p = 0.042$, relative to non-stress ovariectomized mice. There were no significant group differences in pp38 (C), pJNK (D), and pSTAT3 (E) expression. Data in means + standard error of the mean. OVX, ovariectomized; ERK, extracellular signal-regulated kinase; JNK, c-Jun NH₂-terminal kinase. Data in means + standard error of the mean.

PSD-95 expression was not significantly affected by ovariectomy or sub-chronic stress exposure

Expression of the postsynaptic scaffolding protein PSD-95 was utilized as a synaptic marker and a proxy measure of excitatory synapse density (Wainwright et al., 2016a). PSD-95 expression was previously found to be reduced by approximately 40% in the PFC of individuals with depression (Feyissa et al., 2009). Here, PSD-95 expression in the frontal cortex was not significantly affected by stress condition, ovarian status, nor their interaction (all p's >0.4; Table 3.5).

Group	PSD-95 (signal/ug protein)
Sham, non-stress	376.11 ± 53.53
Sham, stress	412.56 ± 54.68
OVX, non-stress	415.88 ± 31.09
OVX, stress	445.36 ± 18.38

Table 3.5 Postsynaptic density protein-95 expression in the prefrontal cortex. Data in means ± standard error of the mean. OVX, ovariectomized; PSD-95, postsynaptic density protein-95.

Reduced latency to immobility in the forced swim test was associated with increased IL-6:IL-10 in the frontal cortex and hippocampus

Pearson's correlations were performed between behavioral measures and markers of neuroinflammation that were significantly affected by stress exposure in an ovarian status-dependent manner. We found that reduced latency to immobility in the forced swim test was significantly associated with increased IL-6:IL-10 in the frontal cortex ($r = -0.43$, $p = 0.048$; Figure 3.10A) and hippocampus ($r = -0.43$, $p = 0.044$; Figure 3.10B). However, there were no significant correlations between latency to immobility and IL-1 β concentrations in the frontal cortex ($r = -0.11$, $p = 0.6$) or hippocampus ($r = 0.04$, $p = 0.85$).

Cell signaling phosphoproteins were positively correlated with cytokines in the frontal cortex

As expected, we found significant positive correlations between several cytokines and cell signaling phosphoproteins in the frontal cortex, after correcting for multiple comparisons. Pearson's correlation coefficients and p values are provided in Table 3.6.

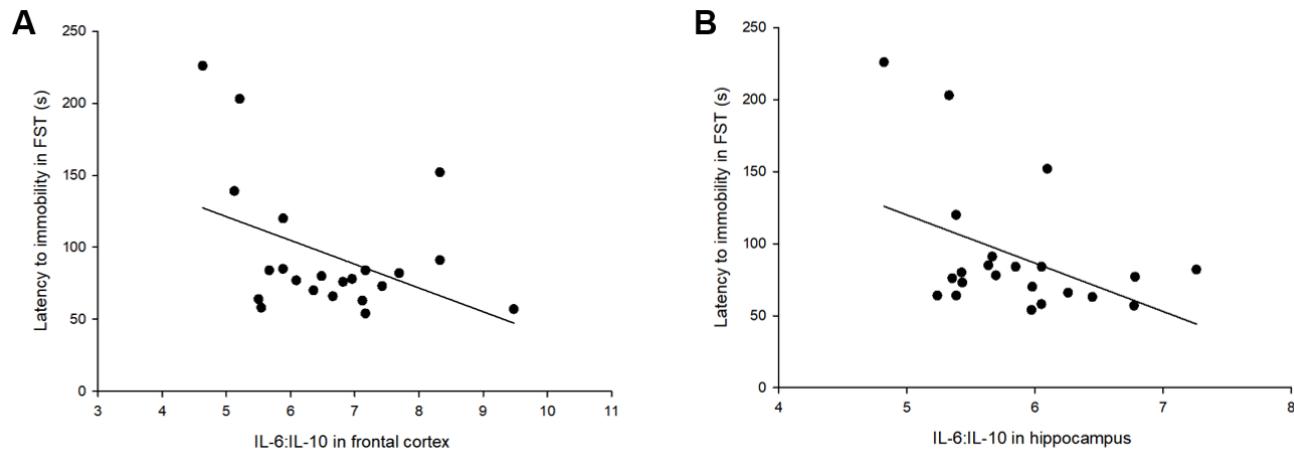


Figure 3.10 Correlation between passive-coping behavior and pro- to anti-inflammatory cytokine balance in the frontal cortex (A) and hippocampus (B). Reduced latency to immobility was significantly associated with increased IL-6:IL-10 in the frontal cortex (A; $r = -0.43$, $p = 0.048$) and hippocampus (B; $r = -0.43$, $p = 0.044$). FST, forced swim test; IL, interleukin.

	pERK1/2	pMEK1/2	pp38	pJNK	pSTAT3
Frontal cortex					
IL-1β	-0.30 p=0.12	0.16 p=0.40	-0.09 p=0.63	0.25 p=0.192	-0.09 p=0.65
IL-2	0.51 p=0.005	0.69* p<0.001	0.79* p<0.001	0.57 p=0.002	0.71* p<0.001
IL-4	0.17 p=0.40	0.08 p=0.67	0.25 p=0.19	0.10 p=0.60	0.11 p=0.59
IL-5	0.39 p=0.04	0.49 p=0.008	0.67* p<0.001	0.40 p=0.034	0.65* p<0.001
IL-6	0.20 p=0.30	0.53 p=0.004	0.46 p=0.014	0.49 p=0.009	0.46 p=0.015
IL-10	0.45 p=0.021	0.64* p<0.001	0.69* p<0.001	0.48 p=0.013	0.56 p=0.003
IL-12p70	0.55 p=0.002	0.65* p<0.001	0.81* p<0.001	0.489 p=0.008	0.68* p<0.001
TNF-α	0.09 p=0.67	0.49 p=0.008	0.45 p=0.017	0.54 p=0.003	0.30 p=0.12
IFN-γ	0.43 p=0.026	0.70* p<0.001	0.72* p<0.001	0.60* p=0.001	0.61* p=0.001
CXCL1	0.41 p=0.03	0.70* p<0.001	0.73* p<0.001	0.80* p<0.001	0.58* p=0.001

Table 3.6 Correlations between cell signaling phosphoproteins and cytokine concentrations in the frontal cortex. Pearson's correlation coefficients (r) and p values. * and grey background indicate significant correlations after correcting for multiple comparisons. IL, interleukin; CXCL1, chemokine (C-X-C motif) ligand 1; TNF- α , tumor necrosis factor- α ; IFN- γ , Interferon- γ .

3.4 Discussion

Here, we report profound effects of long-term ovariectomy on the central cytokine milieu, evidenced by reduced cytokine concentrations in the frontal cortex but increased cytokine concentrations in the hippocampus, in middle-aged female mice. Long-term ovariectomy also reduced phosphorylated ERK1/2 and MEK1/2 expression in the frontal cortex in middle age. Along with these neural changes in the frontal cortex and hippocampus, long-term ovariectomy resulted in a modest increase in depressive-like behavior under non-stress conditions in middle age. We further show ovarian status-dependent effects of sub-chronic stress exposure on cytokine concentrations in the frontal cortex and hippocampus, as seen by increased IL-1 β and a shift toward a pro-inflammatory cytokine bias (IL-6:IL-10) in sham-operated mice only. Sub-chronic stress exposure also decreased expression of phosphorylated ERK1/2 in the frontal cortex in sham-operated mice only. Importantly, this was coupled with a greater behavioral susceptibility to sub-chronic stress in sham-operated mice. These data suggest that ovarian hormones exert a powerful influence on the neuroimmune environment in a region-dependent manner, and may dictate certain behavioral and neuroinflammatory consequences of sub-chronic stress exposure.

Long-term ovariectomy modifies the cytokine signature under non-stress conditions and in response to sub-chronic stress exposure

We observed brain region-specific effects of long-term ovariectomy (9 months) on cytokine concentrations, with reductions in the frontal cortex and modest elevations in the hippocampus. Indeed, more than 50% of the variance within the frontal cortex cytokine data was explained by Principal Component 1 (PC1), which appeared to be largely accounted for by ovarian status. Ovarian status also affected the hippocampal cytokine milieu, although to a lesser extent than in

the frontal cortex. Overall, the principal component loadings indicated 7 overlapping cytokines were important contributors to the first principle component (PC1) that was modified by long-term ovariectomy albeit in opposing directions between the frontal cortex and hippocampus (IL-2, IL-4, IL-5, IL6, IL-10, IL-12p70, TNF- α). In terms of individual ANOVA analyses, more distinct sets of cytokines were affected by long-term ovariectomy in the two brain regions, with the exception of IL-6 which was increased in the hippocampus but decreased in the frontal cortex. The effects of sub-chronic stress exposure on cytokine concentrations in the frontal cortex and hippocampus were less pronounced. This is not surprising given the sub-chronic nature of the stress paradigm (6 days), thus we would expect longer durations of stress exposure to result in larger alterations in the cytokine milieu. However, component loadings indicate IL-1 β , TNF- α and the chemokine CXCL1 as important contributors to PC2 in the hippocampus, which showed significant effects of sub-chronic stress exposure. Overall, we do not observe a clear division of pro-inflammatory or anti-inflammatory cytokines, nor of T helper (Th) 1 and Th2 type cytokines, in the contribution to PCs. This may not be surprising, as although cytokine responses can be biphasic (i.e. an initial pro-inflammatory response followed by an anti-inflammatory response), simultaneous pro- and anti-inflammatory responses can also be observed. Indeed, a meta-analysis indicated that individuals with depression display increased concentrations of IL-10, an anti-inflammatory Th2 type cytokine, in addition to increased pro-inflammatory cytokines (Köhler et al., 2017), perhaps representing a compensatory effect. It is also important to note that all mice underwent behavioral testing, including the forced swim test which is an acute stressor. Therefore, it is plausible that exposing non-stress controls to behavioral testing may have masked some of the effects of sub-chronic stress exposure on cytokine concentrations.

An intriguing finding was that ovarian status impacted the neuroinflammatory consequences of sub-chronic stress exposure, with overall more pronounced effects in sham-operated mice. Specifically, in the frontal cortex and hippocampus of sham-operated mice only, stress exposure increased concentrations of the pro-inflammatory IL-1 β and resulted in a shift toward a pro-inflammatory cytokine bias (IL-6:IL-10). The exaggerated neuroinflammatory response to stress in sham-operated mice was observed in tandem with a greater behavioral susceptibility to sub-chronic stress exposure (discussed below). Our findings in sham-operated females corroborate studies in male subjects, in which IL-1 β was increased by chronic stress exposure in the brain, and further found to be directly implicated in the behavioral consequences of stress exposure (Goshen et al., 2008; Goshen and Yirmiya, 2009; Wohleb et al., 2014). Further, the role of IL-6 in depression and stress-based models is well-established (reviewed in Hodes et al., 2016), and a shift towards a higher IL-6: IL-10 ratio is associated with passive-coping in response to social defeat stress in male rats (Wood et al., 2015). Thus, our findings suggest that in middle-aged females, ovarian hormones may potentiate the inflammatory consequences of stress exposure. Given that ovariectomy was performed in young-adulthood, sham-operated groups more closely approximate what occurs in normal transition to reproductive senescence. Thus, the greater susceptibility to sub-chronic stress exposure in sham-operated mice may be linked to the endogenous changes in ovarian hormones at this time. Indeed, this mirrors the human condition in which the transition to menopause is associated with increased risk for depression (Freeman et al., 2004; Cohen et al., 2006).

Ovarian hormones, especially 17 β -estradiol but also progesterone, have well established anti-inflammatory properties (Bruce-Keller et al., 2000; He et al., 2004; Vegeto et al., 2008; Lei et al., 2014). Consistent with this are the observed effects of long-term ovariectomy to increase

IL-6:IL-10 ratio in the frontal cortex and IL-6 concentrations in the hippocampus under non-stress conditions. Given the role of IL-6 in MDD (Dowlati et al., 2010; Liu et al., 2012), these observations may be related to the modest increase in depressive-like behavior induced by long-term ovariectomy under non-stress conditions, which is in line with findings in humans (Schmidt et al., 2015; Wilson et al., 2018). However, in the frontal cortex we also found that ovariectomy reduced concentrations of other proinflammatory cytokines including IFN- γ and TNF- α . Further, in both the frontal cortex and hippocampus we observed an exaggerated neuroinflammatory response to stress in sham-operated, rather than ovariectomized mice, highlighted by increased IL-1 β and increased IL-6:IL-10 ratio. Importantly, the anti-inflammatory and neuroprotective effects of estrogens have been investigated mostly in younger females and in models of brain injury or stroke, and in response to acute inflammatory challenges or *in vitro* (Selvamani and Sohrabji, 2010; Suzuki et al., 2009; Vegevo et al., 2003). Age is a critical consideration here, as studies using older females have observed neurotoxic effects of estrogens in similar models (Johnson and Sohrabji, 2005; Nordell et al., 2003; Selvamani and Sohrabji, 2010), partially consistent with our finding here that exposure to sub-chronic stress resulted in a larger neuroinflammatory response in sham-operated middle-aged mice. Future studies need to consider age as a variable and should introduce 17 β -estradiol and/or progesterone replacement to further clarify the role of ovarian hormones in the neuroinflammatory outcomes of stress exposure. Additionally, it would be important that future studies investigate whether changes in estrous cyclicity in middle-aged animals can affect the outcomes of stress exposure, as the current study was not powered to directly answer this question.

Long-term ovariectomy and sub-chronic stress exposure interact to affect ERK signaling

Long-term ovariectomy reduced phosphorylated ERK1/2 and its upstream activator MEK1/2 in the frontal cortex under non-stress conditions, and stress exposure reduced pERK1/2 expression in sham-operated mice only. The observed reduction with long-term ovariectomy may not be surprising, as this group had significantly lower circulating concentrations of estradiol, which can rapidly activate ERK1/2 (Bi et al., 2002; Fernandez et al., 2008). Perhaps more intriguing than baseline differences in pERK1/2 expression were the differential effects of stress exposure, as abnormal ERK signaling has been observed in post-mortem brains of individuals with MDD that died of suicide (Dwivedi et al., 2001, 2006; Hsiung et al., 2003; Duric et al., 2010; Labonté et al., 2017). Notably, in human studies there are reports of reduced ERK1/2 expression and activation in the prefrontal cortex and hippocampus of males and females with MDD (Dwivedi et al., 2001, 2006; Hsiung et al., 2003), although data was not stratified by sex. Our results contrast another study in which chronic stress exposure (21 days) increased pERK1/2 in the ventromedial PFC of female mice (Labonté et al., 2017), but this study used young adult females whereas our subjects were middle-aged. Collectively, past studies indicate that dysregulated ERK signaling is implicated in depression and the outcome of stress exposure and our current findings extend previous work to suggest that ovarian hormones can influence the outcomes of stress exposure on ERK signaling in females. Moreover, although we did not test a causal link, we observe significant positive correlations between several cytokines and cell signaling phosphoproteins in the frontal cortex. This is to be expected as not only do cytokines activate all pathways examined in this study, but MAPKs are also important regulators of cytokine production (Johnson and Lapadat, 2002; Cargnello and Roux, 2011). More specifically, cytokines can interact with their respective cell membrane receptors to directly activate ERK signaling (Cargnello and Roux,

2011), and therefore it is possible that changes in pERK observed in this study could be a direct result of alterations in the cytokine milieu. On the other hand, an alternative model linking changes in cytokine profiles and pERK expression could involve the actions of 17 β -estradiol. Indeed, the effects of 17 β -estradiol to ameliorate microglia inflammatory activity *in vitro* are mediated at least in part via MAPK pathways (Baker et al., 2004). Therefore, it is possible that in the current study, group differences in ovarian hormones could have resulted in differential activation of ERK pathway to regulate cytokine expression. Using specific MAPK inhibitors or cytokine antagonists, future experiments could assess the directionality and causality of the observed relationships between cytokines and cell signaling phosphoproteins. In the present study, due to limited tissue, we only examined cell signaling phosphoproteins in the frontal cortex, therefore future studies should examine these signaling pathways in the hippocampus.

Long-term ovariectomy and stress did not significantly affect PSD-95 expression in the frontal cortex in the present study. This finding is consistent with previous studies indicating that although PSD-95 expression is sensitive to transient fluctuations in ovarian hormones (Spencer et al., 2010), it is not modified two weeks post ovariectomy (Waters et al., 2009, 2015a; Zhang et al., 2010; Spencer-Segal et al., 2012). Several studies have found reductions in PSD-95 expression in response to chronic stress exposure (Kallarackal et al., 2013; Kim and Leem, 2016; Pacheco et al., 2017) suggesting that the shorter duration of stress exposure used in this study may not be sufficient to produce significant modifications to PSD-95 expression in the frontal cortex. Future studies should also examine the effects on PSD-95 expression in the hippocampus and with longer durations of stress exposure.

Ovarian status influenced behavior under non-stress conditions and in response to sub-chronic stress exposure

Under non-stress conditions, long-term ovariectomy alone impacted some measures of depressive-like behavior, as seen by reduced latency to immobility in the forced swim test and a trend toward reduced sucrose consumption. These behavioral differences may be linked to the reported effects of long-term ovariectomy on cytokine concentrations in the brain. However, the depressogenic effects of long-term ovariectomy were overall limited, as we did not observe significant differences between sham-operated and ovariectomized mice in any other behavioral measure. This is partially consistent with other studies in which the anxiogenic and depressogenic effects of ovariectomy (Estrada-Camarena et al., 2011; Li et al., 2014) can recover after more prolong periods of ovarian hormone deprivation (de Chaves et al., 2009; Estrada-Camarena et al., 2011).

Exposure to sub-chronic stress reduced latency to immobility in the forced swim test in sham-operated mice only, pointing to a greater susceptibility to stress-induced passive-coping behavior. However, this effect was not seen across all measures, as sub-chronic stress exposure increased anhedonia-like behavior and reduced body mass regardless of ovarian status, suggesting similar susceptibilities in these domains. Further, we do not observe significant group differences in the percentage of time spent immobile in the forced swim test, suggesting overall modest behavioral effects of stress exposure, likely due to the sub-chronic nature of the paradigm (6 days). In the forced swim tests, it is also plausible that ovariectomized mice had reached a floor effect under non-stress conditions, leaving no room for sub-chronic stress exposure to further reduce latency to immobility. However, the effect of stress to reduce latency to

immobility in sham-operated mice was observed in concert with an exaggerated neuroinflammatory response to stress and a reduction in pERK1/2 expression. Therefore, in comparison with ovariectomized mice, we observe more robust effects of sub-chronic stress exposure in sham-operated mice across several measures, perhaps supporting the interpretation of greater overall susceptibility to the effects of sub-chronic stress exposure. Indeed, reduced latency to immobility was significantly correlated with increased IL-6:IL-10 ratio in the frontal cortex and hippocampus, suggesting that the greater susceptibility to stress-induced passive-coping behavior in sham-operated mice may be directly linked to the exaggerated neuroinflammatory response to stress. There are several possible mechanisms by which inflammation can ultimately influence depressive-like behavior. Notably, pro-inflammatory cytokines can affect multiple neurotransmitter systems, including serotonergic, glutamatergic, and dopaminergic systems (Miller et al., 2014; Felger, 2017; Haroon and Miller, 2017). For example, in male mice, IL-1 β has been shown to decrease synaptic availability of serotonin by enhancing serotonin transporter activity, an effect that was directly linked to depressive-like behavior (Zhu et al., 2010). Further, adult hippocampal neurogenesis, which is implicated in stress resilience (Anacker et al., 2018), is reduced by inflammation (Ekdahl et al., 2003; Monje et al., 2003), and regulated directly by IL-1 β (Zunszain et al., 2012). Future studies should investigate whether these mechanisms are implicated in the effects of ovarian hormones to influence stress-induced inflammation and behavior.

Our current findings agree with a previous study in young adult female mice in which exposure to a similar sub-chronic stress paradigm increased passive-coping behavior in sham-operated, but not ovariectomized mice (LaPlant et al., 2009). In contrast, other studies in rats and mice found that long-term ovariectomy increased depressive-like behavior after chronic stress

exposure (Lagunas et al., 2010; Mahmoud et al., 2016a). These dissimilarities may be reconciled by comparing the duration of exposure to stressors, which spanned 4-6 weeks in studies showing increased sensitivity to depressive-like behavior after long-term ovariectomy (Lagunas et al., 2010; Mahmoud et al., 2016a), as opposed to 6 days in the current report and in LaPlant et al (2009), where increased sensitivity to stress is observed in sham-operated females. Thus, together with previous studies our current data indicate that ovarian hormones can confer either risk or resilience, depending on stress paradigm.

3.5 Conclusions

In conclusion, long-term ovarian hormone deprivation significantly altered the neuroimmune environment in a brain region-specific manner, reduced expression of ERK pathway phosphoproteins in the frontal cortex, and modestly increased depressive-like behavior under non-stress conditions, in middle-aged mice. Importantly, ovarian status dictated some of the behavioral and neuroinflammatory outcomes of sub-chronic stress exposure. Specifically, sham-operated mice had a greater neuroinflammatory response to stress as seen by increased concentrations of IL-1 β and increased IL-6:IL-10 in the frontal cortex and hippocampus. We also observed stress-induced reductions in pERK1/2 expression in the frontal cortex of sham-operated mice only. This was coupled with an enhanced behavioral susceptibility to sub-chronic stress exposure in sham-operated mice, as seen by increased passive-coping behavior in the forced swim test. It is important to note ovariectomy was performed in young adulthood and outcomes were examined in middle-age. Therefore, our sham-operated middle-aged mice, tested at a time of transition to reproductive senescence, are perhaps a closer model of the menopausal transition. With this in mind, it may not be surprising that the outcomes of stress exposure were more robust in sham-operated mice, as this mirrors the human condition where the transition to menopause

carries an increased risk for depression. These findings underscore the importance of considering the immunomodulatory properties of ovarian hormones in stress and depression research.

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Chapter 4: Selective activation of estrogen receptors α and β : Implications for depressive-like phenotypes in female mice exposed to chronic unpredictable stress

4.1 Introduction

Major depressive disorder (MDD) is a stress-related psychiatric disorder that affects approximately twice more women than men (Kessler and Bromet, 2013; Salk et al., 2017). Given this disparity in prevalence rates, a focus on female-specific factors that may contribute to depression is warranted. Sex differences in prevalence rates first emerge around puberty (Salk et al., 2017), suggesting that ovarian hormones may increase risk for depression in women.

However, periods associated with declines in ovarian hormones, including the postpartum and perimenopause, are associated with a heightened risk for depression in women (Hendrick et al., 1998; Cohen et al., 2006; Soares, 2014), supporting the notion that ovarian hormones may afford resilience, rather than risk. The complex roles of ovarian hormones in depression may be partly attributed to the diversity of estrogen signaling, which can be loosely categorized into genomic and non-genomic mechanisms (Björnström and Sjöberg, 2005).

Evidence from animal models suggests that 17β -estradiol may interact with classical estrogen receptors (ERs) to regulate affective function. However, this is primarily derived from studies that have used tests of depressive- and anxiety-like behaviour in the absence of any manipulations intended to induce depressive- or anxiety-like phenotypes (e.g. chronic stress). For example, the administration of 17β -estradiol or an ER β but not ER α agonist to ovariectomized rats decreases anxiety- and depressive-like behaviour (Walf et al., 2004; Walf and Frye, 2005; Weiser et al., 2009; Yang et al., 2014). Moreover, the anxiolytic and antidepressant-like effects of 17β -estradiol are not observed in ER β knockout mice (Rocha et al., 2005). Collectively these

findings indicate that 17 β -estradiol's effects on affective behaviours may be primarily mediated via interactions with ER β . Importantly, the effects of ER activation in "healthy" animals may not mirror those in animals displaying depressive-like pathology, and as such although informative, these findings are certainly not conclusive. Indeed, a more recent study found that overexpression of ER α in the nucleus accumbens afforded resilience to the depressive-like outcomes after stress exposure in female mice (Lorsch et al., 2018), but the authors did not test the effects of ER β overexpression. Thus, to date, the mediatory roles of ER α and ER β have not been investigated systematically *and* within an animal model of depression. The current study therefore utilized the chronic unpredictable stress (CUS) model in female mice with the aim of dissecting the contribution of ER α and ER β to the estrogenic modulation of depressive-like pathology.

Endocrine and immune systems engage in extensive crosstalk (Grossman, 1985; Olsen and Kovacs, 1996; Bereshchenko et al., 2018). 17 β -Estradiol in particular possesses immunomodulatory properties that are mediated via interactions with both ER α and ER β (Baker et al., 2004; Saijo et al., 2011; Kovats, 2015). For example, several *in vitro* and *in vivo* studies in females reveal the capacity for 17 β -estradiol and selective ER modulators (SERMs) to ameliorate inflammation and microglial activation in response to inflammatory challenges (Vegeeto et al., 2001, 2008; Baker et al., 2004; Brown et al., 2010; Ishihara et al., 2015). The anti-inflammatory properties of 17 β -estradiol may be of particular interest within the context of depression, as inflammation in MDD has been well-documented and corroborated by animal models of stress exposure (Hodes et al., 2015b; Miller and Raison, 2016). Indeed, meta-analyses show increased proinflammatory cytokines including IL-6 and TNF- α in blood, cerebrospinal fluid, and brains of individuals with MDD (Dowlati et al., 2010; Haapakoski et al., 2015; Enache

et al., 2019). Despite this, there has been insufficient consideration of how sex and sex hormones can influence the association between depression and inflammation (reviewed in Eid et al., 2019b). It is plausible that the immunomodulatory effects of 17 β -estradiol are implicated in depression and the outcomes of stress exposure, yet the receptor mechanisms by which 17 β -estradiol can influence chronic stress-induced inflammation remain unclear.

The hippocampus retains the ability to produce new neurons in adulthood in a variety of species including humans (Kempermann et al., 2018; Moreno-Jiménez et al., 2019; Tobin et al., 2019). Adult hippocampal neurogenesis is reduced by chronic stress, promotes stress resilience, and is implicated in affective function and hypothalamic-pituitary-adrenal (HPA) axis regulation (Surget et al., 2011; Schoenfeld and Gould, 2012; Anacker et al., 2018), although most of this evidence is derived from male rodents. Importantly, 17 β -estradiol influences adult hippocampal neurogenesis in females (reviewed in (Mahmoud et al., 2016b)) and its pro-proliferative effects are mediated via actions on both ER α and ER β , at least acutely (Mazzucco et al., 2006). However, whether chronic activation of ER α and ER β influences the survival of newly produced hippocampal neurons under basal or chronic stress conditions has not been investigated. Beyond hippocampal neurogenesis, both estrogens and stress exposure affect synaptic plasticity in a variety of brain regions, including the hippocampus and frontal cortex (Li et al., 2004; Brinton, 2009; Srivastava and Penzes, 2011; McEwen, 2013; McEwen et al., 2016). 17 β -Estradiol and ER α or β agonists upregulate the expression of synaptic proteins in female rats (Waters et al., 2009), including the postsynaptic density protein 95 (PSD-95), a scaffolding protein which organizes synaptic elements that are important for synaptic function and plasticity (Kim and Sheng, 2004). Importantly, PSD-95 is reduced in the prefrontal cortex (PFC) of individuals with

MDD (Feyissa et al., 2009) and in animal models of stress exposure (Kallarackal et al., 2013; Kim and Leem, 2016; Pacheco et al., 2017). Yet, whether and by which receptor mechanisms 17β -estradiol influences the effects of stress on PSD-95 remains to be elucidated.

Dysregulation of the HPA axis is observed in a subset of individuals with MDD, and includes hypercortisolemia, aberrations in the circadian rhythm of cortisol, and impairments in HPA axis negative feedback function (Ising et al., 2007; Stetler and Miller, 2011). Importantly, the HPA axis interacts bidirectionally with the hypothalamic pituitary gonadal (HPG) axis (Reviewed in Goel et al., 2014). HPA-HPG interactions in females have been primarily investigated at baseline or in response to acute stressors. In general, 17β -estradiol stimulates HPA axis activity and impairs its negative feedback function (Goel et al., 2014), with opposing roles of ER α and ER β (Weiser and Handa, 2009; Weiser et al., 2010). Importantly, these effects of 17β -estradiol may not translate to conditions of chronic stress exposure. Indeed, we have shown that long-term ovarian hormone deprivation in rats increased HPA axis negative feedback impairment under chronic stress exposure (Mahmoud et al., 2016a), which parallels findings in MDD indicating greater negative feedback impairment in women post-menopause (Roy et al., 1986; Young et al., 1993). Therefore, we investigated the roles of ER α and ER β in mediating the effects of estradiol on HPA axis function under chronic stress conditions.

In this study, we sought to determine whether 17β -estradiol conferred resilience in the face of CUS in adult female mice. We further employed a pharmacological approach to disentangle the contribution of ER α and ER β to the effects of 17β -estradiol under non-stress and chronic stress conditions. We examined stress susceptible behaviours, markers of plasticity in the hippocampus and frontal cortex, central immune mediators, and HPA axis function. We

hypothesized that ovariectomy would increase depressive-like endophenotype under non-stress conditions and further exacerbate the depressive-like outcomes of chronic stress exposure. Additionally, we expected estradiol treatment to rescue the effects of ovariectomy and that its actions would be differentially facilitated by ER α and ER β .

4.2 Methods

Animals

Young adult female C57BL6/J mice (Charles River Laboratories; Quebec, Canada) arrived at our facility at approximately 8 weeks of age. All mice were group housed and given *ad libitum* access to food and water. Mice were maintained on a 12-hour light/dark cycle (lights on at 7:00am) in temperature- and humidity-controlled colony rooms ($21 \pm 1^\circ\text{C}$ and $50 \pm 10\%$, respectively). All procedures were conducted in accordance with the ethical guidelines of the Canadian Council on Animal Care and were approved by the Animal Care Committee at the University of British Columbia.

Surgery

All mice received bilateral ovariectomy (OVX) or sham surgery at approximately 3 months of age, adapted from our previously described procedure in rats (Barha et al., 2015; Galea et al., 2018). Briefly, surgery was performed through two lateral skin and muscle incisions under isoflurane anesthesia (5% induction, 1-1.5% maintenance), with ketamine (50mg/kg; intraperitoneally (i.p.)), xylazine (5mg/kg; i.p.), bupivacaine (2mg/kg; local), and Lactated Ringer's Solution (10ml/kg; subcutaneously (s.c.)). Mice received a nonsteroidal anti-inflammatory analgesic at the time of surgery, and at 24- and 48-hours post-surgery (Anafen;

5mg/kg; s.c.) then were allowed to recover for 7-9 days prior to hormone/agonist treatment (see experimental timeline in Figure 4.1).

Hormone and agonist treatment

Ovariectomized mice were randomly assigned to 1 of 4 treatment groups (n=26-28 per group), receiving daily s.c. injections of one of the following: (i) 17 β -estradiol (E2; 0.04 mg/kg), (ii) diarylpropionitrile (DPN; 0.1 mg/kg; ER β -selective agonist), (iii) propylpyrazole-triol (PPT; 0.1 mg/kg; ER α -selective agonist), or (iv) vehicle (sesame oil). To control for injections, sham-operated mice (n=20) also received daily s.c. vehicle injections (sesame oil). We therefore obtained the following 5 treatment groups: E2-treated OVX (E2), DPN-treated OVX (DPN), PPT-treated OVX (PPT), oil-treated OVX (OVX), and oil-treated sham-operated mice (Sham). DPN was chosen as it has a 70-fold greater affinity for ER β over ER α (Meyers et al., 2001), and PPT was chosen as it has a 410-fold greater affinity for ER α over ER β (Stauffer et al., 2000). 17 β -estradiol has an equally high affinity to both ER α and ER β , and its dose was chosen based on previous work and reports showing physiological circulating estradiol concentrations (Ciana et al., 2003; Harburger et al., 2007). Agonist doses were based on reports of behavioural efficacy (Clipperton et al., 2008; Walf et al., 2008). Treatment persisted for 47 days (see Figure 4.1), beginning 14 days prior to chronic unpredictable stress (CUS) exposure. The choice to begin treatment prior to CUS was to examine the potentially protective, rather than therapeutic effects of 17 β -estradiol or agonists.

Bromodeoxyuridine administration

To label dividing progenitor cells and their progeny, 2 injections of bromodeoxyuridine (BrdU; 200mg/kg; i.p; 8 hours apart) were administered to all mice two weeks after hormone treatment

had begun, and one day prior to the initiation of CUS (Figure 4.1). This timing of BrdU administration was intended for the examination of the effects of CUS on the survival of new neurons, independent of effects on cell proliferation.

Chronic Unpredictable Stress

Mice from each treatment group (Sham, OVX, E2, DPN, and PPT) were randomly assigned to CUS or no-CUS conditions, resulting in 10 groups overall ($n = 10-14$). CUS consisted of exposure to 13 stressors, applied twice daily in a pseudo-random order for a duration of 4 weeks (Figure 4.1). The CUS protocol was adapted from published reports (Kreisel et al., 2014; Mahmoud et al., 2016a; Wainwright et al., 2016a) and stressors used are detailed in Table 4.1. Chronic unpredictable stress was chosen as it shows face, construct and predictive validity as an animal model of depression (Willner, 2017). Mice in the no-CUS condition were housed in a separate colony room to avoid the transfer of olfactory cues from CUS-exposed mice (Brechbuhl et al., 2013), and left undisturbed with the exception of daily injections and weekly cage changing.

Stressor	Description
Wet bedding	500 ml of tap water in home cage for 3 hours
No bedding	Bedding removed from home cage for 3 hours
Soiled cage	Placed in a cage soiled by other mice for 3 hours
Tail bleed	Tail blood collection via needle poke
Restraint	Placed in well ventilated 50ml falcon tube for 1 hour
Cage tilting	Cage tilted at a 45° angle for 4 hours
Cage rotation	Housing with unfamiliar mice for 3 hours
Social isolation	Overnight social isolation, 18 hours
Elevated platform	Mice placed individually on a clear plexiglass elevated platform for 5 minutes
Lights off	Lights off for 3 hours during the light cycle
Continuous lighting	Continuous room lighting for two consecutive night cycles
Stroboscopic light	Flashing light (1 flash/sec) in an otherwise dark colony room during the light cycle
Noise	White noise from radio for 1 hour

Table 4.1 Stressors used in the Chronic Unpredictable Stress paradigm.

Behavioural Testing

All mice were assessed on a battery of tests starting the day after CUS. One test was applied per day, and testing occurred in the order depicted in Figure 4.1. Mice were acclimatized to testing rooms for 1 hour prior to each test. All behavioural scoring was performed by experimenters blinded to group assignment.

Forced Swim Test. The forced swim test (FST) was used according to previous methods (Can et al., 2012) to assess stress-coping behaviour which is sensitive to estrogens and exposure to stressors (Rocha et al., 2005; Monteiro et al., 2015). Briefly, under dim light conditions, mice were individually subjected to a 6-minute swimming session in a 4L glass beaker containing clean water at $25 \pm 0.5^{\circ}\text{C}$, filled to a depth of 15 cm. The test was filmed, and time spent in

passive-coping behaviour across the test was scored. Mice were considered to display passive-coping behaviour when immobile, with the absence of any movements except those necessary to remain afloat or keep the head above water.

Tail Suspension Test. The tail suspension test (TST) was used as an additional test of stress-coping behaviour, and performed according to standard methods (Can et al., 2011). Briefly, mice were suspended by the tail using laboratory tape (FisherbrandTM labeling tape) placed 2-3 mm from the tip of the tail, in a 3-walled rectangular chamber. Prior to suspension, and to prevent tail-climbing behaviour which is common in this strain, tails were passed through a 4cm hollow rubber tubing to cover a portion of the tail, as per previous methods (Can et al., 2011). The 6-minute duration of the test was filmed, and percent time spent immobile was considered an index of passive-coping behaviour.

Sucrose preference test. Anhedonia-like behaviour was assessed in the sucrose preference test which was adapted from published methods (Pothion et al., 2004). Mice were acclimatized to two water bottles in the home cage for 24 hours and individually housed approximately 4 hours prior to testing. Testing occurred during the dark phase (19:00-07:00) in which mice were presented with two bottles, one containing 1% sucrose and the other tap water. The right-left position of sucrose was counterbalanced between mice in each group. Sucrose preference was calculated as the percentage of sucrose consumed over total liquid consumed.

Novelty Suppressed feeding. Anxiety-like behaviour was assessed using the novelty suppressed feeding test (NSF), adapted from previously published methods (Samuels and Hen, 2011). Behaviour in this test is sensitive to chronic stress exposure and ovarian status (Stedenfeld et al., 2011; Mahmoud et al., 2016a), and chronic antidepressant treatment produces an anxiogenic

effect in a neurogenesis-dependent manner in males (Santarelli et al., 2003). Briefly, mice were deprived of food for 24 hours, then individually placed in one corner of an open arena (50x50x20cm) containing a froot loop[©] in the centre, and latency to start feeding was measured. Testing was performed under bright light conditions, and the arena was cleaned with 70% EtOH between mice. If a mouse did not feed within 10 minutes, it was removed from the arena and given a latency of 600 seconds in the analysis. Mice were returned to their home cage following testing, and food consumed within 5 minutes in the home cage was measured to account for potential appetite differences. Mice were re-grouped after the test. To avoid a neophobic reaction to the food, froot loops[©] were introduced to the home cage for 3 days prior to the test.

Dexamethasone suppression and HPA axis stress reactivity tests

Disrupted HPA axis negative feedback inhibition is an endocrine hallmark of MDD (Ising et al., 2007; Stetler and Miller, 2011), and estrogens regulate HPA negative feedback function (Goel et al., 2014). Therefore, half the animals from each group were subjected to the dexamethasone (DEX) suppression test to assess the integrity of the glucocorticoid-dependent negative-feedback function of the HPA axis. DEX (i.p.; 100 ug/kg) was administered 15 minutes prior to the regular treatment (E2, DPN, PPT, or oil). Mice were subjected to a 30-min restraint stressor starting 90 minutes after DEX administration, and blood was collected via tail vein nick at the end of restraint. To investigate HPA axis reactivity to an acute stressor, the other half of animals from each group received a vehicle injection (0.9% saline) in place of DEX, with the remainder of the procedure (restraint and blood collection) being identical. Blood samples were placed at 4°C overnight, then centrifuged for 15 min at 1000 x g and serum aliquots were stored at -20°C until processing.

Tissue harvesting and processing

Mice were euthanized approximately 2 hours after the DEX/HPA axis stress reactivity test. To obtain fixed brain tissue for immunohistochemistry, approximately half the mice in each group were deeply anesthetized with sodium pentobarbital (i.p.) then perfused with 10mL of cold 0.9% saline followed by 20mL of cold 4% paraformaldehyde (PFA). Brains were extracted immediately and post-fixed at 4°C in 4% PFA for 24 hours, then transferred to a 30% sucrose solution (in 0.1 M Phosphate Buffer; pH 7.4) and kept at 4°C until sectioning. To obtain fresh tissue for electrochemiluminescence immunoassays, the other half of mice in each group were euthanized by rapid decapitation and brains were removed immediately and micro-dissected on a cold surface. Specifically, the entire rostral-caudal extent of the hippocampus was dissected, and the frontal cortex was collected anterior to the genu of the corpus callosum (Bregma 1.42mm). Tissue was flash-frozen on dry ice and stored at -80°C until homogenized. Blood was collected via cardiac puncture from perfused mice, and trunk blood was collected from decapitated mice. In both cases, blood was collected into EDTA-coated tubes, centrifuged for 15 minutes at 4°C at 1000 x g, then plasma aliquots were stored at -80°C until processing.

Electrochemiluminescence immunoassays

In preparation for electrochemiluminescence immunoassays (ECLIA), frontal cortices and hippocampi were homogenized individually in cold lysis buffer (200µl and 150µL respectively) using an Omni Bead Ruptor (Omni international, Kennesaw, GA). Homogenates were immediately centrifuged at 2600rpm and 4°C for 10 minutes, and aliquots were stored at -80°C until processing. ECLIA kits from Meso Scale Discovery (MSD; Rockville MD) were used for

the quantification of cytokines and PSD-95. All assays were performed in accordance with manufacturer's instructions, with samples run in duplicates. Plates were read using a Sector Imager 2400 (MSD; Rockville MD), and data were analyzed using the Discovery Workbench 4.0 software (MSD). ECLIA values were normalized to total protein concentrations, which were measured in tissue homogenates in triplicates using the Pierce Micro BCA Protein Assay Kits (ThermoFisher Scientific) according to manufacturer instructions.

Cytokine quantification: Because inflammation is implicated in MDD (Dowlati et al., 2010; Haapakoski et al., 2015; Enache et al., 2019), several immune mediators were measured in the hippocampus and frontal cortex. The V-PLEX Proinflammatory Panel 1 kit (mouse) from MSD (catalogue no. K15048D) was used, which allows for the concurrent quantification of interleukin-1beta (IL-1 β), interleukin-2 (IL-2), interleukin-4 (IL-4), interleukin-6 (IL-6), interleukin-10 (IL-10), interferon-gamma (IFN- γ), tumor necrosis factor- α (TNF- α), chemokine (C-X-C motif) ligand 1 (CXCL1), granulocyte-macrophage colony-stimulating factor (GM-CSF), and vascular endothelial growth factor (VEGF), thus provides a comprehensive picture of the inflammatory state. The following lower limits of detection (LLODs) were observed: IL-1 β : 0.74-0.88 pg/ml; IL-2: 2.1-2.6 pg/ml; IL-4: 0.35-0.45 pg/ml; IL-6: 5.6-6.2 pg/ml; IL-10: 1.1-1.8 pg/ml; IFN- γ : 0.07-0.11 pg/ml; TNF- α : 0.88-1.32 pg/ml; CXCL1: 0.17-0.36 pg/ml; GM-CSF: 0.09- 0.13 pg/ml; and VEGF: 0.31-0.38 pg/ml. Values below the LLOD were assigned 0pg/mL, as published previously (Bodnar et al., 2017; Eid et al., 2019a).

PSD-95 quantification: PSD-95 was quantified because its expression is regulated by E2, DPN and PPT in the hippocampus of female rats and mice (Li et al., 2004; Waters et al., 2009) and because it's reduced in the PFC of individuals with MDD (Feyissa et al., 2009) and with chronic

stress exposure in rodents (Kallarackal et al., 2013; Kim and Leem, 2016; Pacheco et al., 2017).

PSD-95 protein expression was quantified in hippocampus and frontal cortex samples using a PSD-95 kit from MSD (catalogue no. K150QND).

Immunohistochemistry

For immunohistochemical staining, PFA-fixed brains were sliced into 30 μ m coronal sections using a Leica SM2000R Microtome (Richmond Hill, Ontario, Canada). Sections were stored at -20 °C in a cryoprotective solution containing 20% glycerol (Sigma-Aldrich, St. Louis, MO, USA) and 30% ethylene glycol (Sigma-Aldrich) in 0.1 M phosphate-buffer (PB, pH 7.4). Tissue was rinsed in 0.1M phosphate-buffered saline (PBS; pH 7.4; 3 x 10 min) to remove the cryoprotectant prior to staining and between all incubations. Unless otherwise noted, all incubations were performed on a rotator and at room temperature.

BrdU/NeuN: We examined the co-expression of BrdU with the neuronal marker NeuN to determine the proportion of BrdU immunoreactive (ir) cells that are neurons. Sections were incubated for 24 hours at 4°C in a primary antibody solution containing mouse anti-NeuN (catalogue no. MAB377; Millipore, Billerica, MA, USA) diluted 1:250 in 0.3% Triton-X and 3% normal donkey serum (NDS) in 0.1M PBS. Sections were then transferred to a secondary antibody solution containing 1:200 donkey anti-mouse Alexa Fluor 488 (Invitrogen, Burlington, ON, Canada) and incubated for 24 hours at 4°C. Sections were fixed in 4% PFA (in 0.1 PBS; 1 x 10 mins) followed by 0.9% saline (2 x 10 mins), before being transferred to 2N HCL for 30 mins at 37°C. Sections were then incubated for 24 hours at 4°C in a primary antibody solution containing rat anti-BrdU (catalogue no. ab6326; Abcam, Cambridge, UK) diluted 1:500 in 0.3% Triton-X and 3% NDS in 0.1M PBS. Sections were transferred to a secondary antibody solution

containing 1:500 donkey anti-rat Alexa Fluor 594 (Jackson ImmunoResearch) in 0.1M PBS for 24 hours. Sections were finally mounted onto glass slides (Superfrost Plus; Fisher scientific, Pittsburgh, PA, USA) and cover-slipped with polyvinyl alcohol (PVA)-DABCO (Sigma-Aldrich).

Ionized calcium binding adaptor molecule-1 (Iba-1): Iba-1 is a calcium-binding protein commonly utilized as a microglial marker (Korzhevskii and Kirik, 2016). Microglia were investigated as positron emission tomography studies provide evidence of increased microglial activation in MDD, and this effect is associated with illness duration and correlated with depression severity (Setiawan et al., 2015, 2018). Hippocampal and ventromedial prefrontal cortex (vmPFC) sections were incubated for 25 min in 0.3% hydrogen peroxide (in dH₂O) then blocked for 1 h with 10% normal goat serum (NGS) and 0.5% Triton-X in 0.1M PBS. Sections were transferred to a primary antibody solution containing rabbit anti-Iba-1 (1:1000; catalogue no. 019-19741; Wako, Osaka, Japan) in 10% NGS and 0.4% Triton-X in 0.1M PBS and incubated at 4°C for 20 hours. Next, sections were incubated in a secondary antibody solution for 1 hour, consisting of 1:500 biotinylated goat anti-rabbit (Vector Laboratories) in 2.5% NGS and 0.4% Triton X in PBS. Sections were then incubated for 1 h in ABC (Elite kit; 1:50, Vector Laboratories) in 0.4% Triton-X in PBS, then immunoreactants were visualized with a 7 min diaminobenzidine (DAB) reaction (Vector Laboratories). Sections were mounted on glass slides, allowed to dry, then dehydrated in increasing graded ethanol solutions, cleared with xylene, and cover-slipped with Permount (Fisher Scientific).

Microscopy, cell quantification, and optical density

BrdU- ir cell quantification was performed by an experimenter blinded to group assignment under a 100x objective on an Olympus BX51 brightfield microscope equipped with epifluorescence. A representative photomicrograph is depicted in Figure 4.4C. BrdU-ir cells were exhaustively quantified in every 10th section of the hippocampus specifically within the granule cell layer (GCL) and the subgranular zone (SGZ), defined as the 50µm band between the GCL and hilus. Cells were quantified separately in the dorsal and ventral hippocampus as these regions have distinctive functions (Fanselow and Dong, 2010). Raw counts were multiplied by 10 to obtain an estimate of the total number of BrdU-ir cells in the hippocampus (Mahmoud et al., 2016a; Pan et al., 2012). BrdU-ir cells were examined for NeuN co-expression to estimate the proportion of BrdU-ir cells that are neurons (calculated as BrdU/NeuN co-labeled cells divided by the total number of BrdU-ir cells). Then, to obtain a neurogenesis index for each animal, the estimated total number of BrdU-ir cells was multiplied by the ratio of BrdU/NeuN co-labeled cells.

Optical density of Iba-1 expression was measured using ImageJ in images obtained using CellSens software (Olympus) at 10x with fixed gain and exposure settings, as we have done previously (Workman et al., 2015; Wainwright et al., 2016a). Six sections were sampled per animal, including two sections each for the dorsal hippocampus (Bregma -1.66 to -2.35mm), ventral hippocampus (Bregma -3.18 to -3.58mm), and vmPFC (Bregma 1.54 to 1.94mm, with infralimbic (IL) and prelimbic (PL) regions sampled separately). Background grey value was obtained in each image from an average of open ellipses placed randomly in 10 non-immunolabeled areas, and threshold for immunoreactivity was set at 3-times the mean background grey value. In hippocampal slices, the GCL, SGZ and an approximately 50µm band of the molecular layer (ML) were traced and mean grey value per area was calculated. In vmPFC

slices, mean grey value per area was measured in two 0.85mm² regions placed within the IL and PL regions.

Vaginal cytology for estrous cycle staging

Behaviour, immune, and neural measures can be affected by estrous cycle stage (Woolley et al., 1990; Beagley and Gockel, 2003; Meziane et al., 2007). Therefore, vaginal cells were collected by lavage from sham-operated mice on all behavioural testing days and prior to euthanasia, and estrous cycle stage was determined according to previous methods (Cora et al., 2015). To account for potential effects of the procedure, all ovariectomized mice were also lavaged.

Radioimmunoassays for corticosterone quantification

Corticosterone concentrations were measured in serum samples obtained from HPA axis stress reactivity and DEX suppression tests. Corticosterone double-antibody radioimmunoassay kits (MP biomedicals, Solon, OH) were used according to manufacturer's instructions and the inter- and intra-assay coefficients of variation were < 10%.

Statistical analyses

Statistica software (Tulsa, OK) was used for all analyses. Behavioural measures (FST, TST, NSF, and SPT), cytokines, and CORT concentrations were each analyzed by factorial analysis of variance (ANOVA) with treatment (Sham, OVX, E2, DPN, PPT) and stress condition (CUS, no-CUS) as the between-subject factors. Neurogenesis index, proportion of BrdU/NeuN co-labeled cells, PSD-95 expression, and Iba-1 optical density were each analyzed using repeated measures ANOVA with treatment (Sham, OVX, E2, DPN, PPT) and stress condition (CUS, no-CUS) as the between-subject factors and brain region (dorsal and ventral hippocampus, or

hippocampus and frontal cortex) as the within-subject factors. Given that 10 cytokines were measured in each brain region, Principal Component Analyses (PCAs) were used as a dimensionality reduction tool (Jolliffe, 2002; Ringner, 2008). PCA reduces the number of potentially correlated variables (in this case cytokines) into a small set of uncorrelated variables called Principal Components (PCs). These components are ordered such that the first PC (PC1) accounts for most of the variance within the data. This approach was used to derive information about the amount of variance in the data that can be accounted for by potential cytokine networks. Because PCA does not take into account group membership, ANOVAs were subsequently applied to individual PC scores as we have published previously (Eid et al., 2019a), in order to explore the effects of treatments and CUS exposure on cytokine networks. DEX was used as a covariate in all cytokine analyses as DEX was administered to half the animals on tissue collection day, and because glucocorticoids have anti-inflammatory effects (Bereshchenko et al., 2018). Body mass was used as a covariate when analyzing immobility in FST. Estrous cycle stage was used as a covariate for all analyses. Covariate effects are only reported when significant. Neuman-Keul's comparisons were used for post-hoc analyses and any *a priori* comparisons employed were subjected to Bonferroni correction. Outliers that fell more than 2.5 standard deviations away from the mean were removed from analyses. Significance was set at $\alpha=0.05$. Where appropriate, effect sizes are given as partial η^2 or Cohen's d.

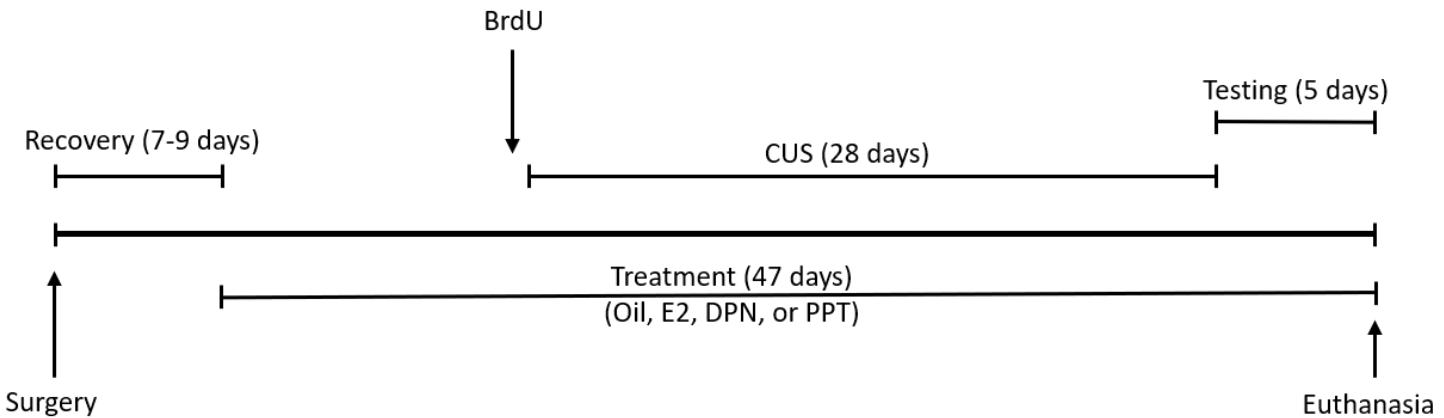


Figure 4.1 Timeline depicting the sequence of experimental events. Ovariectomy or sham surgery was performed at approximately 3 months of age. Following 7-9 days of recovery, all mice received daily subcutaneous treatment (oil, E2, DPN, or PPT) until experimental endpoint (47 days total). On treatment day 14 all mice received two BrdU injections. Starting on treatment day 15, half the mice from each treatment group were subjected to CUS (28 days). Behavioural tests were administered starting the day after CUS in the following order (one test/day): forced swim test, sucrose preference test, novelty suppressed feeding test, and tail suspension test. On the final day of the experiment mice were subjected to the dexamethasone suppression test or the hypothalamic-pituitary-adrenal axis stress reactivity test before tissue harvesting. BrdU, bromodeoxyuridine; CUS, chronic unpredictable stress; E2, 17 β -estradiol; DPN, diarylpropionitrile; PPT, propylpyrazole-trioli.

4.3 Results

TST: 1) CUS increased immobility, and this effect was primarily driven by ER agonists groups. 2) Ovariectomy increased immobility, and this effect was prevented by estrogenic treatments only under no-CUS Conditions.

As expected, CUS significantly increased immobility in the TST (main effect of CUS condition: $F(1, 111)=11.96$, $p<0.001$, $\eta^2_p=0.097$; Figure 4.2A₁). This effect of CUS to increase immobility was clearly driven by the ER agonist groups (PPT and DPN; both p 's < 0.0052, Cohen's d = 0.88-1.1, relative to no-CUS counterparts; Figure 4.2A₂₋₃), and to a lesser extent by the E2 group ($p=0.10$, relative to no-CUS counterparts; *a priori* comparisons: treatment by CUS condition interaction: $p=0.19$). Further, ovariectomy increased immobility in the TST relative to Sham and

DPN groups (p 's <0.03, Cohen's d = 0.7-1.0; main effect of treatment: $F(4, 111)=3.06$, $p=0.02$, $\eta^2_p = 0.099$; Figure 4.2A₂). Upon closer examination, this ovariectomy-induced increase in immobility was prevented by E2, DPN, and PPT treatments under no-CUS conditions only (p 's <0.04; *a priori* comparisons, treatment by CUS condition interaction: $p=0.19$; Figure 4.2A₃). In an additional exploratory analysis of TST data, we performed a median split and categorized mice into low immobility or “resilient”, versus high immobility or “susceptible” phenotypes, adapted from previous reports (Wells et al., 2017). We then performed a Chi-square test to compare the frequency of mice that were susceptible versus resilient between groups under CUS conditions: $\chi^2(9) = 16.17$, $p = 0.06$ (frequencies reported in Table 4.2). In the CUS exposed mice only, OVX mice displayed a bias toward a susceptible phenotype, whereas sham-operated mice displayed a bias toward a resilient phenotype. Further, a bias toward a susceptible phenotype is seen in PPT-treated groups, whereas E2- and DPN-treated groups showed an even split of resilient and susceptible mice after CUS.

Ovariectomy reduced latency to feed in NSF regardless of CUS exposure, and this effect was prevented by E2 treatment

Regardless of CUS condition, OVX significantly reduced latency to feed in NSF in comparison to Sham ($p=0.029$, Cohen's $d = 0.63$; main effect of treatment: $F(4, 111)=3.99$, $p=0.005$, $\eta^2_p = 0.13$; Figure 4.2B). This effect of OVX to reduce latency to feed was prevented by E2 ($p=0.92$, relative to Sham) but not by DPN or PPT treatments (p 's <0.05, relative to Sham). There was no significant main effect of CUS condition nor a treatment by CUS condition interaction (p 's >0.15). There were also no significant group differences in home cage food consumption post-NSF (all p 's >0.4; data not shown).

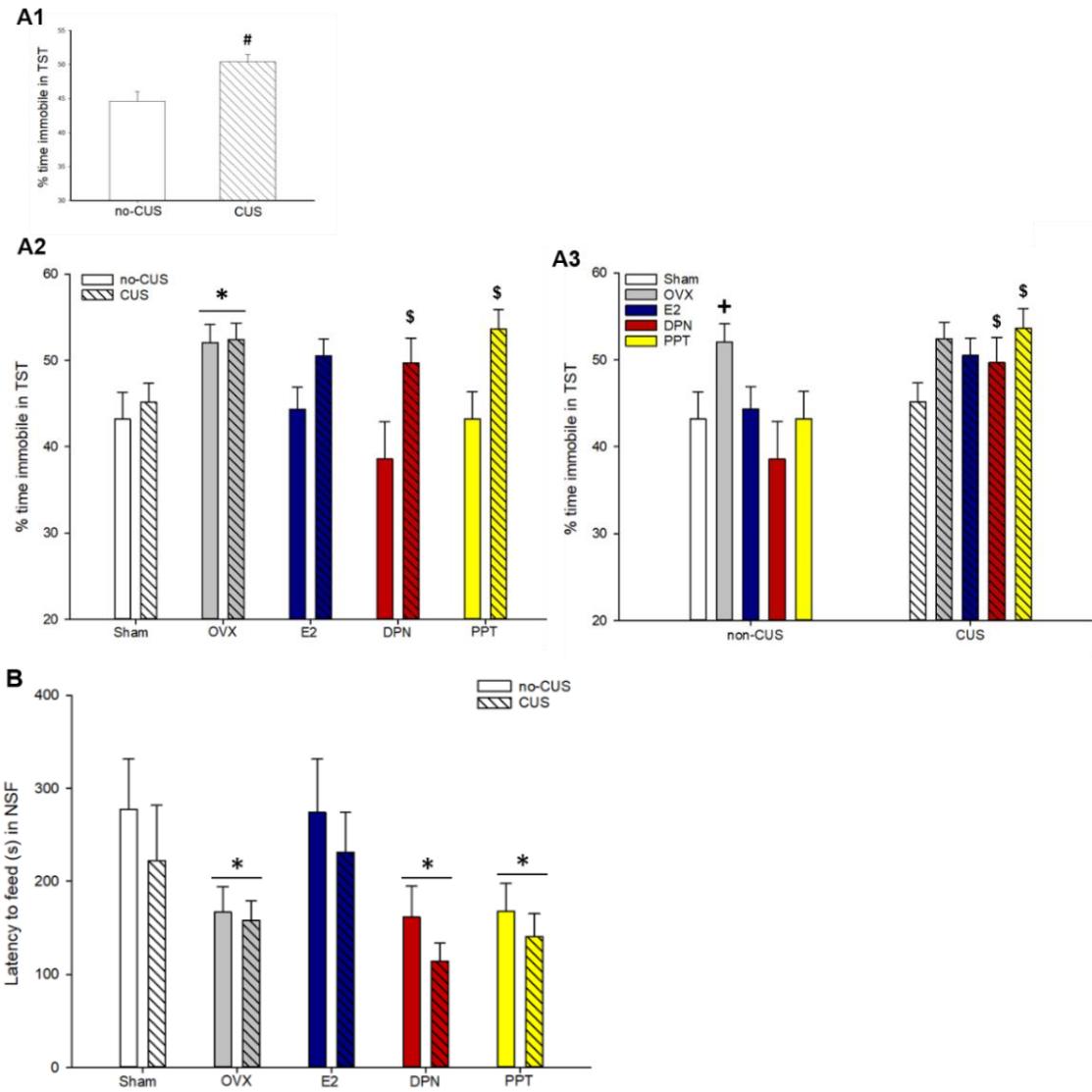


Figure 4.2 Percentage of time spent immobile in the tail suspension test (A1-3), and latency to feed in the novelty suppressed feeding test (B). Figures A2 and A3 depict the same data, organized differently across the x-axis for clarity of effects. (A1) CUS significantly increased immobility in TST; # indicates $p < 0.001$, main effect of CUS condition. (A2, A3) The effect of CUS to increase immobility was primarily driven by DPN- and PPT-treated groups; \$ indicate $p < 0.0052$, significantly higher than no-CUS treatment counterparts. (A2) Ovariectomy significantly increased immobility, * indicates $p < 0.03$, relative to Sham and DPN. (A3) the effect of ovariectomy to increase immobility was prevented by all estrogenic treatments only under non-CUS conditions only; + indicates $p < 0.05$, relative to all non-CUS groups. (B) Ovariectomy significantly decreased latency to feed in

NSF, and this effect was prevented by E2 treatment only, * indicate $p < 0.05$, relative to sham. TST, tail suspension test; NSF, novelty suppressed feeding; CUS, chronic unpredictable stress; OVX, ovariectomized; E2, 17 β -estradiol; DPN, diarylpropionitrile; PPT, propylpyrazole-triol. Data in means + standard error of the mean.

There were no significant group differences in FST immobility or sucrose preference

There were no significant group differences in % time spent immobile in the FST or % sucrose preference (p 's > 0.11 for main effects of CUS condition and treatment and their interaction; Table 4.2).

Group	% time spent immobile in FST	% sucrose preference	# of resilient in TST (low immobility)	# of susceptible in TST (high immobility)
no-CUS				
Sham	47.75 ± 3.49	82.24 ± 6.49	7	3
OVX	45.69 ± 5.11	84.62 ± 3.37	4	10
E2	51.60 ± 4.97	89.64 ± 1.51	9	5
DPN	48.27 ± 4.28	87.79 ± 2.25	8	3
PPT	41.35 ± 3.81	86.26 ± 1.93	9	5
CUS				
Sham	41.92 ± 3.49	90.92 ± 2.26	7	3
OVX	42.38 ± 3.86	90.98 ± 0.81	4	9
E2	47.84 ± 2.97	89.86 ± 3.84	6	7
DPN	41.72 ± 3.35	92.75 ± 1.30	6	7
PPT	42.94 ± 4.15	81.06 ± 3.95	3	10

Table 4.2 Percent time spent immobile in the forced swim test, percent sucrose preference, and frequency of resilient versus susceptible mice in the tail suspension test. No significant group differences were observed. Data show as mean \pm standard error of the mean. CUS, chronic unpredictable stress; Sham, sham-operated; OVX, ovariectomized; 17 β -E2, estradiol; DPN, diarylpropionitrile; PPT, propylpyrazole-triol; FST, forced swim test; NSF, novelty suppressed feeding; TST, tail suspension test.

E2 treatment reduced CORT response to acute restraint stress

In the HPA axis stress reactivity test, E2 treatment reduced post-restraint stress CORT concentrations relative to all other treatment groups (p 's <0.025, Cohen's d = 0.6-1.8; main effect of treatment: $F(4, 49)=3.96$, $p=0.007$, $\eta^2_p = 0.24$; Figure 4.3A). There was no significant main effect of CUS condition nor a treatment by CUS condition interaction (p 's >0.4).

CUS enhanced the dexamethasone suppression of CORT in most treatment groups

In the DEX suppression test, CUS exposure significantly reduced post-restraint stress CORT concentrations in Sham ($p=0.0015$, Cohen's d = 1.8), OVX ($p=0.00057$, Cohen's d = 2.0), and DPN ($p=0.011$, Cohen's d = 2.5) groups, with a trend in PPT ($p=0.06$), but not in E2 ($p=0.9$; Figure 4.3B; treatment by CUS condition interaction: $F(4, 51)=3.42$, $p=0.015$, $\eta^2_p = 0.21$). Further, E2 treated mice had significantly lower CORT concentrations relative to all treatment groups in the no-CUS condition ($p<0.01$; Figure 4.3B) except PPT ($p=0.15$). There were also significant main effects of treatment and stress condition (p 's <0.001).

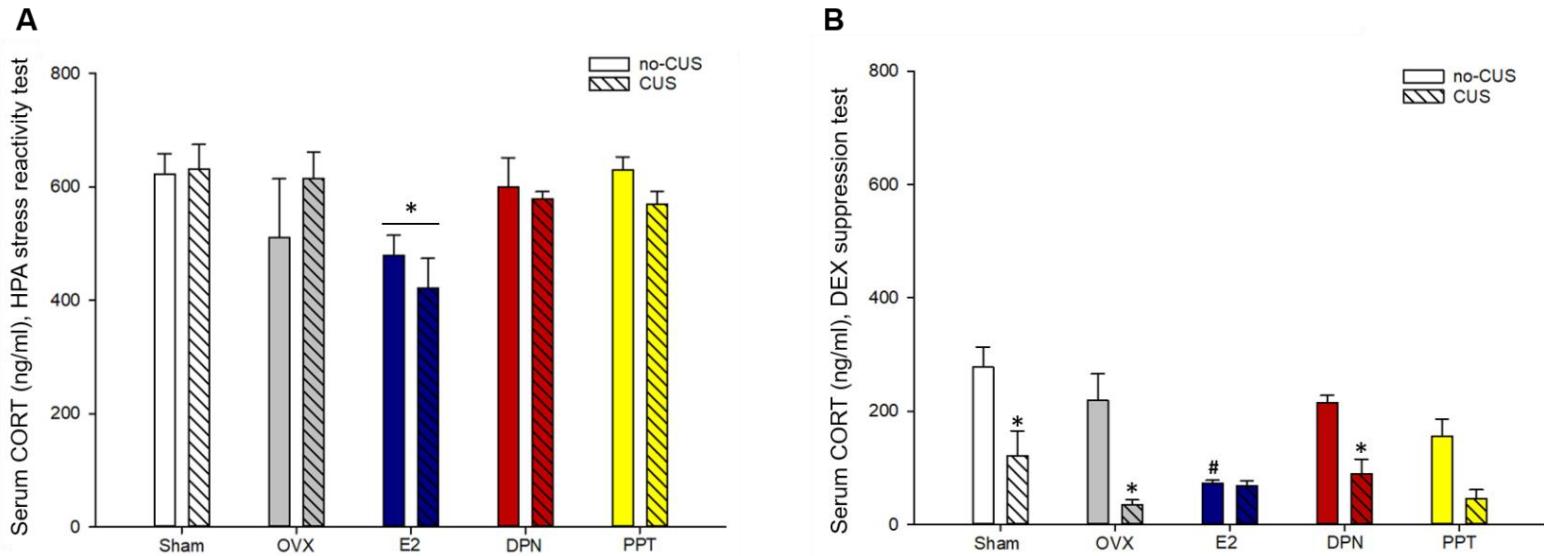


Figure 4.3 Serum corticosterone concentrations in the hypothalamic-pituitary-adrenal (HPA) axis stress reactivity test (A) and dexamethasone (DEX) suppression test (B). (A) E2 treatment reduced serum corticosterone concentrations immediately following an acute restraint stressor; * indicates $p < 0.025$ relative to all other treatment groups. (B) CUS exposure significantly enhanced dexamethasone suppression of corticosterone in Sham, OVX and DPN groups, and a similar but non-significant trend was detected with PPT treatment (* indicates $p < 0.02$ relative to no-CUS counterparts). Further, under no-CUS conditions E2 treatment reduced corticosterone concentrations (# indicates $p < 0.01$ relative to Sham, OVX, and DPN treated no-CUS groups). CUS, chronic unpredictable stress; OVX, ovariectomized; 17 β -E2, estradiol; DPN, diarylpropionitrile; PPT, propylpyrazole-triol.

Data in means + standard error of the mean.

CUS exposure reduced the proportion of BrdU-ir cells that expressed NeuN in ventral dentate gyrus

In the ventral dentate gyrus only, CUS exposure reduced the proportion of BrdU/NeuN co-labeled cells ($p = 0.04$, Cohen's $d = 0.4$; region by CUS condition interaction: $F(1, 53) = 5.73$, $p = 0.02$; Table 4.3). Further, the proportion of BrdU/NeuN co-labeled cells was overall higher in the dorsal, compared to the ventral, dentate gyrus (main effect of region: $F(1, 53) = 14.508$,

$p=0.0004$. $\eta^2_p = 0.2$). There was also a trend for a region by treatment interaction ($p=0.08$), where, intriguingly, E2 increased the proportion of dorsal BrdU-ir cells that also expressed NeuN, suggesting a promotion of cell fate toward a neuronal phenotype with chronic E2, regardless of stress condition (compared to OVX and Sham $p < 0.008$; a priori comparisons). There were no other significant main effects or interactions (p 's > 0.2).

Group	Dorsal ratio	Ventral ratio
no-CUS		
Sham	0.60 ± 0.079	0.70 ± 0.028
OVX	0.73 ± 0.067	0.65 ± 0.052
E2	$0.81 \pm 0.028^{\$}$	0.73 ± 0.031
DPN	0.73 ± 0.052	0.72 ± 0.031
PPT	0.70 ± 0.046	0.64 ± 0.067
CUS		
Sham	0.69 ± 0.042	0.61 ± 0.094
OVX	0.67 ± 0.052	0.62 ± 0.084
E2	$0.83 \pm 0.017^{\$}$	0.56 ± 0.062
DPN	0.79 ± 0.023	0.64 ± 0.064
PPT	0.77 ± 0.055	0.70 ± 0.014

Table 4.3 Proportion of BrdU/NeuN co-labeled cells in dorsal and ventral dentate gyrus. * indicates CUS exposure significantly reduced the proportion of BrdU/NeuN co-labeled cells only in the ventral dentate gyrus, $p = 0.04$. \\$ indicates E2 increased the proportion of BrdU/NeuN co-labeled cells only in the dorsal dentate gyrus, but regardless of CUS condition, $p < 0.008$, compared to OVX and Sham groups. BrdU: bromodeoxyuridine CUS, chronic unpredictable stress; Sham, sham-operated; OVX, ovariectomized; 17 β -E2, estradiol; DPN, diarylpropionitrile; PPT, propylpyrazole-triol.

CUS reduced neurogenesis in the ventral dentate gyrus and E2 increased neurogenesis in the dorsal dentate gyrus

Irrespective of CUS exposure, E2 significantly increased neurogenesis index in the dorsal GCL only, relative to all other treatments (all p's <0.0003, Cohen's $d = 0.7\text{-}1.2$; region by treatment interaction: $F(4, 53)=3.37$, $p=0.016$, $\eta^2_p = 0.2$; Figure 4.4A). CUS exposure reduced neurogenesis index in the ventral dentate gyrus ($p=0.05$, Cohen's $d = 0.5$) but not in the dorsal dentate gyrus ($p=0.28$; region by CUS condition interaction: $F(1, 53)=7.48$, $p=0.008$, $\eta^2_p = 0.12$; Figure 4.4B). There were also significant main effects of treatment and region (p's <0.04) but no other main effects or interactions (p's >0.5).

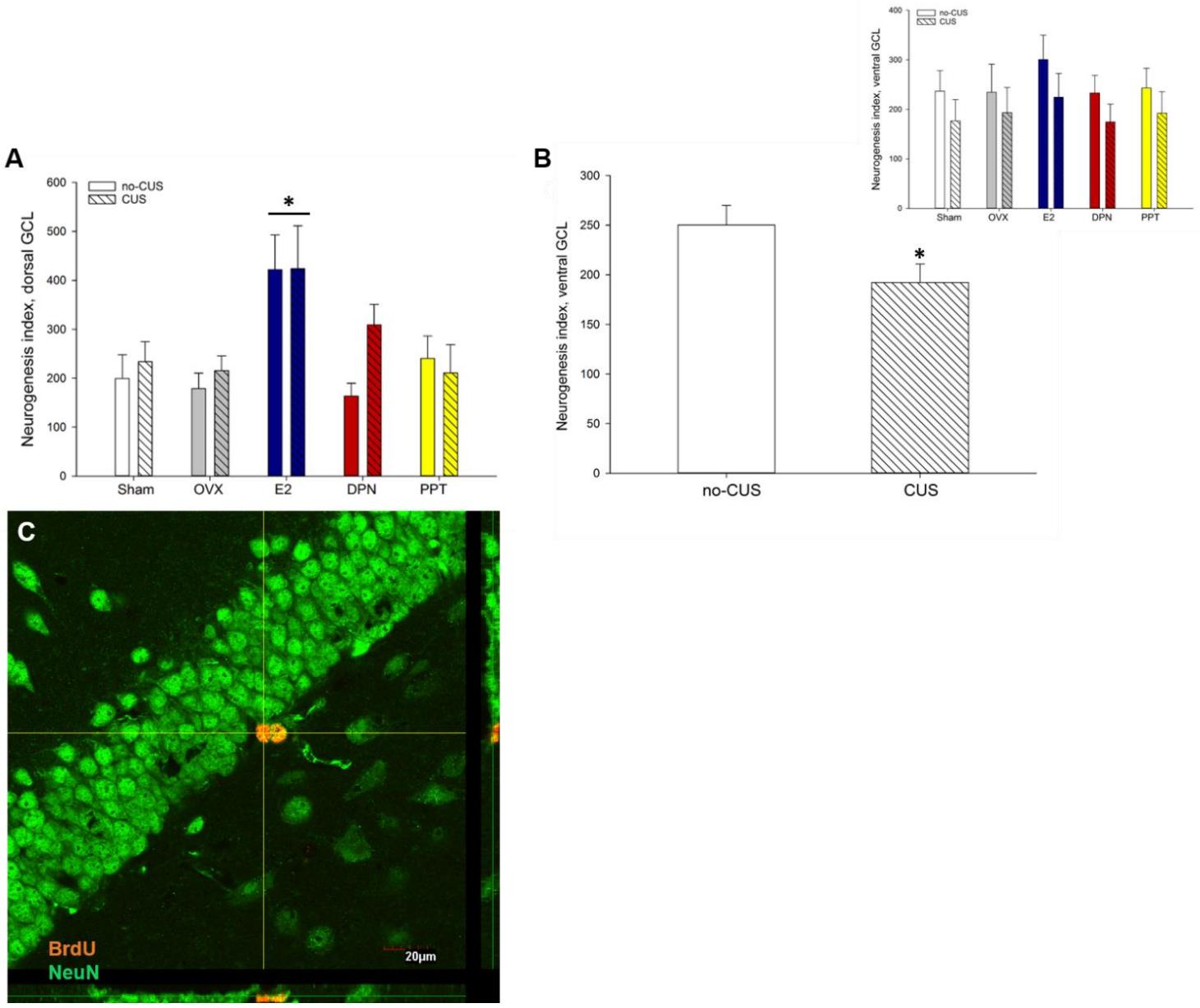


Figure 4.4 Neurogenesis index in the dorsal (A) and ventral (B) dentate gyrus. (A) Estradiol treatment significantly increased neurogenesis in the dorsal granule cell layer (GCL); * indicates $p < 0.0003$ relative to all other treatment groups. (B) CUS exposure significantly reduced neurogenesis in the ventral GCL; * indicates $p = 0.05$ relative to no-CUS. Inset graph depicts the data by treatment and CUS condition. (C) Representative photomicrographs of the granule cell layer (GCL) of the dentate gyrus, obtained at 60x magnification showing two BrdU-ir cells (orange) co-expressing NeuN (green). GCL, granule cell layer
BrdU, bromodeoxyuridine; CUS, chronic unpredictable stress; OVX, ovariectomized; 17 β -E2, estradiol; DPN, diarylpropionitrile; PPT, propylpyrazole-triol. Data in means + standard error of the mean.

CUS exposure decreased PSD-95 expression in the hippocampus and frontal cortex, and this effect was observed primarily in DPN- and PPT-treated groups

CUS exposure significantly decreased PSD-95 expression in the hippocampus and frontal cortex (main effect of CUS condition: $F(1, 45)=7.89$, $p=0.007$, $\eta^2_p = 0.15$; Figure 4.5A). The effects of CUS to decrease PSD-95 were largely driven by PPT and DPN groups (p 's <0.05 , relative to non-CUS counterparts; CUS condition by treatment interaction: $F(4, 45)=2.67$, $p=0.044$, $\eta^2_p = 0.19$). Further, PSD-95 expression was overall significantly higher in the hippocampus than the frontal cortex (main effect of region: $F(1, 45)=98.18$, $p<0.001$ $\eta^2_p=0.69$; Figure 4.5B). There were no other main effects or interactions (all p 's >0.1).

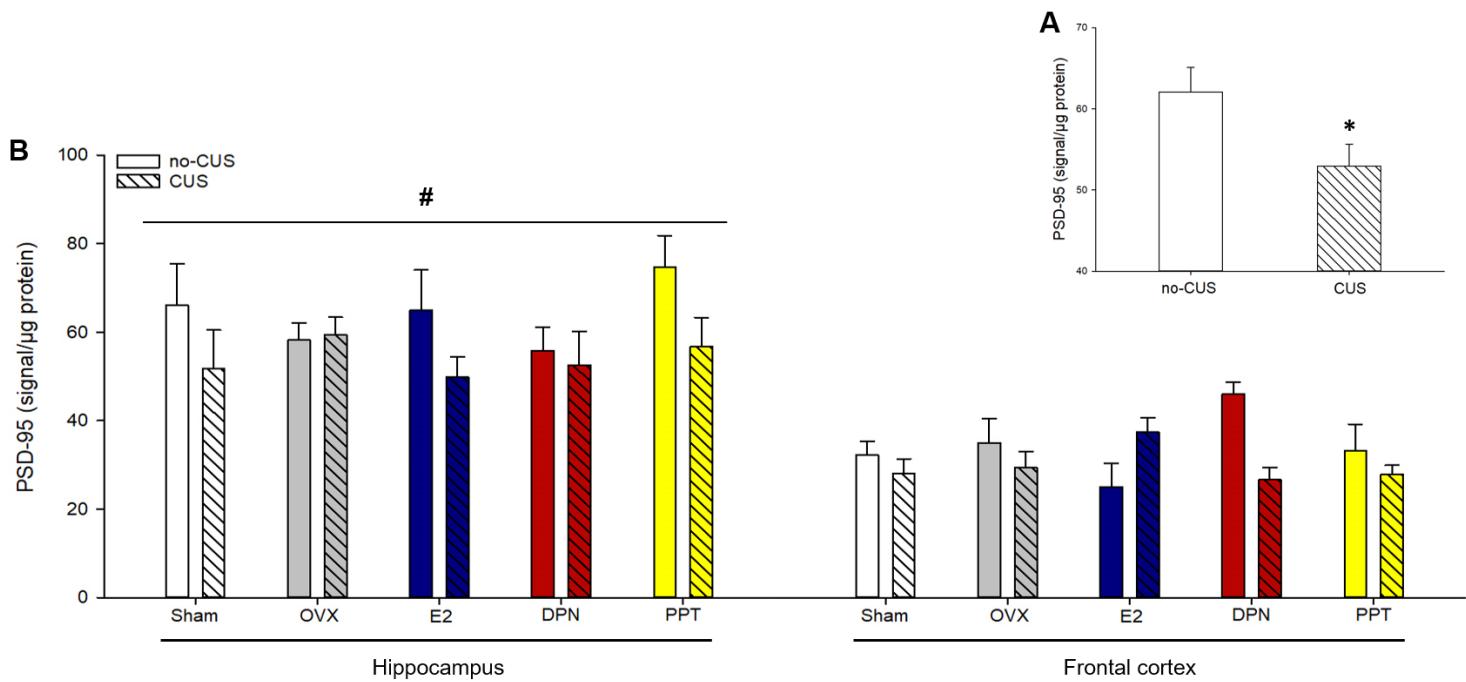


Figure 4.5 Postsynaptic density protein 95 expression in the hippocampus and frontal cortex, normalized by total protein concentrations. (A) CUS exposure decreased PSD-95 expression in both regions; * indicate $P=0.007$, main effect of CUS condition. (B) PSD-95 expression was significantly higher in the hippocampus in comparison with the frontal cortex. # indicates $p < 0.001$, main effect of region. PSD-95, postsynaptic density-95; CUS, chronic unpredictable stress; OVX, ovariectomized; E2, 17 β -estradiol; DPN, diarylpropionitrile; PPT, propylpyrazole-triol. Data in means + standard error of the mean.

Cytokine PCA results

CUS increased PC1 scores in the hippocampus, an effect driven primarily by Sham-operated and DPN-treated groups, and Sham-operated mice had lower PC2 scores

The model generated two principal components, accounting for 57.5% of the variance within the dataset, with variance explained by principal component 1 (PC1) = 39.7%, and PC2 = 17.8%.

Factor loadings are shown in Table 4.4. ANOVA results reveal that CUS exposure increased PC1 scores (main effects of CUS condition: $F(1, 53)=6.2920$, $p=0.015$, $\eta^2_p=0.11$; Figure 4.6A), a significant covariate effect of DEX to reduce scores ($p=0.002$), but no significant main effect of treatment ($p=0.45$). The effect of CUS exposure to increase PC1 scores appears to be driven by Sham and DPN groups ($p=0.027$ and 0.017 , relative to no-CUS counterparts; *a priori* comparisons, CUS condition by treatment interaction: $F(4, 53)=1.63$, $p=0.18$). PC2 scores were lower in Sham relative to all other treatment groups (p 's <0.02; main effect of treatment: $F(4, 52)=3.39$, $p=0.016$, $\eta^2_p=0.21$; Figure 4.6B), but this missed significance in comparison to PPT treatment ($p=0.08$). There was also trend toward significance for CUS to reduce PC2 scores ($p=0.07$), but no significant CUS condition by treatment interaction ($F(4, 52)=1.3093$, $p=0.28$).

CUS increased PC1 scores in the frontal cortex, an effect driven primarily by PPT treatment

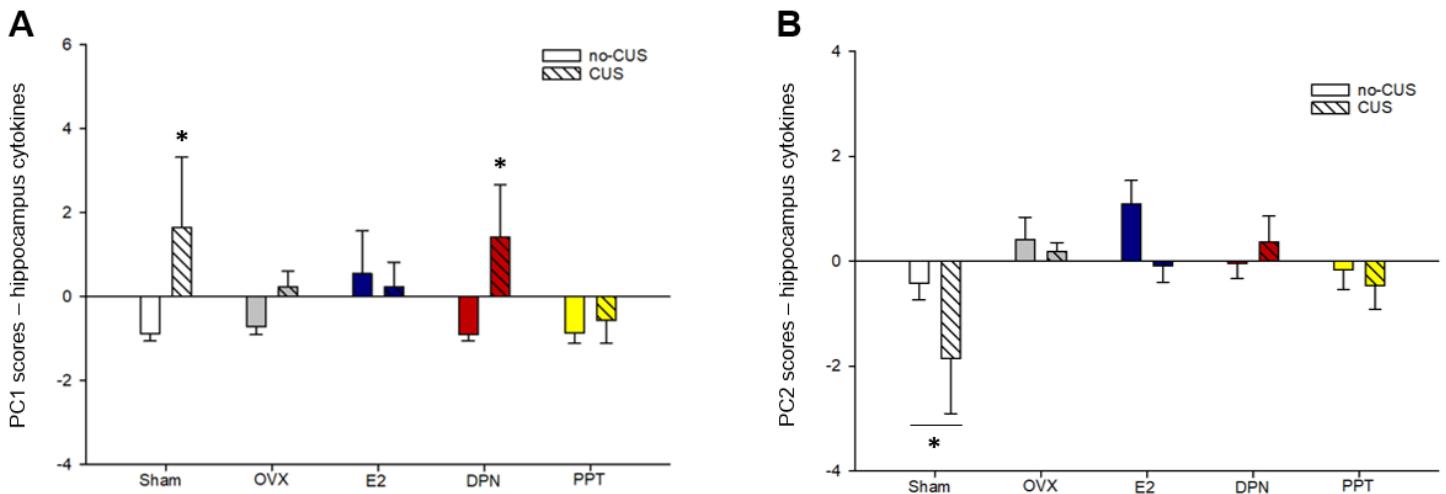
The model generated two principal components, accounting for 71.4% of the variance within the dataset, with variance explained by PC1 = 53.2% and PC2 = 18.2%. Factor loadings are shown in Table 4.4. ANOVA results show that CUS exposure significantly increased PC1 scores (main effect of CUS condition: $F(1, 45)=13.43$, $p=0.00065$, $\eta^2_p=0.23$; Figure 4.6C). This effect appears to be driven by PPT-treated mice ($p=0.003$; relative to no-CUS counterparts) with a trend toward

significance in OVX ($p=0.068$; *a priori* comparisons; treatment by CUS condition interaction: $F(4, 45)=1.12$, $p=0.36$). There was also a significant covariate effect of DEX ($p=0.024$), but no significant main effect of treatment ($p=0.14$). PC2 scores were not significantly affected by treatment, CUS condition, nor their interaction (p 's >0.1 ; Figure 4.6D), but there was a significant covariate effect of DEX ($p=0.009$).

Variable	Hippocampus		Frontal cortex	
	PC1	PC2	PC1	PC2
GM-CSF	0.52	-0.18	0.20	0.81
IFN-γ	0.71	0.38	0.78	0.20
IL-10	0.90	-0.06	0.89	0.15
IL-1 β	0.87	-0.30	0.90	-0.07
IL-2	0.59	-0.63	0.80	0.14
IL-4	0.64	0.30	0.73	0.15
IL-6	0.41	0.56	0.91	-0.05
CXCL1	0.11	0.64	-0.22	0.68
TNF-α	0.82	-0.12	0.94	-0.13
VEGF	0.15	0.52	-0.37	0.76

Table 4.4 Principle component analyses loading table. High loadings (> 0.4) indicated in bold text. CUS, chronic unpredictable stress; Sham, sham-operated; OVX, ovariectomized; E2, 17β -estradiol; DPN, diarylpropionitrile; PPT, propylpyrazole-triol; IL, Interleukin; IFN- γ , Interferon-gamma; TNF- α , tumor necrosis factor- α ; CXCL1, chemokine (C-X-C motif) ligand 1; GM-CSF, Granulocyte-macrophage colony-stimulating factor; VEGF, vascular endothelial growth factor

Hippocampus



Frontal cortex

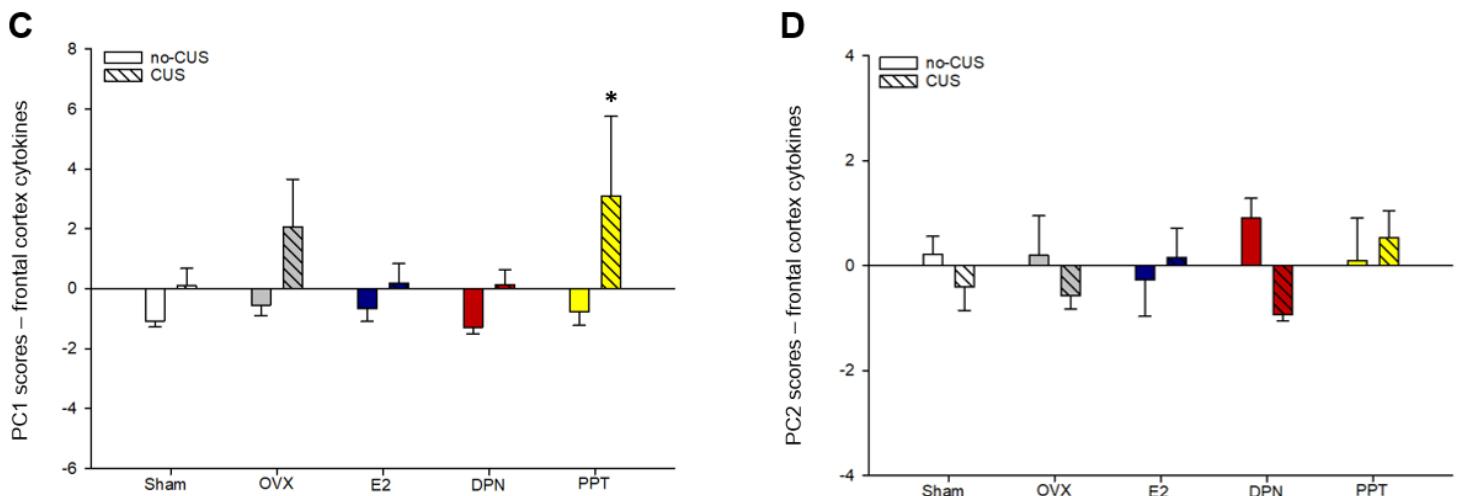


Figure 4.6 Principal component scores in hippocampus (A-B) and frontal cortex (C-D) analyses of cytokine data. (A) In the hippocampus, CUS exposure significantly increased principal component (PC) 1 scores, and this effect was driven by Sham and DPN groups only; * indicate $p < 0.03$, relative to no-CUS counterparts. (B) Sham-operated groups had significantly lower PC2 scores in the hippocampus; * indicates $p < 0.02$, in comparison with all treatments except PPT ($p = 0.08$). (C) In the frontal cortex, CUS exposure significantly increased PC1 scores, and this effect was driven by PPT treatment; * indicates $p = 0.003$ relative to no-CUS counterparts. (D) There were no significant group differences in frontal cortex PC2 scores. PC, principal component; CUS, chronic unpredictable stress; OVX, ovariectomized; E2, 17 β -estradiol; DPN, diarylpropionitrile; PPT, propylpyrazole-triol. Data in means + standard error of the mean.

Individual cytokine results: CUS increased several cytokines in the hippocampus and frontal cortex, but the effects of estrogens were limited to VGEF and CXCL1 in the hippocampus

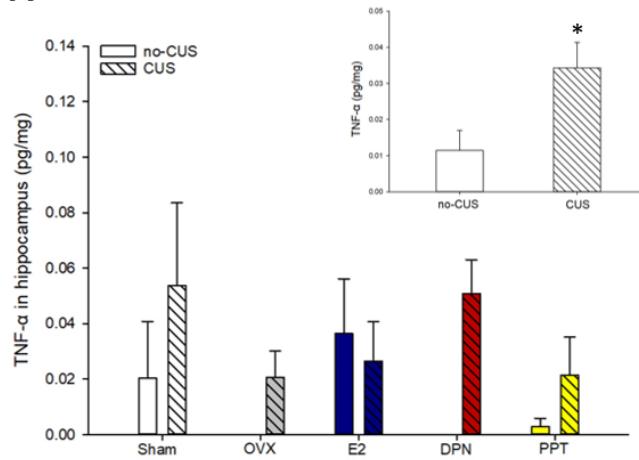
In the hippocampus, CUS exposure significantly increased TNF- α , IL-1 β , and IL-10 concentrations (main effects of CUS: $F(1, 53)=6.84$, $p=0.012$, $\eta^2_p=0.11$; $F(1, 51)=12.87$, $p=0.0007$, $\eta^2_p=0.20$; and $F(1, 53)=7.38$, $p=0.0009$, $\eta^2_p=0.12$, respectively; Figure 4.7A-C), but decreased CXCL1 concentrations ($F(1, 52)=4.01$, $p=0.05$; Figure 4.7D). However, IL-2, IL-4, IL-6, IFN- γ , VGEF, and GM-CSF were not significantly affected by CUS exposure (p 's > 0.16). Estrogens only significantly influenced two proteins: VEGF and CXCL1. Specifically, the ER β agonist, DPN, increased hippocampal VEGF concentration relative to Sham and PPT groups (p 's < 0.03; main effect of treatment: $F(4, 53)=3.55$, $p=0.012$, $\eta^2_p=0.21$; Figure 4.7E), and E2 significantly increased hippocampal CXCL1 concentrations relative to Sham mice ($p = 0.0045$; main effect of treatment: $F(4, 52)=3.57$, $p=0.012$, $\eta^2_p=0.22$; Figure 4.7D). There were no other significant main or interaction effects for any other cytokine in the hippocampus (p 's > 0.083; Table 4.5). Finally, there was a significant covariate effect of DEX to reduce IL-1 β , IL-6, IL-10, and TNF- α concentrations (p 's < 0.05) and trends toward significance to reduce IL-2 ($p=0.051$) and GM-CSF ($p=0.07$) concentrations. DEX was not a significant covariate for concentrations of IFN- γ , VGEF, CXCL1, IL-4 (p 's > 0.14).

In the frontal cortex, CUS exposure significantly increased TNF- α , IL-10, IL-1 β , IL-2, IL-4, and IL-6 concentrations (main effects of CUS condition: $F(1, 48)=10.59$, $p=0.0021$, $\eta^2_p=0.18$; $F(1, 46)=4.00$, $p=0.05$, $\eta^2_p=0.08$; $F(1, 42)=15.63$, $p=0.0003$, $\eta^2_p=0.27$; $F(1, 44)=7.47$, $p=0.009$, $\eta^2_p=0.15$; $F(1, 46)=4.79$, $p=0.034$, $\eta^2_p=0.09$; and $F(1, 44)=9.57$, $p=0.0034$, $\eta^2_p=0.18$,

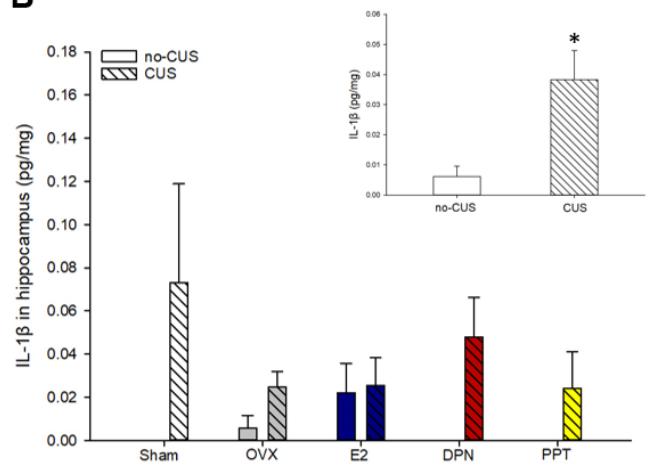
respectively; Figure 4.7F-K). However, GM-CSF, IFN- γ , VEGF, and CXCL1 concentrations were not significantly affected by CUS exposure ($p's > 0.09$; Table 4.5). Estrogens did not significantly influence any cytokine in the frontal cortex (all $p's > 0.13$), nor were there any significant CUS condition by treatment interactions for any cytokine ($p's > 0.055$). Finally, there was a significant covariate effect of DEX to reduce IL-1 β , IL-6, and VGEF in the frontal cortex ($p' < 0.02$), and a trend toward significance for reduced TNF- α ($p = 0.065$), but not for any other cytokine examined ($p's > 0.13$).

Hippocampus

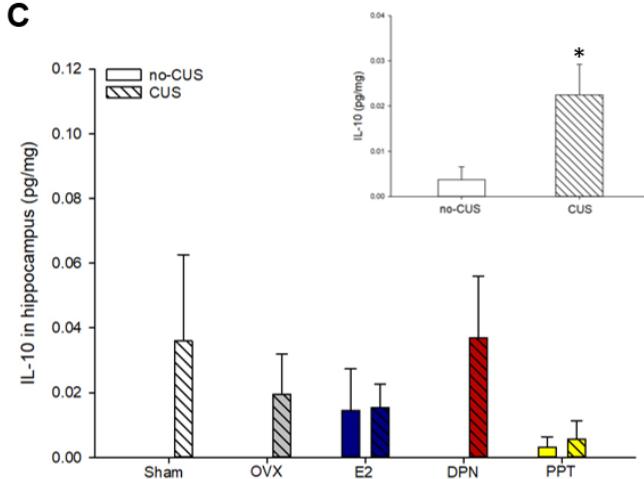
A



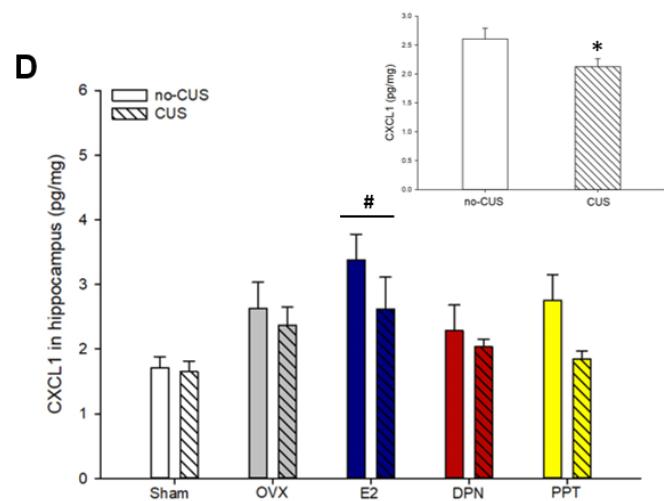
B



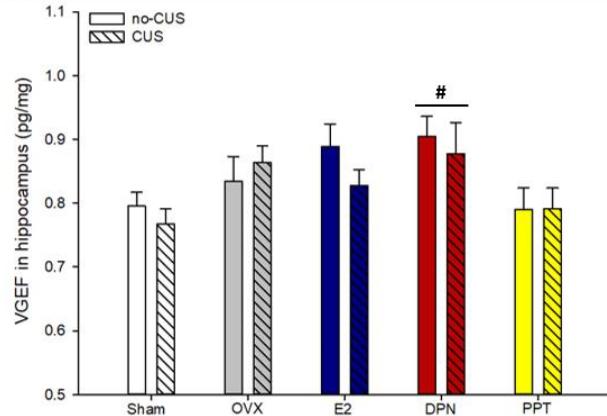
C



D



E



Frontal Cortex

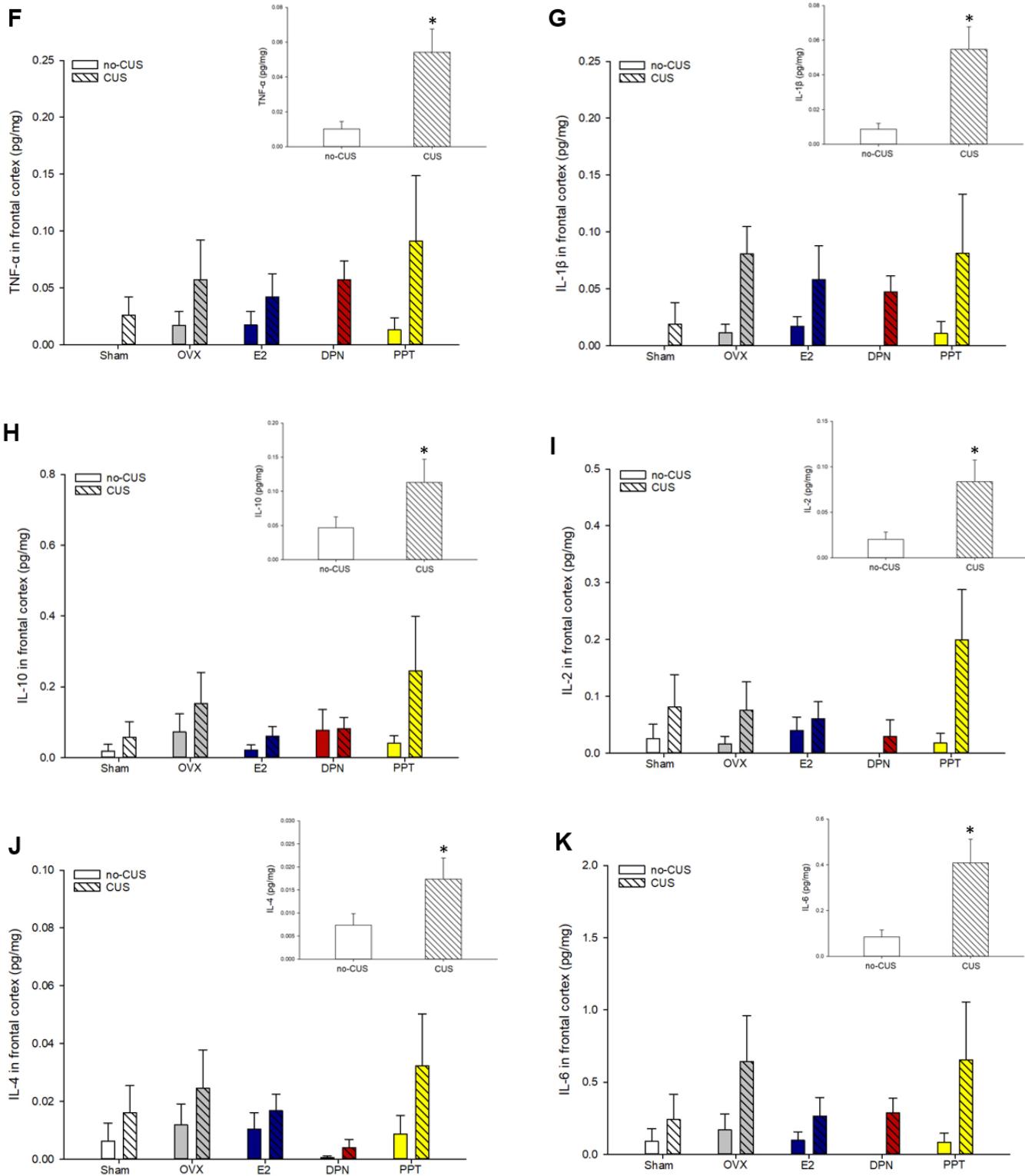


Figure 4.7 Protein concentrations of immune mediators in the hippocampus (A-E) and frontal cortex (F-K). CUS exposure increased TNF- α (A), IL-1 β (B), and IL-10 (C) concentrations in the hippocampus; * indicates $p < 0.015$, main effect of CUS condition. (D) CUS exposure decreased and E2 treatment increased CXCL1 concentrations in the hippocampus; * indicates $p=0.05$, main effect of CUS exposure, and # indicates $p=0.0045$ relative to Sham. (E) DPN treatment significantly increased VEGF concentrations in the hippocampus; * indicates $p < 0.03$, relative to Sham and PPT groups. CUS exposure increased TNF- α (F), IL-1 β (G), IL-10 (H), IL-2 (I), IL-4 (J), and IL-6 (K) concentrations in the frontal cortex* indicates $p \leq 0.05$, main effect of CUS condition. IL, Interleukin; TNF- α , tumor necrosis factor- α ; CXCL1, chemokine (C-X-C motif) ligand 1; VEGF, vascular endothelial growth factor; CUS, chronic unpredictable stress; OVX, ovariectomized; E2, 17 β -estradiol; DPN, diarylpropionitrile; PPT, propylpyrazole-triol. Data in means + standard error of the mean.

Hippocampus						Frontal cortex				
Group	GM-CSF	IFN- γ	IL-2	IL-4	IL-6	GM-CSF	IFN- γ	CXCL1	VEGF	
no-CUS										
Sham	0.014 ± 0.0017	ND	ND	0.0029 ± 0.0029	0.17 ± 0.14	0.022 ± 0.005	ND	1.37 ± 0.10	1.02 ± 0.05	
OVX	0.015 ± 0.0011	ND	ND	0.0066 ± 0.0047	0.29 ± 0.18	0.023 ± 0.006	ND	1.99 ± 0.64	0.89 ± 0.10	
E2	0.014 ± 0.0008	0.0008 ± 0.0008	0.0095 ± 0.0095	0.0123 ± 0.0047	0.28 ± 0.10	0.016 ± 0.005	0.0002 ± 0.0002	2.18 ± 0.39	0.82 ± 0.15	
DPN	0.016 ± 0.0012	ND	0.0176 ± 0.0176	ND	0.06 ± 0.03	0.030 ± 0.006	ND	2.34 ± 0.67	1.12 ± 0.03	
PPT	0.013 ± 0.0011	ND	0.0265 ± 0.0193	0.0036 ± 0.0025	0.12 ± 0.06	0.019 ± 0.004	ND	2.33 ± 0.62	0.87 ± 0.13	
CUS										
Sham	0.017 ± 0.0024	ND	0.0970 ± 0.0706	0.0112 ± 0.0051	0.19 ± 0.14	0.020 ± 0.004	0.0008 ± 0.0008	1.09 ± 0.09	0.82 ± 0.05	
OVX	0.014 ± 0.0015	ND	0.0068 ± 0.0068	0.0103 ± 0.0048	0.50 ± 0.23	0.021 ± 0.004	0.0002 ± 0.0002	1.47 ± 0.28	0.94 ± 0.10	
E2	0.015 ± 0.0025	ND	0.0345 ± 0.0227	0.0097 ± 0.0055	0.25 ± 0.13	0.017 ± 0.003	0.0002 ± 0.0002	2.46 ± 0.79	0.95 ± 0.08	
DPN	0.014 ± 0.0022	0.0012 ± 0.0008	0.0114 ± 0.0114	0.0127 ± 0.0089	0.34 ± 0.10	0.015 ± 0.002	ND	1.15 ± 0.13	0.81 ± 0.04	
PPT	0.013 ± 0.0024	0.0002 ± 0.0002	ND	0.0038 ± 0.0038	0.13 ± 0.13	0.027 ± 0.006	0.0044 ± 0.0037	1.30 ± 0.08	0.87 ± 0.07	

Table 4.5 Protein concentrations of immune mediators in the hippocampus and frontal cortex, normalized to total protein concentrations (pg/mg). No significant group differences were observed. Data in mean ± standard error of the mean. ND, non-detectable; CUS, chronic unpredictable stress; Sham, sham-operated; OVX, ovariectomized; E2, 17 β -estradiol; DPN, diarylpropionitrile; PPT, propylpyrazole-triol; IL, Interleukin; IFN- γ , Interferon-gamma; CXCL1, chemokine (C-X-C motif) ligand 1; GM-CSF, Granulocyte-macrophage colony-stimulating factor; VEGF, vascular endothelial growth factor.

DPN treatment increased Iba-1 optical density in the dorsal GCL

Irrespective of CUS condition, DPN treatment increased Iba-1 optical density in the dorsal GCL in comparison to OVX and E2-treated mice (p 's <0.05; main effect of treatment: $F(4, 53)=2.98$, $p=0.027$, $\eta^2_p=0.18$; Figure 4.8A). There were no group differences in Iba-1 optical density in the ventral GCL, IL-mPFC, or PL-mPFC (all p 's >0.2; no significant main effects or interactions; Table 4.6).

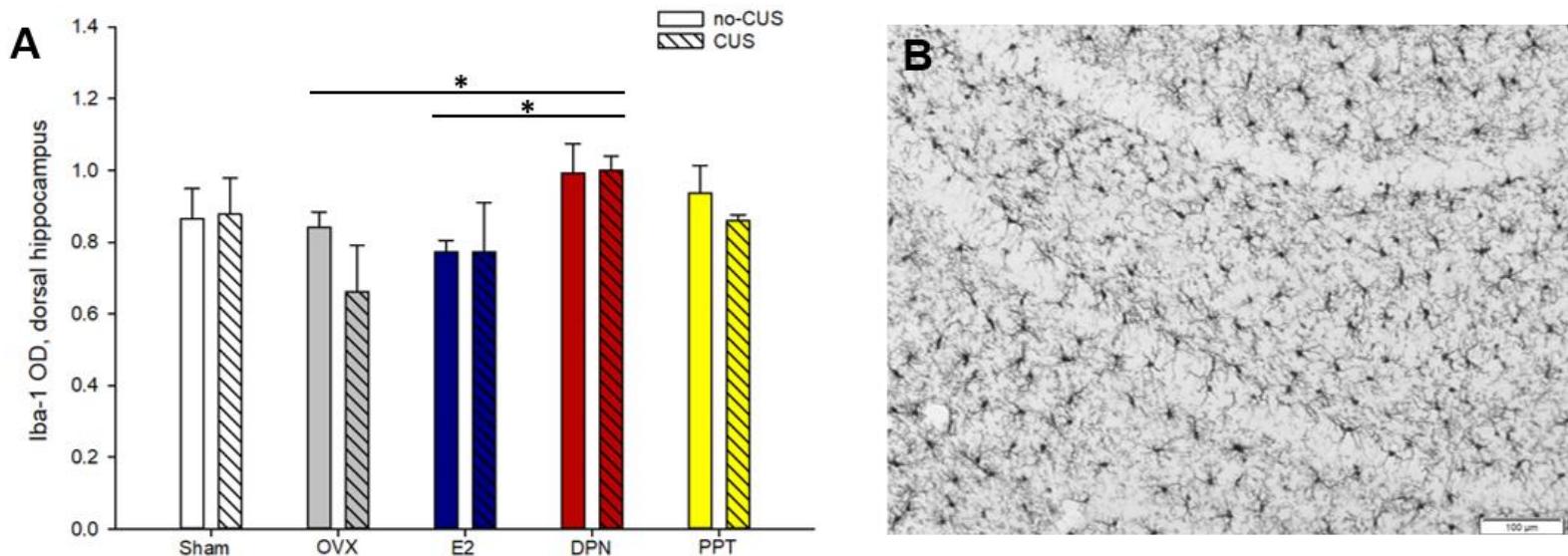


Figure 4.8 Iba-1 optical density in the dorsal dentate gyrus. (A) DPN treatment increased Iba-1 optical density in the granule cell layer of the dorsal dentate gyrus, irrespective of CUS exposure; * indicates p 's <0.05, significantly higher than OVX and E2 groups. (B) representative photomicrograph of the dentate gyrus obtained at 10x magnification showing Iba-1-immunoreactive cells. OD, optical density; CUS, chronic unpredictable stress; OVX, ovariectomized; E2, 17 β -estradiol; DPN, diarylpropionitrile; PPT, propylpyrazole-triol. Data in means + standard error of the mean.

Group	vGCL	PL vmPFC	IL vmPFC
no-CUS			
Sham	0.49 ± 0.02	0.74 ± 0.04	0.75 ± 0.05
OVX	0.45 ± 0.02	0.71 ± 0.03	0.74 ± 0.04
E2	0.51 ± 0.03	0.74 ± 0.02	0.74 ± 0.02
DPN	0.47 ± 0.05	0.75 ± 0.03	0.80 ± 0.02
PPT	0.45 ± 0.04	0.71 ± 0.04	0.73 ± 0.05
CUS			
Sham	0.51 ± 0.02	0.77 ± 0.02	0.78 ± 0.02
OVX	0.48 ± 0.02	0.72 ± 0.03	0.69 ± 0.03
E2	0.47 ± 0.03	0.74 ± 0.03	0.77 ± 0.06
DPN	0.53 ± 0.02	0.73 ± 0.04	0.76 ± 0.03
PPT	0.43 ± 0.01	0.81 ± 0.06	0.81 ± 0.06

Table 4.6 Iba-1 optical density in the ventral dentate gyrus and ventromedial prefrontal cortex. No significant group differences were observed. Data in mean ± standard error of the mean. CUS, chronic unpredictable stress; Sham, sham-operated; OVX, ovariectomized; E2, 17 β -estradiol; DPN, diarylpropionitrile; PPT, propylpyrazole-triol; vGCL, ventral granule cell layer; vmPFC, ventromedial prefrontal cortex; PL, prelimbic; IL, infralimbic

4.4 Discussion

This study employed a pharmacological approach to investigate the role of the classical estrogen receptors, ER α and ER β , in the behavioural, neuroplastic and neuroinflammatory consequences of chronic stress exposure in female mice. CUS exposure increased immobility in the TST, increased concentrations of neuroinflammatory mediators, reduced PSD-95 expression, and reduced neurogenesis in the ventral hippocampus. The effects of ovarian status and estrogenic treatments were overall less robust than those of CUS. We do, however, observe interesting effects of estrogenic treatments that in some instances depended on CUS exposure. We found

that irrespective of CUS exposure, ovarian hormone deprivation increased passive-coping behaviour in the TST in comparison with sham-operated mice. Intriguingly, E2, DPN, and PPT treatments rescued this depressive-like outcome of ovariectomy under non-CUS conditions, but the effect of CUS exposure to increase depressive-like behaviour was primarily driven by the same treatments. On the other hand, ovarian hormone deprivation produced an anxiolytic effect in the novelty suppressed feeding test regardless of CUS exposure, and this was prevented by 17 β -estradiol, but not DPN or PPT treatment. Further, the overall effects of CUS to alter the cytokine milieu and to reduce PSD-95 expression in the hippocampus and frontal cortex appear to be driven, at least in part, by DPN and PPT treatments, respectively. Finally, 17 β -estradiol increased neurogenesis in the dorsal hippocampus. Combined, these findings shed light on the complexities of estrogen signaling in modulating behavioural, neuroplastic, and neuroinflammatory states under non-stress and chronic stress conditions. These findings further suggest that contrary to our expectations, chronic selective activation of ER α or β does not impart resilience in the face of CUS exposure. These findings could have implications for the development of selective estrogen-based therapies for stress-related disorders.

Ovarian status and estrogenic treatments produce distinct behavioural profiles depending on CUS condition and behavioural test

Irrespective of CUS condition, ovariectomy increased passive-coping behaviour in the TST, and under non-CUS conditions, this effect was prevented by E2, DPN, and PPT treatments. This finding is consistent with previous reports showing that ovarian hormone deprivation alone increases depressive-like behaviour in both rats and mice, at least in the short term (Bekku and Yoshimura, 2005; Li et al., 2014). Importantly, our findings implicate both ER α and β in

mediating the effects of estradiol to promote active-coping behaviour under non-stress conditions. This counters the findings of past studies that have administered ER agonists acutely to suggest a role of ER β , but not ER α , in depressive-like behaviour under non-stress conditions (Walf et al., 2004; Yang et al., 2014). Our current findings are important, as acute treatments with ER agonists provide only limited information regarding the role of ERs in affective behaviour. Interestingly, although CUS exposure did not significantly affect passive-coping behaviour in the TST in sham-operated and ovariectomized mice, we report that CUS significantly increased passive-coping behaviour in DPN- and PPT-treated mice, with trends in the same direction with E2 treatment. This is somewhat in line with our analysis of resilience versus susceptibility to a depressive-like phenotype in TST, in which CUS exposure resulted in a resilient phenotype in the Sham group but a susceptible phenotype in OVX and PPT-treated groups, while no bias was observed in E2- or DPN-treated groups. This suggests that chronic, selective activation of ER α or ER β , is protective under non-stress conditions but not under CUS exposure. This seemingly contradictory finding could perhaps be interpreted in light of the “healthy cell bias of estrogen action” hypothesis (Brinton, 2008), put forth to explain observations in which the actions of estrogens result in disparate outcomes in health versus pathology. That is, under “healthy”, non-stress conditions, 17 β -estradiol and ER agonists protect against ovariectomy-induced depressive-like behaviour, yet under “pathological” chronic stress conditions the same treatments were not protective. A “pathological” state under chronic stress conditions could be related to consequences of increased HPA axis activity, including increased concentrations of corticotropin-releasing hormone (CRH) and corticosterone. This finding is important as it underscores the need for using a model of depression when investigating estrogens’ regulation of affective function.

It is important to note that the effects observed in the TST were not consistent across the entire battery of behavioural tests used in this study. For example, we do not see significant group differences in the forced swim test or the sucrose preference test. It is plausible that testing order could affect behaviour on subsequent tests, an issue that can be addressed by counterbalancing testing order in future studies. Further, unlike the observed effect of ovariectomy to increase passive-coping behaviour in the TST, we found an anxiolytic effect of ovariectomy in the novelty suppressed feeding test. This was seen irrespective of CUS exposure, and was prevented by E2, but not DPN or PPT treatment. This indicates that targeting ER α or ER β alone was not sufficient to return anxiety-like behaviour to levels similar to that of intact mice. On the other hand, E2 treatment mimicked the phenotype of intact mice, indicating that concurrent activation of both receptor subtypes or possibly the G protein coupled estrogen receptor 1 (GPER) may be involved in this response. In line with our current findings, estradiol benzoate treatment in ovariectomized C57BL/6 or Swiss Webster mice, administered chronically via subcutaneous capsule, increased anxiety-like behaviour in comparison to vehicle treatment across several tests of anxiety-like behaviour (Morgan and Pfaff, 2001, 2002). However, more studies find anxiogenic effects of ovariectomy and anxiolytic effects of acute, sub-chronic, or chronic estradiol treatment in ovariectomized rats or mice (Walf et al., 2009b; Walf and Frye, 2010; Kastenberger et al., 2012). Further, reduced anxiety-like behaviour is observed in proestrus, during which endogenous 17 β -estradiol is elevated (Frye et al., 2000; Walf et al., 2009a). Importantly, few studies have investigated the effects of ovarian hormones in the novelty suppressed feeding test, which targets motivated behaviour in addition to anxiety-like behaviour. In middle aged rats, we previously found increased anxiety like-behaviour in NSF with long-term ovariectomy and chronic stress exposure (Mahmoud et al., 2016a). Another study in young

adult rats found no effect of ovariectomy or chronic 17 β -estradiol treatment (18 days) on behaviour in NSF (Gogos et al., 2018). It is therefore difficult to synthesize the existing literature, but it appears that length of ovarian hormone deprivation, 17 β -estradiol dose and treatment regimen, species, strain, age, and test of anxiety-like behaviour can all contribute to the differences in findings.

In this study we investigate the corticosterone response to an acute stressor with and without a dexamethasone challenge (discussed ahead), but we did not explore changes in baseline HPA activity across the experimental timeline. Thus, in order to clarify the complexities and inconsistencies in the literature surrounding the role of ovarian hormones in anxiety- and depressive-like behaviour, it would be important to consider changes CRH and corticosterone as a result of ovariectomy and estrogenic treatments under both non-stress and chronic stress conditions. These hormones are regulated by estrogens (Goel et al., 2014; Oyola and Handa, 2017) and are key effectors of the primary neuroendocrine stress response system with notable effects on anxiety- and depressive-like behaviours, and known perturbations in MDD (Binder and Nemeroff, 2010; Stetler and Miller, 2011).

Ovarian status and ER agonists influenced the neuroinflammatory consequences of CUS exposure in a brain region-specific manner

The neuroinflammatory effects of chronic stress exposure are well documented in males (Kubera et al., 2011; Kreisel et al., 2014). Here, we also observe robust neuroinflammatory effects of CUS exposure in the frontal cortex and hippocampus in young adult female mice. Notably CUS increased concentrations of IL-1 β , IL-6 and TNF- α . This mirrors meta-analyses indicating increased concentrations of the same cytokines in the blood and cerebral spinal fluid of

individuals with MDD (Dowlati et al., 2010; Liu et al., 2012; Haapakoski et al., 2015; Wang and Miller, 2018). We also demonstrate that ovarian status and selective ER activation can influence the neuroinflammatory response to stress in a brain region-dependent manner. Specifically, PCA analyses indicate that the neuroinflammatory effects of CUS were largely driven by PPT-treated groups in the frontal cortex, and by Sham-operated and DPN-treated groups in the hippocampus. This suggests that chronic ER α and ER β activation potentiated the neuroinflammatory consequences of stress exposure in the frontal cortex and hippocampus, respectively. This regional specificity also agrees with our observed effects of the ER β agonist DPN to increase Iba-1 optical density in the dorsal dentate gyrus. It is possible that the exaggerated neuroinflammatory response to CUS in DPN and PPT groups could contribute to the enhanced behavioural susceptibility to CUS in the tail suspension test, however the current experiment cannot confirm this link. Previous studies provide support for a role of ER α and ER β in the anti-inflammatory and neuroprotective actions of 17 β -estradiol (Lewis et al., 2008; Brown et al., 2010; Smith et al., 2011; Chakrabarti et al., 2014), however these conclusions are largely derived from studies using acute inflammatory challenges *in vivo* or from *in vitro* studies. Therefore, our current findings provide insight into the immunomodulatory actions of 17 β -estradiol in the context of chronic stress exposure. Although estrogens themselves possess immunomodulatory actions, future studies should also consider how estrogens may influence the neuroinflammatory milieu indirectly via the regulation of the HPA axis, especially under conditions of chronic stress. This is important as CRH can produce anti-inflammatory effects indirectly via the actions of glucocorticoids, but can also produce pro-inflammatory effects (Bellavance and Rivest, 2014; Bereshchenko et al., 2018; Elenkov et al., 1999). Therefore, considering well

known HPA-HPG interactions (Goel et al., 2014) may shed light on how differing backgrounds of ovarian hormones can influence the neuroinflammatory consequences of stress exposure.

17 β -estradiol increased neurogenesis in the dorsal hippocampus

Chronic 17 β -estradiol treatment increased the number of BrdU-ir cells in the dorsal dentate gyrus and increased the proportion of BrdU-ir cells that also expressed the mature neuronal marker (NeuN). This finding is interesting considering the functional heterogeneity along the dorsal-ventral axis of the hippocampus, in which the dorsal region is predominantly implicated in cognitive function (Fanselow and Dong, 2010; Strange et al., 2014). Few studies to date have examined the effects of chronic 17 β -estradiol treatment in ovariectomized females on the survival of new neurons in the hippocampus, but the available evidence indicates that the effects depend on the timing of 17 β -estradiol exposure in relation to that of cell proliferation. The survival of new neurons was found to be increased in cell populations that had proliferated after the initiation of estradiol treatment (McClure et al., 2013), but reduced in cell populations that proliferated prior to the initiation of estradiol treatment (Barker and Galea, 2008; Chan et al., 2014). Our current data corroborate this past literature, as 17 β -estradiol treatment here was initiated two weeks prior to BrdU administration. Therefore, our current findings provide further support for the notion that a 17 β -estradiol rich vs deficient environment during cell proliferation can largely influence the effects of chronic 17 β -estradiol treatment on the survival of new neurons. Further, as several studies report pro-proliferative effects of 17 β -estradiol that depend on dose and timing of treatment (Tanapat et al., 1999, 2005; Ormerod and Galea, 2001; Barha et al., 2009), the effect of E2 to increase BrdU-ir cells in the current study could be attributed to increased cell proliferation. To our knowledge, the effects ER α and β agonists on hippocampal

cell survival have not been investigated prior to this study. We found that unlike E2 treatment, DPN and PPT did not increase neurogenesis in the dorsal dentate gyrus, despite previous data showing that acute administration of DPN or PPT enhances cell proliferation, albeit at a different dose (Mazzucco et al., 2006). Therefore, promoting the survival of cells proliferating in an estradiol rich environment may require the concurrent engagement of ER α and β or alternatively GPER, which is achieved by 17 β -estradiol but not PPT or DPN. It is important to note that although NeuN remains to be the gold standard for identifying neuronal fate of adult born granule cells, NeuN immunoreactivity and antibody binding can differ between neurons in other brain regions and is influenced by a variety of factors including protein phosphorylation (Gusel'nikova and Korzhevskiy, 2015; Lind et al., 2005). Thus, the possibility exists that hormonal treatments in this study may have affected NeuN immunoreactivity and colocalization with BrdU without having any significant change in neuronal number. However, in the current study we did not detect any change in NeuN intensity between groups.

CUS exposure reduced markers of neuroplasticity (PSD-95 expression and neurogenesis)

CUS significantly reduced neurogenesis in the ventral, but not dorsal, hippocampus, and impacted cell fate by reducing the proportion of new cells becoming neurons. This is in line with the finding that neurogenesis in this region promotes stress resilience in males (Anacker et al., 2018) and with an overall role of the ventral hippocampus in stress regulation (Fanselow and Dong, 2010; Bagot et al., 2015; Padilla-Coreano et al., 2016). We also observe an overall effect of CUS to reduce PSD-95 in both the hippocampus and frontal cortex, pointing to a reduction in excitatory synapse number and/or stability. Importantly, this parallels findings of reduced expression of PSD-95, synapse loss, and reduced expression of synapse-related genes in the PFC

and hippocampus of individuals with MDD (Feyissa et al., 2009; Kang et al., 2012; Duric et al., 2013). Interestingly, CUS-induced reductions in PSD-95 expression were largely driven by DPN and PPT treated groups. This indicates that chronic DPN or PPT treatment in tandem with CUS exposure may result in remodeling of neuronal circuits in regions important for mood and stress regulation, and this could be linked to the observed behavioural alterations under CUS exposure in the tail suspension test. This effect may be driven by stress-induced alterations in the HPA axis, including possible elevations in basal levels of CRH and corticosterone, a possibility that should be explored in future studies. On the other hand, we found limited effects of estrogenic treatments on PSD-95 expression under non-CUS conditions. In contrast, previous studies report increased PSD-95 expression in the hippocampus of female rats after acute treatment with estradiol, PPT, and to a lesser extent DPN (Waters et al., 2009) and with 17 β -estradiol treatment in vitro (Akama and McEwen, 2003), therefore the differences between these findings and our current observations are likely due to treatment duration.

Chronic 17 β -estradiol treatment blunted the corticosterone response to an acute stressor and CUS exposure increased HPA axis negative feedback sensitivity

Chronic 17 β -estradiol treatment, regardless of CUS exposure, blunted the corticosterone response to an acute restraint stressor. This partially contrasts previous studies which in general have indicated that ovarian hormones, and 17 β -estradiol in particular, potentiate acute stress-induced activation of the HPA axis (Carey et al., 1995; Figueiredo et al., 2007; Serova et al., 2010). Importantly, most past studies have used shorter durations of estradiol replacement than in the current study, which likely contributes to the differences in findings. It is important to keep in mind that although the dose of 17 β -estradiol used here produces proestrus-like circulating

concentrations, the daily treatment regimen does not mimic natural estradiol cyclicity in the mouse, but rather is informative to potential effects of chronic estrogen-based therapies. Our current findings therefore extend the past literature to suggest that longer durations of 17 β -estradiol replacement may suppress the HPA axis response to stressors, which could represent a protective effect.

Studies in humans generally report a failure of dexamethasone to suppress cortisol release in a subpopulation of individuals with MDD (Ising et al., 2007; Stetler and Miller, 2011), pointing to impaired HPA axis negative feedback inhibition. In the current study, however, we observed enhanced dexamethasone suppression of corticosterone in mice exposed to CUS, pointing to increased sensitivity of negative feedback inhibition. This effect was present in all treatment groups except for E2, however this could be explained by the above-mentioned suppression of corticosterone in response to acute restraint stress in absence of dexamethasone. This unexpected enhancement of negative feedback inhibition with CUS could be an adaptive response to prevent prolonged exposure to glucocorticoids under chronic stress conditions. Interestingly, a similar phenotype is reported in clinical populations of trauma exposure and post-traumatic stress disorder (PTSD), in individuals with comorbid MDD and PTSD (Yehuda et al., 1993; Griffin et al., 2005), and in women with atypical MDD (Levitian et al., 2002), suggesting that this profile can be seen in certain stress-related psychiatric disorders, including MDD with atypical features, which is more prevalent in women than in men (Marcus et al., 2008).

4.5 Conclusions

In conclusion, we observe depressive-like, neuroinflammatory, and neuroplastic effects of CUS in young adult female mice that were largely independent of ovarian status and estrogenic treatments. However, our findings also point to complex effects of ovariectomy and 17 β -estradiol/ER agonist replacement that in some instances were dependent on chronic stress condition. Specifically, ovariectomy increased passive-coping behaviour in the tail suspension test regardless of CUS exposure, and this effect was rescued by 17 β -estradiol and ER agonists only under non-CUS conditions, suggesting that active-coping behaviour is promoted by ER α and ER β . Intriguingly, the effects of CUS exposure to increase passive-coping behaviour in the TST were largely driven by the same treatments. In agreement with the increased behavioural susceptibility to CUS exposure, the effects of CUS to modify the cytokine milieu and reduced PSD-95 expression appear to be driven in part by DPN and PPT. In addition, although ovariectomy increased passive-coping behaviour in the TST, it reduced anxiety-like behaviour in the NSF test regardless of CUS exposure. This effect was only prevented by 17 β -estradiol treatment suggesting that the concurrent activation of ER α , ER β and GPER produced an anxiogenic effect in this paradigm. We further observe opposing effects of estradiol and CUS exposure on neurogenesis along the dorsal-ventral axis of the hippocampus, such that 17 β -estradiol increased and CUS decreased neurogenesis in the dorsal and ventral regions, respectively. Finally, CUS exposure resulted in increased HPA axis negative feedback sensitivity in most groups, pointing to stress-induced adaptations in HPA axis function. Taken together, these data highlight that estrogenic regulation of behaviour, neuroplasticity, and neuroinflammation is complex and at times depends on chronic stress condition. Moreover, our data indicate that chronic selective activation of ER α or ER β is not protective under chronic

stress exposure, which could have implications for the development of estrogen-based therapies for stress-related disorders such as depression.

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Declarations of interest

None.

Chapter 5: General discussion

5.1 Summary of major findings

The experiments presented in this thesis aimed to clarify the role of ovarian hormones in influencing depressive-like phenotypes in stress-based rodent models. In addition to examining behavioural outcomes, the experiments explored neuroplastic and neuroimmune correlates as potential targets for the interaction between stress and ovarian hormones. The major findings of this thesis are that: 1) in middle-aged rats, long-term ovariectomy increased depressive- and anxiety-like behaviour and impaired HPA axis negative feedback function under CUS exposure. Further, fluoxetine showed little behavioural efficacy irrespective of ovarian status, but showed efficacy for neural and endocrine outcomes (Chapter 2: Mahmoud et al., 2016); 2) in middle-aged mice, long-term ovariectomy modestly increased depressive-like behaviour under non-stress conditions, and this was coupled with reduced pERK expression and cytokine concentrations in the frontal cortex, but increased cytokine concentrations in the hippocampus. Moreover, intact mice showed a greater behavioral susceptibility to the depressive-like effects of sub-chronic stress exposure, coupled with a stress-induced reduction in pERK expression in the frontal cortex, and an exaggerated neuroinflammatory response to stress in the frontal cortex and hippocampus (Chapter 3: Eid et al., 2020); and 3) in young adult mice, CUS increased depressive-like behaviour, reduced PSD-95 expression and increased cytokine concentrations in the hippocampus and frontal cortex, effects that were largely seen in groups treated with ER α and ER β agonists. Independent of CUS exposure, 17 β -estradiol increased neurogenesis in the dorsal hippocampus, blunted the corticosterone response to an acute stressor, but increased anxiety-like behaviour (Chapter 4: Eid et al., 2020).

Collectively, this work indicates that ovarian hormones are important determinants of depressive-like phenotypes. However, the data do not consistently support either one of the hypotheses that: 1) ovarian hormones increase risk or 2) ovarian hormones confer resilience, to depressive-like behaviour. There were notable methodological differences between the studies presented in each chapter (outlined in Table 5.1). In section 5.2, I discuss several factors that may contribute to variability in the behavioural effects of ovarian hormones, including stress background, the characteristics of the stress paradigm, the length of ovarian hormone deprivation, age, and species. Importantly, the experiments presented in this thesis provide important insights into the role of ovarian hormones in regulating depressive-like phenotypes using valid animal models of MDD, an approach that has been rarely taken by previous research yet is critical to our understanding of the role of ovarian hormones in depression. Further, the results of this thesis also provide novel information to suggest that ovarian hormones influence neuroimmune and neuroplastic measures under non-stress conditions and in response to stress exposure. These neuroplastic and neuroimmune effects may provide insight into the complex regulation of behaviour, as will be discussed in sections 5.3-5.4.

Chapter	Species	Age at ovariectomy	Age at stress exposure	Duration of stress exposure	Age at behavioural testing and tissue collection
2	Rat	5 months	9 months	6 weeks	~ 10.5
3	Mouse	2 months	11 months	6 days	~ 11.5
4	Mouse	3 months	~ 4 months	4 weeks	~ 5.5

Table 5.1 Summary of important methodological differences between studies presented in Chapters 2-4.

5.2 Factors that may contribute to the variable behavioural effects of ovarian hormones

5.2.1 Stress background

The findings of this thesis indicate that ovariectomy influences the expression of depressive-like behaviour in a manner that is dependent on stress background. Notably, in Chapter 3, long-term ovariectomy *increased* depressive-like behaviour under non-stress conditions but *mitigated* the depressive-like outcomes of sub-chronic stress exposure in middle-aged rodents. The effects observed under non-stress conditions corroborate several other studies showing that ovariectomy, without any other manipulations, increases depressive-like behaviour in rats and mice (Bernardi et al., 1989; Rachman et al., 1998; Bekku and Yoshimura, 2005; Bekku et al., 2006; de Chaves et al., 2009; Li et al., 2014). However, the effects of long-term ovariectomy to increase depressive-like behaviour under non-stress conditions were not robust, as we only observe a reduction in latency to immobility in FST, and a trend toward reduced sucrose consumption, but no significant effect in the total time spent immobile. This suggests that under non-stress conditions, longer durations of ovarian hormone deprivation may result in a partial recovery of depressive-like behaviour, in line with previous studies (de Chaves et al., 2009; Walf et al., 2009b; Estrada-Camarena et al., 2011). Intriguingly, even though ovariectomized mice displayed more depressive-like behaviour under non-stress conditions, it was the intact group that displayed greater susceptibility to the depressive-like outcomes, at least after sub-chronic stress exposure. Specifically, the effects of sub-chronic stress exposure to reduce latency to immobility in FST were only observed in intact mice. As an alternative explanation, it is possible that a floor effect occurred in ovariectomized mice under non-stress conditions, leaving no room for sub-chronic stress exposure to further reduce latency to

immobility. However, our findings are in agreement with another study in young adult female mice, where exposure to a similar sub-chronic stress paradigm increased depressive-like behaviour in intact, but not ovariectomized mice, as measured by increased total time spent immobile in the FST (LaPlant et al., 2009). Thus, together with previous work, findings from Chapter 3 suggest that ovarian hormones may produce opposite effects in regulating depressive-like behaviour, depending on stress background.

An important contribution of the current thesis is the exploration of mediating roles of ER subtypes using chronic agonist treatment and within a valid animal model of MDD. To date, studies examining ER mechanisms implicated in estrogenic regulation of depressive- and anxiety-like behaviour have typically used acute ER agonist administration (Walf et al., 2004; Walf and Frye, 2005; Weiser et al., 2009; Yang et al., 2014). Further, these studies assessed animals on tests of depressive- or anxiety-like behaviour without the use of a valid model to induce MDD-like pathology. Findings from these studies attributed the acute effects of 17 β -estradiol in reducing depressive- and anxiety- like behaviour to its actions on ER β , but not ER α (Walf et al., 2004; Rocha et al., 2005; Walf and Frye, 2005; Weiser et al., 2009; Yang et al., 2014). Our findings in Chapter 4 confirm a role ER β but provide novel information by extending the previous literature and demonstrating that ER α is also implicated in regulating depressive-like behaviour, when activated chronically. This was concluded from the finding that under non-stress conditions, chronic treatment with E2, DPN, or PPT, all reversed the ovariectomy-induced increase in TST immobility. In further support of a role of ER α , a recent study showed that overexpression of ER α in the nucleus accumbens (NAc) increased resilience to the depressive-like outcomes of stress exposure in female mice (Lorsch et al., 2018). Importantly, we demonstrate that the actions of ER agonists in TST did not translate to conditions of CUS

exposure. Indeed, the depressive-like effects of CUS exposure were primarily attributed to DPN- and PPT-treated groups. This finding, together with findings from Chapter 3, suggest that estrogenic signaling can differentially affect depressive-like behaviour based on stress background. Therefore, findings derived from non-stress conditions cannot be assumed to mirror those under conditions of stress exposure. These data are novel and underscore the importance of using valid animal models of MDD when examining the effects of estrogens, an approach that is not consistently adopted in the literature (Walf et al., 2004; Rocha et al., 2005; Walf and Frye, 2005; Weiser et al., 2009; Yang et al., 2014). An additional important consideration is the timing and regimen of hormone or agonist administration in Chapter 4. In this study, 17 β -estradiol and agonists were administered via daily injections and thus do not mimic the natural pattern of hormone release in the estrous cycle of mice. It is therefore possible that a more naturalistic treatment regimen could be protective or produce different outcomes. Further, these treatments were administered to young adult mice, therefore our findings cannot be assumed to hold true for aged female mice, as previous studies demonstrate that sensitivity or response to estrogens may differ in aged versus younger adult females (Selvamani and Sohrabji, 2010; Barha and Galea, 2011).

In Chapter 2, all groups were exposed to CUS as we aimed to investigate whether ovarian status influenced antidepressant efficacy. In humans, the mood-enhancing effects of antidepressants are not observed in individuals without MDD, therefore we chose to model MDD in all groups for translational relevance. As such, we are not able to derive conclusions regarding the effects of ovarian hormones under non-stress versus stress conditions from this chapter. Nonetheless, under CUS conditions, we consistently report increased anxiety-, depressive-, and anhedonia-like behaviour in ovariectomized compared to intact rats, suggesting that long-term

ovariectomy may increase susceptibility to CUS exposure. Indeed, this is in agreement with another study in mice, where long-term (4 months) but not short-term (2 weeks) ovariectomy enhanced the effects of CUS exposure on depressive-like behaviour (Lagunas et al., 2010). Our findings in Chapter 2 are therefore partially contrary to those of Chapter 3, in which long-term ovariectomy mitigated the outcomes of sub-chronic stress exposure in mice. Below I discuss important methodological differences between the chapters that may contribute to the variable outcomes of long-term ovariectomy.

5.2.2 Characteristics of the stress paradigm

The duration of the stress paradigm and the nature of stressors used are important considerations. The sub-chronic variable stress procedure used in Chapter 3, was not only shorter in duration (6 days), but also included stressors that were primarily physiological/psychological in nature (restraint, tail suspension, and foot shock). Conversely, in Chapters 2 and 4, CUS procedures was much longer in duration (4-6 weeks), and in addition to psychological/psychological stressors, included stressors that were social in nature. Examples of this include overnight social isolation, periods of housing with non-cage mates, and placement in a cage soiled by other animals. It is therefore interesting that long-term ovariectomy increased depressive-like behaviour under conditions of CUS (Chapter 2) but mitigated depressive-like behaviour under conditions of sub-chronic stress (Chapter 3). Clearly, future experiments would be required to determine the effects of duration versus nature of stressors, but it's plausible that one or both factors may contribute to the differences in findings. Indeed, the findings of Chapters 2 and 3 are in line with previously mentioned studies using similar paradigms (LaPlant et al., 2009; Lagunas et al., 2010). Specifically, ovariectomy increased the depressive-like effects of a

4-week CUS procedure that included physiological, psychological and social stressors (Lagunas et al., 2010), but mitigated the depressive-like effects of a 6-day sub-chronic stress procedure that included physiological/psychological, but not social stressors (LaPlant et al., 2009). Importantly, estradiol influences social behaviour and social memory in rats and mice (Tang et al., 2005; Garcia et al., 2017). Further, stress exposure interacts with social housing in a sex-dependent manner, to differentially affect outcomes including hippocampal cell proliferation and Fos expression in the PVN (Westenbroek et al., 2003, 2004). Therefore, it is certainly possible that differences in the ovarian hormone milieu may result in different sensitivities to social stressors.

5.2.3 Species

Other important considerations when synthesizing findings from this thesis are species and age. The experiments presented here used middle-aged rats (Chapter 2), middle-aged mice (Chapter 3), and young-adult mice (Chapter 4). The switch from rats to mice stemmed from plans to use transgenic models available only in mice (on C57bL/6 background), however for reasons not within our control, these plans did not materialize. Nonetheless, we can leverage the use of the two species to gain insight into potential differences between mice and rats. For example, differences in the effects of long-term ovariectomy on the outcomes of stress exposure between Chapters 2 and 3 could be partly attributed to species differences. However, given the abovementioned methodological differences between the studies, it is not possible to confirm this without further experiments. Overall, it is important to consider some fundamental differences between rats and mice when interpreting behaviour, not only in this thesis, but in the literature at large. Of relevance to FST, for example, basic swimming behaviour differs between

mice and rats. Rats are better swimmers than mice, display qualitatively different behaviours than mice including diving , and generally show lower levels of immobility at baseline (Kalynchuk et al., 2004; Can et al., 2012; Li et al., 2014; Chen et al., 2015; Wainwright et al., 2016a). Indeed, many strains of mice, including C57BL/6 strains, display very high levels of immobility (~50-87%) under non-stress conditions (Can et al., 2012). Although widely used in stress-based models, these strains may therefore not be ideal for this purpose as there is little room for any manipulations to increase passive-coping behaviour. Overall, it is therefore conceivable that different experimental manipulations can interact with baseline differences in swimming behaviour between rats and mice to produce differing outcomes.

5.2.4 Age

The experiments presented in this thesis used rodents of different ages: Chapters 2 and 3 use middle-aged rats and mice, respectively, and Chapter 4 used young-adult mice. Age is an important factor, as increasing age has been shown alter depressive- and anxiety-like behaviour at baseline or in response to other manipulations including immune activation or CUS exposure (Godbout et al., 2008; Moretti et al., 2011; Shoji et al., 2016; Lotan et al., 2018). Few studies have directly compared the effects of stress exposure on depressive-like phenotypes in adult and aged animals, especially in females. One study found age-dependent effects of CUS exposure in female mice, such that 8 weeks of CUS exposure reduced anxiety-like behaviour and enhanced cognitive performance in young adults (3 months), but impaired cognitive performance in old mice (20 to 23 months) (Lotan et al., 2018). In males mice exposed to social defeat stress, aging is associated with increased depressive-like behaviour and an exaggerate inflammatory response (Kinsey et al., 2008; Oizumi et al., 2019). Age differences in the effects of stress exposure may

not be surprising, as ageing itself is characterized by a constellation of changes, some that markedly overlap with the effects of stress exposure, including substantial reductions in many forms of neuroplasticity, reductions in neurotrophic support, alterations in HPA axis function, and immune senescence (Otte et al., 2005; Burke and Barnes, 2006; Nikolich-Žugich, 2018). In addition, the aged female brain responds differently to estrogens than the young-adult female brain. For example, 17β -estradiol increases hippocampal cell proliferation in young, but not middle-aged nulliparous rats (Chiba et al., 2007; Barha et al., 2009; Barha and Galea, 2011). Further, 17β -estradiol produces anti-inflammatory and neuroprotective effects in younger, but not older, females in models of stroke (Johnson and Sohrabji, 2005; Selvamani and Sohrabji, 2010a). Although we used animals of different ages between studies, attributing differences in findings to age would be confounded by the length of ovarian hormone deprivation, an issue that is discussed below.

5.2.5 Age at ovariectomy and length of ovarian hormone deprivation

In addition to the abovementioned differences between Chapters 2 and 3 in terms of species and the characteristics of the stress paradigm, there were also important differences between the two chapters in relation to the age at ovariectomy and the length of ovarian hormone deprivation relative to stress exposure (detailed in Table 5.1). More specifically, in Chapter 3 mice were ovariectomized at 2 months of age and exposed to stressors at 11 months of age, but in Chapter 2 rats were ovariectomized at 5 months of age and exposed to stressors at 9 months of age. Therefore, prior to stress exposure, ovariectomized mice in Chapter 3 experienced approximately 9 months of ovarian hormone deprivation, whereas ovariectomized rats in Chapter 2 experienced approximately 4 months of ovarian hormone deprivation. It is certainly possible that differences in cumulative exposure to ovarian hormones may have contributed to the

differences in findings between Chapters 2 and 3. Indeed, there is evidence from the human literature to support the notion that increased cumulative exposure to estrogens, measured by variables including age at menarche and menopause, is associated with a reduced risk for depression in perimenopause and post-menopause (Georgakis et al., 2016; Marsh et al., 2017). Based on this evidence, one might predict a more resilient phenotype in ovariectomized rats in Chapter 2 as these groups experienced shorter durations of ovarian hormone deprivation, but this was not the case, although as the length of CUS was not consistent across studies a direct comparison is not possible. Importantly, however, in each chapter ovariectomized animals were compared to sham-operated groups, and there may have been differences between the ovarian status of these groups between Chapters 2 and 3. Specifically, in addition to varying durations of ovarian hormone deprivation, middle-aged sham-operated groups in Chapters 2 and 3 may have been tested at different stages of transition to reproductive senescence. In Chapter 2, sham-operated rats still displayed regular estrous cycles, whereas more than 50% of sham-operated mice in Chapter 3 were irregularly cycling. It is therefore possible that stress exposure in Chapter 3 occurred at a critical window of transition to reproductive senescence in sham-operated mice, leading to greater vulnerability/less protection in the context of stress exposure. Indeed, this mirrors the human condition in which there is an increased risk for first onset and recurrent depression in women during the transition to menopause (Freeman et al., 2004, 2006, 2014; Cohen et al., 2006; Bromberger et al., 2007). On the other hand, in Chapter 2 our sham-operated rats were still cycling, therefore it's possible that the resiliency that these groups showed under chronic stress conditions may be due to protective effects of more stable patterns of ovarian hormone production. Therefore, not only is the length of ovarian hormone deprivation an important factor, but the ovarian status of sham-operated control groups should be considered

especially in middle-aged animals. Given that there were other notable differences between Chapters 2 and 3, it is not possible to attribute differences between the studies solely to the duration of ovarian hormone deprivation or the ovarian status of sham-operated groups, and these issues require further exploration.

5.3 Ovarian hormones influence neuroinflammatory signatures under non-stress conditions: links to behaviour?

There is growing evidence for a role of inflammation in MDD (Dowlati et al., 2010; Liu et al., 2012; Haapakoski et al., 2015; Wang and Miller, 2018), and it is recognized that ovarian hormones possess immunomodulatory properties (Vegezo et al., 2008; Villa et al., 2016). Yet, few studies have addressed whether and how ovarian hormones can modulate immune dysregulation following stress exposure or in MDD. The experiments presented in this thesis targeted this notable gap in the literature and provide novel information about the immunomodulatory effects of ovarian hormones both under non-stress conditions and in the context of sub-chronic and chronic stress exposure. Our findings indicate that ovarian hormones have potent effects on the central cytokine milieu and influence the neuroinflammatory consequences of stress exposure. In chapter 3, we report robust effects of long-term ovariectomy on cytokine concentrations in the hippocampus and frontal cortex. These effects were largely independent of sub-chronic stress exposure but depended on brain region, with generally reduced cytokine concentrations in the frontal cortex and increased concentrations in the hippocampus. Of interest was the finding that long-term ovariectomy increased IL-6:IL-10 ratio in the frontal cortex and increased IL-6 concentrations in the hippocampus. In light of the prominent role of IL-6 in MDD and in depressive-like behaviour in animal models (Dowlati et al., 2010; Liu et al., 2012; Hodes et al., 2014), these modifications may be linked to the modest increase in

depressive-like behaviour in ovariectomized mice under non-stress conditions. It is important to note that the effects of IL-6 may depend on tissue type or disease state, as IL-6 is a highly pleiotropic cytokine (Scheller et al., 2011). More specifically, classic signaling of IL-6 is mediated via interactions with membrane bound IL-6 receptors to produce anti-inflammatory effects, and trans signaling is mediated via interactions with the soluble IL-6 receptors to produce pro-inflammatory effects (Scheller et al., 2011). However, In the context of MDD and its stress-based models, IL-6 appears to be pro-inflammatory, and substantial evidence directly links elevations of IL-6 to depressive-like phenotypes in animal models (reviewed in Hodes et al., 2016).

An important finding in Chapter 3 was that ovarian status dictated the neuroinflammatory consequences of sub-chronic stress exposure. We report an exaggerated neuroinflammatory response to sub-chronic stress in intact mice, highlighted by increased concentrations of the pro-inflammatory IL-1 β and a shift toward a pro-inflammatory cytokine bias (IL-6:IL-10), in both the frontal cortex and hippocampus. This enhanced neuroinflammatory response to stress in intact mice may be functionally significant, as this group displayed a greater behavioral susceptibility to sub-chronic stress exposure. Importantly, in addition to being reliably increased in MDD (Dowlati et al., 2010; Liu et al., 2012; Haapakoski et al., 2015; Wang and Miller, 2018), IL-1 β and IL-6 have specifically been implicated in the depressive-like outcomes of stress exposure in male subjects (Goshen et al., 2008; Goshen and Yirmiya, 2009; Wohleb et al., 2014; Wood et al., 2015; Hodes et al., 2016). The observation that ovarian hormones may potentiate the inflammatory consequences of sub-chronic stress exposure may be linked to the fact that mice were in middle age. Age can influence the immunomodulatory properties of estrogens (Straub, 2007). For example, in models of stroke, estrogens can be neuroprotective and anti-

inflammatory in younger females, but neurotoxic and pro-inflammatory in older females (Johnson and Sohrabji, 2005; Nordell et al., 2003; Selvamani and Sohrabji, 2010).

In Chapter 4, we did not observe robust effects of ovariectomy or estrogenic treatments on cytokine concentrations under non-stress conditions. However, in that experiment, young adult female mice with a far shorter duration of ovarian hormone deprivation were studied. Taken together with the findings of Chapter 3, this suggests that more pronounced modifications in the central cytokine milieu may result from prolonged periods of ovarian hormone deprivation coupled with increasing age. This may be somewhat akin to post-menopausal states, which are associated with loss of ovarian function and an increase in chronic low-grade inflammation (Brinton et al., 2015; Mishra and Brinton, 2018).

5.4 Stress and Ovarian hormones influence neuroinflammatory signatures: links to behaviour?

The neuroinflammatory effects of long-term CUS exposure in Chapter 4 were robust in both the frontal cortex and hippocampus. Importantly, CUS exposure increased concentrations of IL-1 β , IL-6 and TNF- α , cytokines that are reliably increased blood and cerebral spinal fluid of individuals with MDD (Dowlati et al., 2010; Liu et al., 2012; Haapakoski et al., 2015; Wang and Miller, 2018). These findings are an important contribution to the literature as most previous studies investigating the neuroinflammatory consequence of stress exposure have used male rodents. Our PCA analyses also indicated that estrogenic treatments differentially contribute to the neuroinflammatory effects of CUS, such that the effects of CUS were primarily driven by PPT-treated groups in the frontal cortex, but by DPN-treated groups in the hippocampus. These findings suggest that chronic, selective activation of ER α and ER β may potentiate, rather than

mitigate, the neuroinflammatory consequences of CUS exposure in a brain region-dependent manner. To our knowledge this is the first report examining the roles of ER α and ER β in stress-induced changes in the central cytokine milieu. These results appear to be linked to greater behavioural susceptibility to CUS at least in the TST, however, clearly, this requires further exploration. Thus, overall, in Chapters 3 and 4, groups that showed an exaggerated neuroinflammatory response to stress also displayed a greater behavioural susceptibility to stress exposure, but only for some and not all behavioural tests.

An interesting comparison between Chapters 3 and 4 is that cytokine concentrations in the frontal cortex and hippocampus were more strongly affected by ovarian status than stress exposure in Chapter 3, but more strongly affect by stress exposure than ovarian status/estrogenic treatments in Chapter 4. This is to be expected given that Chapter 3 examined the effects of *long-term* ovariectomy and a *shorter* stress paradigm (6 days), whereas Chapter 4 examined the effects of *short-term* ovariectomy and a *longer* stress paradigm (4 weeks). Therefore, longer durations of stress exposure or ovarian hormone deprivation each resulted in larger alterations in the cytokine milieu.

Although we did not measure cytokine concentrations in Chapter 2, we did examine microglia number and morphology (length of processes) in the GCL. In groups exposed to CUS but not fluoxetine, we did not observe significant differences in microglia number or morphology between ovariectomized and intact rats. This contrasts the findings of another study where long-term ovariectomy in aged mice increased indices of microglial activation in the hippocampus, including retracted, thick processes, and larger cell bodies (Benedusi et al., 2012). However, this study did not specify the hippocampal subfield examined, and further used 22-month-old mice

that were not exposed to stress, whereas we used approximately 11-month-old rats exposed to CUS. Nonetheless, when viewing microglia data in Chapter 2 together with cytokine data in Chapters 3 and 4, it appears that stress exposure may result in differential cytokine responses depending on ovarian hormone background, but not in significant changes in microglia proliferation or apoptosis, nor in morphology. However, it is important to bear in mind that substantial methodological differences exist between the experiments, and that microglia were only examined in the GCL whereas cytokine concentrations were measured in the entire hippocampus. It would be important to examine other regions and perhaps to employ a more sophisticated analysis of microglia morphology (York et al., 2018).

Collectively, findings from the experiments described in this thesis provide novel insight into how ovarian hormones can modulate the neuroinflammatory consequences of stress exposure. We report that ovarian hormones impacted neuroinflammatory signatures both under non-stress conditions, and, importantly, in response to sub-chronic and chronic stress exposure. In particular, our findings using sub-chronic stress may give us important clues into prodromal states, which is important given evidence in humans suggesting that inflammation may precede and drive MDD (Khandaker et al., 2014; Franscina Pinto and Andrade, 2016). Our findings therefore suggest that the immunomodulatory properties of ovarian hormones are of relevance in the context of stress and therefore possibly in MDD. These findings should encourage clinical researchers to investigate the interplay between ovarian hormones and immune mediators in MDD.

5.5 Ovarian hormones influence the neuroplastic effects of stress and fluoxetine exposure: links to behaviour?

The neuroplastic effects of both stress and estrogens are profound, ranging from regulation of synaptic plasticity, modifications in hippocampal neurogenesis, dendritic remodeling, and alterations in spine density and synapse number (Srivastava and Penzes, 2011; Galea et al., 2013; McEwen, 2014; McEwen et al., 2015, 2016; Sellers et al., 2015; Sheppard et al., 2019). A central hypothesis that resonated across all experiments presented in this thesis was that differing backgrounds of ovarian hormones would influence the neuroplastic outcomes of stress or antidepressant exposure. To this end, we examined adult hippocampal neurogenesis (Chapters 2 and 4), and PSD-95 expression in the frontal cortex and hippocampus (Chapters 3 and 4).

In chapter 2, chronic fluoxetine treatment upregulated cell proliferation, the survival of new neurons, and the expression of PSA-NCAM in the hippocampus, in line with previous studies (Malberg et al., 2000; Guirado et al., 2012; Vega-Rivera et al., 2015). Interestingly, although these effects were overall more robust in ovariectomized rats, fluoxetine showed little behavioural efficacy regardless of ovarian status. Past studies indicate that neurogenesis is required for antidepressant efficacy in NSF but not other behavioural tests (Santarelli et al., 2003; Bessa et al., 2009) at least in male rats and mice. Thus, in Chapter 2, the lack of a clear association between the effects of fluoxetine on neurogenesis and behaviour, especially in NSF, may point to sex differences in the requirement of neurogenesis for the behavioural effects of SSRIs, but this necessitates confirmation in future studies. In addition, despite stronger neurogenic effects of fluoxetine in ovariectomized rats, the effect of fluoxetine to enhance HPA

negative feedback function was only observed in intact rats. In young adult male mice, the ability of fluoxetine to restore HPA function under chronic stress conditions was found to be dependent on adult hippocampal neurogenesis (Surget et al., 2011). Taken together with our findings, this points to potential sex differences in fluoxetine's recruitment of adult hippocampal neurogenesis to restore HPA function, but this remains to be directly tested. In vehicle-treated groups, we do not observe ovarian status differences in terms of cell proliferation, the survival of new neurons, or the expression of PSA-NCAM in the hippocampus. However, we do detect substantial behavioural differences between the two groups, such that ovariectomized rats consistently showed more depressive- and anxiety-like behaviour, further suggesting a dissociation between neurogenesis and behaviour in our middle-aged female rats. Another study in middle-aged female mice also reported a disconnect between neurogenesis and behavioural effects, such that chronic aromatase inhibition significantly increased neurogenesis but did not affect depressive-like behaviour (Chaiton et al., 2019). Collectively, this underscores the need for studying the functional role of adult hippocampal neurogenesis in females.

In Chapter 4, we report hippocampal subregion-specific effects of CUS exposure and 17 β -estradiol on neurogenesis. Irrespective of ovarian status and estrogenic treatments, and independent of effects on cell proliferation, CUS exposure reduced neurogenesis in the ventral hippocampus. Neurogenesis is consistently reduced in animal models of chronic stress or corticosterone exposure (Bessa et al., 2009; David et al., 2009; Brummelte and Galea, 2010a; Wainwright et al., 2011), however only some studies report a similar restriction of this effect to the ventral hippocampus (Tanti and Belzung, 2013). This subregion-specific effect is interesting considering the functional differences across the dorsal-ventral axis of the hippocampus, with the ventral region being more important for anxiety and stress regulation (Fanselow and Dong, 2010;

Bagot et al., 2015; Padilla-Coreano et al., 2016). On the other hand, and irrespective of CUS condition, 17 β -estradiol increased neurogenesis only in the dorsal hippocampus, a region that plays a prominent role in cognitive functions (Fanselow and Dong, 2010). Importantly, 17 β -estradiol influences cognition (Luine, 2014; Hamson et al., 2016), and neurogenesis is required for some forms of hippocampus-dependent learning and memory (Toda and Gage, 2018). Therefore, the pro-neurogenic effect of 17 β -estradiol may have implications for hippocampus-dependent learning and memory, but this was not explored in Chapter 4.

In Chapter 3, we did not detect significant effects of long-term ovariectomy or sub-chronic stress exposure on PSD-95 expression in the frontal cortex. A previous study indicates that the post-ovariectomy interval influences the effect of ovarian hormone depletion on PSD-95 expression in the hippocampus of rats, such that reductions are observed at 1, but not 2 weeks after ovariectomy (Zhang et al., 2010). Together with our findings this suggests that PSD-95 expression recovers after prolonged periods of ovarian hormone deprivation. The lack of a stress effect in Chapter 3 is likely attributed to the sub-chronic nature of the stress paradigm (6 days). Indeed in Chapter 4, we do observe significant reductions in PSD-95 expression in frontal cortex and hippocampus following 4 weeks of CUS, in keeping with several other studies using chronic stress paradigms (Kallarackal et al., 2013; Kim and Leem, 2016; Pacheco et al., 2017). Interestingly, the effect of CUS to reduce PSD-95 expression in the frontal cortex and hippocampus was largely driven by DPN- and PPT-treated groups. This suggests that estrogenic signaling may influence the effects of CUS to reduce the number and/or stability of excitatory synapses in these regions. This finding further demonstrates that chronic selective activation of ER α or β was not protective, and this could be linked to the observed behavioural effects in the TST, although this cannot be confirmed from the current experiments.

5.6 Caveats and future directions

I discussed earlier that age, species, length of ovarian hormone deprivation, and the characteristics of the stress paradigm may all influence the effects of ovarian hormones on depressive-like phenotypes. However, the experiments presented in this thesis were not designed to systematically investigate these factors. As such, it is important that future studies are designed to disentangle the contribution of each factor alone. For example, age at stress exposure and testing can be controlled for while manipulating the length of ovarian hormone deprivation. In other experiments, animals can be tested at various ages while controlling for the length of ovarian hormone deprivation. Finally, both rats and mice can be used in stress paradigms of varying lengths and using stressors of different characteristics (i.e. social, physiological, or psychological stressors). It would also be important to use strains of mice other than C57bL/6, which were used in this thesis based on plans to use transgenic mice on this background. C57bL/6 stains are resilient to stress exposure, therefore it may be more appropriate to use more susceptible stains such as BALB/cJ, or outbred strains like CD-1 which may produce larger individual variability in resilience/susceptibility to stress exposure (Malki et al., 2015). Overall, a careful dissection of the influence of the abovementioned factors will clarify inconsistencies not only in experiments presented here, but in the literature as a whole.

This thesis examined the effects of ovarian hormone deprivation, and treatment with 17 β -estradiol or ER agonists, but did not directly consider potential roles of progesterone. Withdrawal from progesterone is thought to be implicated PMDD and PPD (Hantsoo and Epperson, 2015; Schiller et al., 2015). This is corroborated by studies in female rats and mice demonstrating that progesterone withdrawal increases depressive- and anxiety-like behaviour

(Smith et al., 2006; Li et al., 2012). There is further evidence to suggest that the progesterone metabolite allopregnanolone, a potent positive allosteric modulator of the GABA_A receptor, may play a role in depression and anxiety (Schüle et al., 2014). Indeed, the United States Food and Drug Administration recently approved brexanolone, an allopregnanolone analogue, for the treatment of PPD after successful clinical trials (Kanes et al., 2017; Meltzer-Brody et al., 2018). Future studies should examine progesterone replacement, alone or in concert with 17 β -estradiol, in order to clarify the role of ovarian hormones in models of MDD.

Chapter 4 aimed to elucidate the receptor mechanisms by which 17 β -estradiol can influence depressive-like phenotypes. To that end, the experiment explored ER α and ER β , but did not consider potential roles of GPER. The choice to target classical ERs was backed by evidence that estradiol interacts with ER β to acutely influence depressive- and anxiety-like behaviours (Walf et al., 2004; Rocha et al., 2005; Walf and Frye, 2005; Weiser et al., 2009; Yang et al., 2014). However, it is certainly plausible that GPER may also be implicated in the effects of 17 β -estradiol on depressive- and anxiety-like behaviour. Few studies to date have examined this possibility and the findings are somewhat conflicting. One study found that acute systemic administration of the GPER agonist G1 produced an anxiogenic effect in ovariectomized mice (Kastenberger et al., 2012). On the other hand, and also using ovariectomized mice, G1 administration to the basolateral amygdala following an acute stressor prevented an increase in anxiety-like behaviour (Tian et al., 2013). In order to clarify the role of GPER in mediating the actions of 17 β -estradiol on depressive- and anxiety-like behaviour, future studies should examine the effects of chronic G1 administration within a valid model of MDD.

The complex roles of ovarian hormones in MDD and depressive-like phenotypes across the literature and in the experiments presented in this thesis point to individual differences in estrogen sensitivity. That is, it likely that some women may be more sensitive than others to the effects of fluctuations or reductions in ovarian hormones on mood. There is good evidence for this idea from human studies that have employed pharmacological manipulations of hormones. For example, estradiol withdrawal after three weeks of treatment significantly increased depressive symptoms only in women with a history of perimenopausal MDD (Schmidt et al., 2015). Further, withdrawal from ovarian hormone-simulated pregnancy significantly increased depressive symptoms only in women with a prior history of PPD (Bloch et al., 2000). Intriguingly, one study identified patterns of gene expression in the blood of pregnant women that predicted PPD with 88% accuracy, and found an over-representation of transcripts that were linked to estrogen signaling (Mehta et al., 2014). Importantly, this study did not find group differences in circulating concentrations of estradiol or estrone during pregnancy or postpartum, pointing to increased estrogen sensitivity, rather than differences in absolute concentration, in women that go on to develop PPD (Mehta et al., 2014). It is therefore imperative that future studies in rodents investigate factors that may contribute to differences in estrogen sensitivity, and in particular targeting underlying genetic factors could prove fruitful.

One exploratory approach could be to take advantage of the many available in-bred mouse strains to probe for genetic predisposition to depressive-like behaviour in response to manipulations in ovarian hormones. Using 37 inbred mouse strains, one study has already demonstrated strain-dependent effects of ovariectomy to increase anxiety-like behaviour in the open field test (Schoenrock et al., 2016). This study did not find significant strain differences in the effects of ovariectomy in FST, but it would be important to examine effects in the TST,

which we find to be more sensitive to ovariectomy than the FST (Chapter 4). Characterizing behavioural differences as Schoenrock et al. (2016) would be the first step, and this could be followed by RNA-sequencing to analyze transcriptional profiles associated with vulnerability to ovarian hormone deprivation. Co-expression network analyses can then be used to identify “hub” genes associated with vulnerability to the depressive-like effects of ovariectomy. In strains that are identified as resilient/susceptible to the depressogenic effects of ovariectomy, the expression of identified hub genes can subsequently be down- or up-regulated using viral vectors. The effects of these manipulations in shifting behaviour toward a resilient/susceptible phenotype can be tested following ovariectomy. To further examine gene × environment interactions, a similar approach could be taken in tandem with exposure to chronic stressors. Such experiments can shed light on genetic factors that may contribute to estrogen sensitivity and may provide targets for the development of more personalized treatments.

Besides the question addressed in Chapter 4 regarding the mediating roles of ER subtypes, another question that remains is with respect to the cellular target of 17 β -estradiol. ERs are expressed on both neurons and glia, including microglia (McEwen et al., 2001; Sierra et al., 2008; Ishihara et al., 2015). Therefore, direct actions of 17 β -estradiol on any number of neural cell types can mediate their effects in MDD or depressive-like behaviour. Future studies should determine the neural cell type/s through which 17 β -estradiol could produce its effects on depressive-like phenotypes. The Cre/loxP system can be used to induce cell-specific ER deletions, and this can be done in an inducible manner, such that deletions would take effect in adulthood to avoid developmental perturbations. Building on findings from this thesis showing that ovarian hormones can dictate the inflammatory outcomes of stress exposure, it would be important to target ERs on microglia, the primary orchestrators of the inflammatory response in

the brain. Following cell-specific ER deletions, experiments should assess the risk/resilience to developing depressive-like phenotypes after CUS exposure.

The overall aim of this work was to clarify the role of ovarian hormones in stress-based models of MDD, and as such we only included female subjects. However, estrogenic signaling may certainly affect depressive-like phenotypes in males. Indeed, androgens serve as precursors to several other steroids hormones that can activate ERs. Notably, testosterone can be aromatized to 17β -estradiol, and dihydrotestosterone can be metabolized to 3β -diol, which binds to ER β (Pak et al., 2005). Importantly, hypogonadism in men is associated with MDD (Shores et al., 2005; McIntyre et al., 2006; Westley et al., 2015), and testosterone replacement therapies show some efficacy in alleviating symptoms, particularly in hypogonadal men (Seidman and Rabkin, 1998; Shores et al., 2009; Zarrouf et al., 2009). Studies in rats substantiate the human literature, as gonadectomized males are more susceptible to the depressogenic effects of chronic stress than intact males (Wainwright et al., 2011). Furthermore, testosterone shows some efficacy in reversing depressive-like phenotypes after chronic stress in gonadectomized male rats (Carrier and Kabbaj, 2012; Wainwright et al., 2016b). The protective and therapeutic effects of testosterone in males could certainly be mediated, in part, by estrogenic metabolites. There is some evidence in support of this notion, as one study found that an aromatase inhibitor blocked the antidepressant-like effects of testosterone in male rats (Carrier et al., 2015). Future experiments could take a similar approach to that described in Chapter 4 to dissect the ER receptor mechanisms mediating the effects of estrogenic metabolites in males.

Finally, the experiments presented in this thesis explored two brain regions compromised in MDD: the hippocampus and frontal cortex. We further focus on a few indices of

neuroplasticity (adult hippocampal neurogenesis and PSD-95 expression) and on neuroimmune mediators (cytokines and microglia), as targets of stress exposure, ovarian hormones, or their interaction. However, several other brain regions and neural systems/processes are dysregulated by stress or in MDD, and influenced by ovarian hormones, and therefore should be targeted in future studies. Notably, the NAc, and mesolimbic reward circuits in general, are dysregulated in MDD and stress-based models (Russo and Nestler, 2013; Heshmati and Russo, 2015). The amygdala is also a region that is relevant to MDD (Sibille et al., 2009), shows substantial plasticity in response to stress exposure (McEwen et al., 2016), and is influenced by estrogens (Zeidan et al., 2011; Cover et al., 2014). Ovarian hormones also modulate various neurotransmitter systems, including serotonergic, dopaminergic, and GABAergic systems (Amin et al., 2005; Barth et al., 2015), all of which are perturbed in MDD or its models (Spies et al., 2015; Belujon and Grace, 2017; Fogaça and Duman, 2019). Reductions in neurotrophic factors, particularly brain-derived neurotrophic factor (BDNF), are also thought to contribute to MDD (Duman and Li, 2012). Estrogens regulate BDNF expression (Sohrabji and Lewis, 2006), and therefore it is conceivable that some of the effects of estrogens in MDD may be produced through its regulation of BDNF. The abovementioned regions and systems should be targeted in future experiments investigating the role of ovarian hormones in stress-based models of MDD.

5.7 Conclusions

The work presented in this thesis demonstrates that ovarian hormones are important determinants of depressive-like behaviour. However, ovarian hormone regulation of depressive-like phenotypes is complex, and may depend on several factors including stress background, the characteristics of the stress paradigm, species, age, and length of ovarian hormone deprivation.

In pursuit of a better understanding of the role of ovarian hormones in MDD models, it is imperative that future studies carefully consider the contribution of each of these factors. Importantly, we provide novel data showing that ovarian hormones influence neuroimmune signatures under non-stress conditions and in response to stress exposure, suggesting that the immunomodulatory properties of ovarian may be of relevance to immune dysregulation in MDD. The data presented here also raise important questions regarding the translation of findings from male to female subjects, especially in relation to the functional roles of adult hippocampal neurogenesis. It is also important to consider translational relevance to the human condition. Estrogen receptors might represent an attractive system for drug development in stress-related disorders, including MDD. Indeed, 17 β -estradiol shows some efficacy as a standalone or adjunct treatment in women with MDD (Rasgon et al., 2007; Moses-Kolko et al., 2009). This thesis aimed to determine the receptor mechanisms through which the protective effects of estradiol may be produced, an approach that could shed light on more specific targets for drug development. Although the data should be considered preliminary, our findings suggest that selectively targeting ER α or ER β is not protective under conditions of chronic stress, and therefore possibly in MDD.

References

- Abreu AP, Kaiser UB (2016) Pubertal development and regulation. *Lancet Diabetes Endocrinol.*
- Adzic M, Djordjevic J, Mitic M, Brkic Z, Lukic I, Radojcic M (2015) The contribution of hypothalamic neuroendocrine, neuroplastic and neuroinflammatory processes to lipopolysaccharide-induced depressive-like behaviour in female and male rats: Involvement of glucocorticoid receptor and C/EBP- β . *Behav Brain Res* 291:130–139.
- Ahokas A, Kaukoranta J, Wahlbeck K, Aito M (2001) Estrogen deficiency in severe postpartum depression: Successful treatment with sublingual physiologic 17 β -estradiol: A preliminary study. *J Clin Psychiatry*.
- Akama KT, McEwen BS (2003) Estrogen Stimulates Postsynaptic Density-95 Rapid Protein Synthesis via the Akt/Protein Kinase B Pathway. *J Neurosci*.
- Akil H, Gordon J, Hen R, Javitch J, Mayberg H, McEwen B, Meaney MJ, Nestler EJ (2017) Treatment resistant depression: A multi-scale, systems biology approach. *Neurosci Biobehav Rev*:1–17 Available at: <http://dx.doi.org/10.1016/j.neubiorev.2017.08.019>.
- Aloisi AM, Ceccarelli I, Lupo C (1998) Behavioural and hormonal effects of restraint stress and formalin test in male and female rats. *Brain Res Bull*.
- American Psychiatric Association (2013) Diagnostic and Statistical Manual of Mental Disorders, 5th Edition.
- Amin Z, Canli T, Epperson CN (2005) Effect of estrogen-serotonin interactions on mood and cognition. *Behav Cogn Neurosci Rev*.
- Anacker C, Hen R (2017) Adult hippocampal neurogenesis and cognitive flexibility-linking memory and mood. *Nat Rev Neurosci*.
- Anacker C, Luna VM, Stevens GS, Millette A, Shores R, Jimenez JC, Chen B, Hen R (2018)

Hippocampal neurogenesis confers stress resilience by inhibiting the ventral dentate gyrus.

Nature 559:98–102 Available at: <http://www.nature.com/articles/s41586-018-0262-4>

[Accessed July 12, 2019].

Angst J, Gamma A, Sellaro R, Zhang H, Merikangas K (2002) Toward validation of atypical depression in the community: Results of the Zurich cohort study. *J Affect Disord* 72:125–138.

Anisman H, Ravindran A V., Griffiths J, Merali Z (1999) Endocrine and cytokine correlates of major depression and dysthymia with typical or atypical features. *Mol Psychiatry*.

Arnsten AFT (2009) Stress signalling pathways that impair prefrontal cortex structure and function. *Nat Rev Neurosci*.

Asnis GM, McGinn LK, Sanderson WC (1995) Atypical depression: Clinical aspects and noradrenergic function. *Am J Psychiatry*.

Atkinson HC, Waddell BJ (1997) Circadian variation in basal plasma corticosterone and adrenocorticotropin in the rat: Sexual dimorphism and changes across the estrous cycle. *Endocrinology* 138:3842–3848.

Bagot RC, Parise EM, Peña CJ, Zhang HX, Maze I, Chaudhury D, Persaud B, Cachope R, Bolaños-Guzmán CA, Cheer J, Deisseroth K, Han MH, Nestler EJ (2015) Ventral hippocampal afferents to the nucleus accumbens regulate susceptibility to depression. *Nat Commun*.

Baker AE, Brautigam VM, Watters JJ (2004) Estrogen modulates microglial inflammatory mediator production via interactions with estrogen receptor β . *Endocrinology* 145:5021–5032.

Bale TL (2006) Stress sensitivity and the development of affective disorders. *Horm Behav*.

Banasr M, Hery M, Printemps R, Daszuta A (2004) Serotonin-induced increases in adult cell proliferation and neurogenesis are mediated through different and common 5-HT receptor subtypes in the dentate gyrus and the subventricular zone. *Neuropsychopharmacology*.

Banki CM, Arato M, Papp Z, Rihmer Z, Kovacs Z (1986) Associations among dexamethasone non-suppression and TRH-induced hormonal responses: increased specificity for melancholia. *Psychoneuroendocrinology*.

Barha CK, Brummelte S, Lieblich SE, Galea LAM (2011) Chronic restraint stress in adolescence differentially influences hypothalamic-pituitary-adrenal axis function and adult hippocampal neurogenesis in male and female rats. *Hippocampus*.

Barha CK, Dalton GL, Galea LAM (2010) Low doses of 17 α -estradiol and 17 β -estradiol facilitate, whereas higher doses of estrone and 17 α -and 17 β -estradiol impair, contextual fear conditioning in adult female rats. *Neuropsychopharmacology*.

Barha CK, Galea LAM (2011) Motherhood alters the cellular response to estrogens in the hippocampus later in life. *Neurobiol Aging* 32:2091–2095.

Barha CK, Lieblich SE, Chow C, Galea LAM (2015) Multiparity-induced enhancement of hippocampal neurogenesis and spatial memory depends on ovarian hormone status in middle age. *Neurobiol Aging* 36:2391–2405.

Barha CK, Lieblich SE, Galea LAM (2009) Different forms of oestrogen rapidly upregulate cell proliferation in the dentate gyrus of adult female rats. *J Neuroendocrinol* 21:155–166.

Barker JM, Galea LAM (2008) Repeated estradiol administration alters different aspects of neurogenesis and cell death in the hippocampus of female, but not male, rats. *Neuroscience*.

Barker JM, Galea LAM (2009) Sex and regional differences in estradiol content in the prefrontal cortex, amygdala and hippocampus of adult male and female rats. *Gen Comp Endocrinol*.

Barth C, Villringer A, Sacher J (2015) Sex hormones affect neurotransmitters and shape the adult female brain during hormonal transition periods. *Front Neurosci.*

Basterzi AD, Aydemir Ç, Kisa C, Aksaray S, Tuzer V, Yazici K, Göka E (2005) IL-6 levels decrease with SSRI treatment in patients with major depression. *Hum Psychopharmacol.*

Bath KG, Chuang J, Spencer-Segal JL, Amso D, Altemus M, McEwen BS, Lee FS (2012) Variant brain-derived neurotrophic factor (Valine66Methionine) polymorphism contributes to developmental and estrous stage-specific expression of anxiety-like behavior in female mice. *Biol Psychiatry.*

Beagley KW, Gockel CM (2003) Regulation of innate and adaptive immunity by the female sex hormones oestradiol and progesterone. *FEMS Immunol Med Microbiol.*

Bekku N, Yoshimura H (2005) Animal model of menopausal depressive-like state in female mice: Prolongation of immobility time in the forced swimming test following ovariectomy. *Psychopharmacology (Berl).*

Bekku N, Yoshimura H, Araki H (2006) Factors producing a menopausal depressive-like state in mice following ovariectomy. *Psychopharmacology (Berl).*

Belujon P, Grace AA (2017) Dopamine system dysregulation in major depressive disorders. *Int J Neuropsychopharmacol.*

Belzung C, Le Guisquet AM, Barreau S, Calatayud F (2001) An investigation of the mechanisms responsible for acute fluoxetine-induced anxiogenic-like effects in mice. *Behav Pharmacol.*

Benedusi V, Meda C, Della Torre S, Monteleone G, Vegeto E, Maggi A (2012) A lack of ovarian function increases neuroinflammation in aged mice. *Endocrinology.*

Bereshchenko O, Bruscoli S, Riccardi C (2018) Glucocorticoids, sex hormones, and immunity. *Front Immunol.*

- Berlanga C, Flores-Ramos M (2006) Different gender response to serotonergic and noradrenergic antidepressants. A comparative study of the efficacy of citalopram and reboxetine. *J Affect Disord.*
- Bernardi M, Vergoni A V., Sandrini M, Tagliavini S, Bertolini A (1989) Influence of ovariectomy, estradiol and progesterone on the behavior of mice in an experimental model of depression. *Physiol Behav.*
- Bessa JM, Ferreira D, Melo I, Marques F, Cerqueira JJ, Palha JA, Almeida OFX, Sousa N (2009) The mood-improving actions of antidepressants do not depend on neurogenesis but are associated with neuronal remodeling. *Mol Psychiatry* 14:764–773 Available at: <http://www.nature.com/doifinder/10.1038/mp.2008.119>.
- Bethea CL, Mirkes SJ, Shively CA, Adams MR (1999) Steroid regulation of tryptophan hydroxylase protein in the dorsal raphe of macaques. *Biol Psychiatry.*
- Bi R, Foy MR, Vouimba R-M, Thompson RF, Baudry M (2002) Cyclic changes in estradiol regulate synaptic plasticity through the MAP kinase pathway. *Proc Natl Acad Sci.*
- Bigos KL, Pollock BG, Stankevich BA, Bies RR (2009) Sex differences in the pharmacokinetics and pharmacodynamics of antidepressants: An updated review. *Gend Med* 6:522–543.
- Binder EB, Künzel HE, Nickel T, Kern N, Pfennig A, Majer M, Uhr M, Ising M, Holsboer F (2009) HPA-axis regulation at in-patient admission is associated with antidepressant therapy outcome in male but not in female depressed patients. *Psychoneuroendocrinology* 34:99–109.
- Binneman B, Feltner D, Kolluri S, Shi Y, Qiu R, Stiger T (2008) A 6-week randomized, placebo-controlled trial of CP-316,311 (a selective CRH1 antagonist) in the treatment of major depression. *Am J Psychiatry.*

Birur B, Amrock EM, Shelton RC, Li L (2017) Sex differences in the peripheral immune system in patients with depression. *Front Psychiatry* 8.

Biver F, Goldman S, Delvenne V, Luxen A, De Maertelaer V, Hubain P, Mendlewicz J, Lotstra F (1994) Frontal and parietal metabolic disturbances in unipolar depression. *Biol Psychiatry*.

Bjørnebekk A, Mathé A a, Brené S (2005) The antidepressant effect of running is associated with increased hippocampal cell proliferation. *Int J Neuropsychopharmacol* 8:357–368 Available at: <http://www.ncbi.nlm.nih.gov/pubmed/15769301>.

Bjørnebekk A, Mathé AA, Brené S (2006) Running has differential effects on NPY, opiates, and cell proliferation in an animal model of depression and controls. *Neuropsychopharmacology* 31:256–264.

Björnström L, Sjöberg M (2005) Mechanisms of Estrogen Receptor Signaling: Convergence of Genomic and Nongenomic Actions on Target Genes. *Mol Endocrinol*.

Blanco C, Vesga-López O, Stewart JW, Liu S-M, Grant BF, Hasin DS (2012) Epidemiology of major depression with atypical features: results from the National Epidemiologic Survey on Alcohol and Related Conditions (NESARC). *J Clin Psychiatry* 73:224–232.

Blier P (2003) The pharmacology of putative early-onset antidepressant strategies. *Eur Neuropsychopharmacol*.

Bloch M, Schmidt PJ, Danaceau M, Murphy J, Nieman L, Rubinow DR (2000) Effects of gonadal steroids in women with a history of postpartum depression. *Am J Psychiatry* 157:924–930.

Block TS, Kushner H, Kalin N, Nelson C, Belanoff J, Schatzberg A (2018) Combined Analysis of Mifepristone for Psychotic Depression: Plasma Levels Associated With Clinical

Response. *Biol Psychiatry*.

Bodnar TS, Taves MD, Lavigne KM, Woodward TS, Soma KK, Weinberg J (2017) Differential activation of endocrine-immune networks by arthritis challenge: Insights from colony-specific responses. *Sci Rep* 7:1–14 Available at: <http://dx.doi.org/10.1038/s41598-017-00652-4>.

Bodnoff SR, Suranyi-Cadotte B, Aitken DH, Quirion R, Meaney MJ (1988) The effects of chronic antidepressant treatment in an animal model of anxiety. *Psychopharmacology (Berl)*.

Bogren M, Brådvik L, Holmstrand C, Nöbbelin L, Mattisson C (2017) Gender differences in subtypes of depression by first incidence and age of onset: a follow-up of the Lundby population. *Eur Arch Psychiatry Clin Neurosci*:1–11.

Boldrini M, Fulmore CA, Tartt AN, Simeon LR, Pavlova I, Poposka V, Rosoklija GB, Stankov A, Arango V, Dwork AJ, Hen R, Mann JJ (2018) Human Hippocampal Neurogenesis Persists throughout Aging. *Cell Stem Cell*:589–599.

Boldrini M, Hen R, Underwood MD, Rosoklija GB, Dwork AJ, Mann JJ, Arango V (2012) Hippocampal Angiogenesis and Progenitor Cell Major Depression. *Biol Psychiatry*.

Boldrini M, Santiago AN, Hen R, Dwork AJ, Rosoklija GB, Tamir H, Arango V, John Mann J (2013) Hippocampal granule neuron number and dentate gyrus volume in antidepressant-treated and untreated major depression. *Neuropsychopharmacology*.

Bollinger JL, Bergeon Burns CM, Wellman CL (2016) Differential effects of stress on microglial cell activation in male and female medial prefrontal cortex. *Brain Behav Immun* 52:88–97.

Bonfanti L (2006) PSA-NCAM in mammalian structural plasticity and neurogenesis. *Prog Neurobiol*.

Bostwick JM, Pankratz VS (2000) Affective disorders and suicide risk: A reexamination. *Am J Psychiatry*.

Brailoiu E, Dun SL, Brailoiu GC, Mizuo K, Sklar LA, Oprea TI, Prossnitz ER, Dun NJ (2007) Distribution and characterization of estrogen receptor G protein-coupled receptor 30 in the rat central nervous system. *J Endocrinol*.

Brambilla DJ, Matsumoto AM, Araujo AB, McKinlay JB (2009) The effect of diurnal variation on clinical measurement of serum testosterone and other sex hormone levels in men. *J Clin Endocrinol Metab* 94:907–913.

Brechbuhl J, Moine F, Klaey M, Nenniger-Tosato M, Hurni N, Sporkert F, Giroud C, Broillet M-C (2013) Mouse alarm pheromone shares structural similarity with predator scents. *Proc Natl Acad Sci*.

Bredemann TM, McMahon LL (2014) 17β Estradiol increases resilience and improves hippocampal synaptic function in helpless ovariectomized rats. *Psychoneuroendocrinology* 42:77–88.

Brinton RD (2008) The healthy cell bias of estrogen action: mitochondrial bioenergetics and neurological implications. *Trends Neurosci*.

Brinton RD (2009) Estrogen-induced plasticity from cells to circuits: predictions for cognitive function. *Trends Pharmacol Sci*.

Brinton RD, Yao J, Yin F, Mack WJ, Cadenas E (2015) Perimenopause as a neurological transition state. *Nat Rev Endocrinol*.

Brkic Z, Petrovic Z, Franic D, Mitic M, Adzic M (2016) Male-specific effects of lipopolysaccharide on glucocorticoid receptor nuclear translocation in the prefrontal cortex of depressive rats. *Psychopharmacology (Berl)* 233:3315–3330.

- Bromberger JT, Matthews KA, Schott LL, Brockwell S, Avis NE, Kravitz HM, Everson-Rose SA, Gold EB, Sowers MF, Randolph JF (2007) Depressive symptoms during the menopausal transition: The Study of Women's Health Across the Nation (SWAN). *J Affect Disord.*
- Bromet E et al. (2011) Cross-national epidemiology of DSM-IV major depressive episode. *BMC Med* 9.
- Brown CM, Mulcahey TA, Filipek NC, Wise PM (2010) Production of proinflammatory cytokines and chemokines during neuroinflammation: Novel roles for estrogen receptors α and β . *Endocrinology*.
- Bruce-Keller AJ, Keeling JL, Keller JN, Huang FF, Camondola S, Mattson MP (2000) Antiinflammatory effects of estrogen on microglial activation. *Endocrinology* 141:3646–3656.
- Brummelte S, Galea LAM (2010a) Chronic high corticosterone reduces neurogenesis in the dentate gyrus of adult male and female rats. *Neuroscience* 168:680–690.
- Brummelte S, Galea LAM (2010b) Depression during pregnancy and postpartum: Contribution of stress and ovarian hormones. *Prog Neuro-Psychopharmacol Biol Psychiatry*.
- Burger HG, Dudley EC, Robertson DM, Dennerstein L (2002) Hormonal changes in the menopause transition. *Recent Prog Horm Res* 57:257–275 Available at: <http://www.ncbi.nlm.nih.gov/pubmed/12017547> [Accessed July 10, 2019].
- Burgess LH, Handa RJ (1992) Chronic estrogen-induced alterations in adrenocorticotropin and corticosterone secretion, and glucocorticoid receptor-mediated functions in female rats. *Endocrinology*.
- Burgess LH, Handa RJ (1993) Estrogen-induced alterations in the regulation of

mineralocorticoid and glucocorticoid receptor messenger RNA expression in the female rat anterior pituitary gland and brain. *Mol Cell Neurosci*.

Burke SN, Barnes CA (2006) Neural plasticity in the ageing brain. *Nat Rev Neurosci*.

Byrne SE, Rothschild AJ (1998) Loss of antidepressant efficacy during maintenance therapy: Possible mechanisms and treatments. *J Clin Psychiatry* 59:279–288.

Calcia MA, Bonsall DR, Bloomfield PS, Selvaraj S, Barichello T, Howes OD (2016) Stress and neuroinflammation: A systematic review of the effects of stress on microglia and the implications for mental illness. *Psychopharmacology (Berl)* 233:1637–1650.

Cameron HA, Woolley CS, McEwen BS, Gould E (1993) Differentiation of newly born neurons and glia in the dentate gyrus of the adult rat. *Neuroscience*.

Can A, Dao DT, Arad M, Terrillion CE, Piantadosi SC, Gould TD (2012) The mouse forced swim test. *J Vis Exp*:e3638 Available at:

[http://www.ncbi.nlm.nih.gov/pubmed/22314943%5Cnhttp://www.ncbi.nlm.nih.gov/ar](http://www.ncbi.nlm.nih.gov/pubmed/22314943)
[ticlerender.fcgi?artid=PMC3353513](http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3353513/).

Can A, Dao DT, Terrillion CE, Piantadosi SC, Bhat S, Gould TD (2011) The Tail Suspension Test. *J Vis Exp* Available at: <http://www.jove.com/details.php?id=3769>.

Carey MP, Deterd CH, De Koning J, Helmerhorst F, De Kloet ER (1995) The influence of ovarian steroids on hypothalamic-pituitary-adrenal regulation in the female rat. *J Endocrinol.*

Cargnello M, Roux PP (2011) Activation and Function of the MAPKs and Their Substrates, the MAPK-Activated Protein Kinases. *Microbiol Mol Biol Rev*.

Carney RM, Freedland KE (2017) Depression and coronary heart disease. *Nat Rev Cardiol*.

Carrier N, Kabbaj M (2012) Testosterone and imipramine have antidepressant effects in socially

- isolated male but not female rats. *Horm Behav* 61:678–685.
- Carrier N, Saland SK, Duclot F, He H, Mercer R, Kabbaj M (2015) The Anxiolytic and Antidepressant-like Effects of Testosterone and Estrogen in Gonadectomized Male Rats. *Biol Psychiatry*.
- Carroll BJ (1982) The dexamethasone suppression test for melancholia. *Br J Psychiatry*.
- Chaiton JA, Wong SJ, Galea LA (2019) Chronic aromatase inhibition increases ventral hippocampal neurogenesis in middle-aged female mice. *Psychoneuroendocrinology* 106:111–116 Available at: <https://doi.org/10.1016/j.psyneuen.2019.04.003>.
- Chakrabarti M, Haque A, Banik NL, Nagarkatti P, Nagarkatti M, Ray SK (2014) Estrogen receptor agonists for attenuation of neuroinflammation and neurodegeneration. *Brain Res Bull*.
- Chan M, Chow C, Hamson DK, Lieblich SE, Galea LAM (2014) Effects of chronic oestradiol, progesterone and medroxyprogesterone acetate on hippocampal neurogenesis and adrenal mass in adult female rats. *J Neuroendocrinol*.
- Chen C, Grigoriadis DE (2005) NBI 30775 (R121919), an orally active antagonist of the corticotropin-releasing factor (CRF) type-1 receptor for the treatment of anxiety and depression. *Drug Dev Res*.
- Chen L, Faas GC, Ferando I, Mody I (2015) Novel insights into the behavioral analysis of mice subjected to the forced-swim test. *Transl Psychiatry* 5.
- Chesney E, Goodwin GM, Fazel S (2014) Risks of all-cause and suicide mortality in mental disorders: A meta-review. *World Psychiatry*.
- Chiba S, Suzuki M, Yamanouchi K, Nishihara M (2007) Involvement of granulin in estrogen-induced neurogenesis in the adult rat hippocampus. *J Reprod Dev*.

Chow C, Epp JR, Lieblich SE, Barha CK, Galea LAM (2013) Sex differences in neurogenesis and activation of new neurons in response to spatial learning and memory.

Psychoneuroendocrinology.

Christian KM, Song H, Ming G (2014) Functions and Dysfunctions of Adult Hippocampal Neurogenesis. *Annu Rev Neurosci*.

Chrousos GP (2009) Stress and disorders of the stress system. *Nat Rev Endocrinol* 5:374–381 Available at: <http://www.ncbi.nlm.nih.gov/pubmed/19488073> [Accessed December 11, 2013].

Ciana P, Raviscioni M, Mussi P, Vegeto E, Que I, Parker MG, Lowik C, Maggi A (2003) In vivo imaging of transcriptionally active estrogen receptors. *Nat Med*.

Clipperton AE, Spinato JM, Chernets C, Pfaff DW, Choleris E (2008) Differential effects of estrogen receptor alpha and beta specific agonists on social learning of food preferences in female mice. *Neuropsychopharmacology*.

Cohen LS, Soares CN, Vitonis AF, Otto MW, Harlow BL (2006) Risk for New Onset of Depression During the Menopausal Transition The Harvard Study of Moods and Cycles. *Arch Gen Psychiatry* 63:385–390.

Cole MA, Kim PJ, Kalman BA, Spencer RL (2000) Dexamethasone suppression of corticosteroid secretion: Evaluation of the site of action by receptor measures and functional studies. *Psychoneuroendocrinology* 25:151–167.

Conference Board of Canada (2016) Healthy Brains at Work: Estimating the Impact of Workplace Mental Health Benefits and Programs. In, pp 2016.

Cora MC, Kooistra L, Travlos G (2015) Vaginal Cytology of the Laboratory Rat and Mouse:Review and Criteria for the Staging of the Estrous Cycle Using Stained Vaginal

- Smears. *Toxicol Pathol* 43:776–793.
- Couillard-Despres S, Wuertinger C, Kandasamy M, Caioni M, Stadler K, Aigner R, Bogdahn U, Aigner L (2009) Ageing abolishes the effects of fluoxetine on neurogenesis. *Mol Psychiatry*.
- Coutinho AE, Chapman KE (2011) The anti-inflammatory and immunosuppressive effects of glucocorticoids, recent developments and mechanistic insights. *Mol Cell Endocrinol* 335:2–13.
- Cover KK, Maeng LY, Lebrón-Milad K, Milad MR (2014) Mechanisms of estradiol in fear circuitry: Implications for sex differences in psychopathology. *Transl Psychiatry*.
- Cryan JF, Mombereau C, Vassout A (2005) The tail suspension test as a model for assessing antidepressant activity: Review of pharmacological and genetic studies in mice. *Neurosci Biobehav Rev*:571–625 Available at: www.elsevier.com/locate/neubiorev.
- Csabai D, Wiborg O, Czéh B (2018) Reduced synapse and axon numbers in the prefrontal cortex of rats subjected to a chronic stress model for depression. *Front Cell Neurosci*.
- Dalla C, Antoniou K, Drossopoulou G, Xagoraris M, Kokras N, Sfikakis A, Papadopoulou-Daifoti Z (2005) Chronic mild stress impact: Are females more vulnerable? *Neuroscience*.
- Dalla C, Antoniou K, Kokras N, Drossopoulou G, Papathanasiou G, Bekris S, Daskas S, Papadopoulou-Daifoti Z (2008) Sex differences in the effects of two stress paradigms on dopaminergic neurotransmission. *Physiol Behav*.
- Dalla C, Papachristos EB, Whetstone AS, Shors TJ (2009) Female rats learn trace memories better than male rats and consequently retain a greater proportion of new neurons in their hippocampi. *Proc Natl Acad Sci U S A*.
- Damoiseaux VA, Proost JH, Jiawan VCR, Melgert BN (2014) Sex Differences in the

Pharmacokinetics of Antidepressants: Influence of Female Sex Hormones and Oral Contraceptives. *Clin Pharmacokinet* 53:509–519 Available at:
<http://link.springer.com/10.1007/s40262-014-0145-2>.

David DJ, Samuels BA, Rainer Q, Wang J-W, Marsteller D, Mendez I, Drew M, Craig D a, Guiard BP, Guilloux J-P, Artymyshyn RP, Gardier AM, Gerald C, Antonijevic I a, Leonardo ED, Hen R (2009) Neurogenesis-dependent and -independent effects of fluoxetine in an animal model of anxiety/depression. *Neuron* 62:479–493 Available at:
<http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2759281/> [tool=pmcentrez&rendertype=abstract] [Accessed December 16, 2013].

de Brito R de CCM, Lucena-Silva N, Torres LC, Luna CF, Correia J de B, da Silva GAP (2016) The balance between the serum levels of IL-6 and IL-10 cytokines discriminates mild and severe acute pneumonia. *BMC Pulm Med*.

de Chaves G, Moretti M, Castro AA, Dagostin W, da Silva GG, Boeck CR, Quevedo J, Gavioli EC (2009) Effects of long-term ovariectomy on anxiety and behavioral despair in rats. *Physiol Behav*.

De Groot M, Anderson R, Freedland KE, Clouse RE, Lustman PJ (2001) Association of depression and diabetes complications: A meta-analysis. *Psychosom Med*.

De Kloet ER, Joëls M, Holsboer F (2005) Stress and the brain: From adaptation to disease. *Nat Rev Neurosci*.

Dean J, Keshavan M (2017) The neurobiology of depression: An integrated view. *Asian J Psychiatr* 27:101–111.

Detke MJ, Rickels M, Lucki I (1995) Active behaviors in the rat forced swimming test differentially produced by serotonergic and noradrenergic antidepressants.

- Psychopharmacology (Berl).
- Dickmeis T (2009) Glucocorticoids and the circadian clock. *J Endocrinol* 200:3–22.
- Diorio D, Viau V, Meaney MJ (1993) The role of the medial prefrontal cortex (cingulate gyrus) in the regulation of hypothalamic-pituitary-adrenal responses to stress. *J Neurosci*.
- Dixon ML, Thiruchselvam R, Todd R, Christoff K (2017) Emotion and the prefrontal cortex: An integrative review. *Psychol Bull*.
- Dowlati Y, Herrmann N, Swardfager W, Liu H, Sham L, Reim EK, Lanctôt KL (2010) A Meta-Analysis of Cytokines in Major Depression. *Biol Psychiatry* 67:446–457 Available at: <http://dx.doi.org/10.1016/j.biopsych.2009.09.033>.
- Drevets WC (2000) Functional anatomical abnormalities in limbic and prefrontal cortical structures in major depression. In: *Progress in Brain Research*.
- Drevets WC, Price JL, Furey ML (2008) Brain structural and functional abnormalities in mood disorders: Implications for neurocircuitry models of depression. *Brain Struct Funct*.
- Drysdale AT et al. (2017) Resting-state connectivity biomarkers define neurophysiological subtypes of depression. *Nat Med* 23:28–38 Available at: <http://www.ncbi.nlm.nih.gov/pubmed/27918562> [Accessed April 19, 2018].
- Duarte-Guterman P, Lieblich SE, Chow C, Galea LAM (2015) Estradiol and GPER activation differentially affect cell proliferation but not GPER expression in the hippocampus of adult female rats. *PLoS One* 10.
- Dubbelaar ML, Kracht L, Eggen BJL, Boddeke EWGM (2018) The Kaleidoscope of Microglial Phenotypes. *Front Immunol* 9:1753 Available at: <http://www.ncbi.nlm.nih.gov/pubmed/30108586> [Accessed July 23, 2019].
- Dulawa SC, Hen R (2005) Recent advances in animal models of chronic antidepressant effects:

The novelty-induced hypophagia test. *Neurosci Biobehav Rev* 29:771–783 Available at:
www.elsevier.com/locate/neubiorev.

Duman RS, Aghajanian GK, Sanacora G, Krystal JH (2016) Synaptic plasticity and depression: New insights from stress and rapid-acting antidepressants. *Nat Med*.

Duman RS, Li N (2012) A neurotrophic hypothesis of depression: Role of synaptogenesis in the actions of NMDA receptor antagonists. *Philos Trans R Soc B Biol Sci*.

Duric V, Banasr M, Licznerski P, Schmidt HD, Stockmeier CA, Simen AA, Newton SS, Duman RS (2010) A negative regulator of MAP kinase causes depressive behavior. *Nat Med* 16:1328–1332 Available at: <http://www.nature.com/doifinder/10.1038/nm.2219>.

Duric V, Banasr M, Stockmeier CA, Simen AA, Newton SS, Overholser JC, Jurus GJ, Dieter L, Duman RS (2013) Altered expression of synapse and glutamate related genes in post-mortem hippocampus of depressed subjects. *Int J Neuropsychopharmacol*.

Dwivedi Y, Rizavi HS, Conley RR, Pandey GN (2006) ERK MAP kinase signaling in post-mortem brain of suicide subjects: Differential regulation of upstream Raf kinases Raf-1 and B-Raf. *Mol Psychiatry*.

Dwivedi Y, Rizavi HS, Roberts RC, Conley RC, Pandey GN (2001) Reduced activation and expression of ERK1 / 2 MAP kinase in the post-mortem brain of depressed suicide subjects. :916–928.

Eid RS, Chatton JA, Lieblich SE, Bodnar TS, Weinberg J, Galea LAM (2019a) Early and late effects of maternal experience on hippocampal neurogenesis, microglia, and the circulating cytokine milieu. *Neurobiol Aging* 78:1–17 Available at:
<https://www.sciencedirect.com/science/article/pii/S0197458019300351?via%3Dihub>
[Accessed May 28, 2019].

Eid RS, Gobinath AR, Galea LAM (2019b) Sex differences in depression: Insights from clinical and preclinical studies. *Prog Neurobiol* Available at: <https://www.sciencedirect.com.ezproxy.library.ubc.ca/science/article/pii/S030100821830087X?via%3Dihub> [Accessed March 14, 2019].

Eid RS, Gobinath AR, Galea LAM (2019c) Sex differences in depression: Insights from clinical and preclinical studies. *Prog Neurobiol* 176:86–102.

Ekdahl CT, Claassen J-H, Bonde S, Kokaia Z, Lindvall O (2003) Inflammation is detrimental for neurogenesis in adult brain. *Proc Natl Acad Sci* 100:13632–13637 Available at: <http://www.pnas.org/cgi/doi/10.1073/pnas.2234031100>.

Eliwa H, Belzung C, Surget A (2017) Adult hippocampal neurogenesis: Is it the alpha and omega of antidepressant action? *Biochem Pharmacol* 141:86–99.

Eller T, Vasar V, Shlik J, Maron E (2008) Pro-inflammatory cytokines and treatment response to escitalopram in major depressive disorder. *Prog Neuro-Psychopharmacology Biol Psychiatry*.

Elovainio M, Aalto AM, Kivimäki M, Pirkola S, Sundvall J, Lönnqvist J, Reunanen A (2009) Depression and C-reactive protein: Population-based health 2000 study. *Psychosom Med* 71:423–430.

Enache D, Pariante C, Mondelli V (2019) Markers of central inflammation in major depressive disorder: a systematic review and meta-analysis of studies examining cerebrospinal fluid , positron emission tomography and post-mortem brain tissue. *Brain Behav Immun* Available at: <https://www.sciencedirect.com/science/article/pii/S0889159118312121?via%3Dihub> [Accessed June 16, 2019].

Engler H, Benson S, Wegner A, Spreitzer I, Schedlowski M, Elsenbruch S (2016) Men and

- women differ in inflammatory and neuroendocrine responses to endotoxin but not in the severity of sickness symptoms. *Brain Behav Immun* 52:18–26.
- Eriksson PS, Perfilieva E, Björk-Eriksson T, Alborn AM, Nordborg C, Peterson DA, Gage FH (1998) Neurogenesis in the adult human hippocampus. *Nat Med* 4:1313–1317 Available at: <http://www.ncbi.nlm.nih.gov/pubmed/9809557>.
- Estrada-Camarena E, López-Rubalcava C, Hernández-Aragón A, Mejía-Mauries S, Picazo O (2011) Long-term ovariectomy modulates the antidepressant-like action of estrogens, but not of antidepressants. *J Psychopharmacol*.
- Evans DL, Nemerooff CB (1987) The clinical use of the dexamethasone suppression test in DSM-III affective disorders: Correlation with the severe depressive subtypes of melancholia and psychosis. *J Psychiatr Res.*
- Falconer EM, Galea LAM (2003) Sex differences in cell proliferation, cell death and defensive behavior following acute predator odor stress in adult rats. *Brain Res* 975:22–36.
- Fanselow MS, Dong HW (2010) Are the Dorsal and Ventral Hippocampus Functionally Distinct Structures? *Neuron* 65:7–19 Available at: <http://dx.doi.org/10.1016/j.neuron.2009.11.031>.
- Farooq RK, Isingrini E, Tanti A, Le Guisquet AM, Arlicot N, Minier F, Leman S, Chalon S, Belzung C, Camus V (2012) Is unpredictable chronic mild stress (UCMS) a reliable model to study depression-induced neuroinflammation? *Behav Brain Res* 231:130–137 Available at: <http://dx.doi.org/10.1016/j.bbr.2012.03.020>.
- Felger JC (2017) The role of dopamine in inflammation-associated depression: Mechanisms and therapeutic implications. In: *Current Topics in Behavioral Neurosciences*.
- Felger JC (2019) Role of Inflammation in Depression and Treatment Implications. In: *Handbook of Experimental Pharmacology*, pp 255–286. Springer New York LLC.

Felger JC, Haroon E, Miller AH (2015) Risk and resilience: Animal models shed light on the pivotal role of inflammation in individual differences in stress-induced depression. *Biol Psychiatry*.

Ferbinteanu J, Ray C, McDonald RJ (2003) Both dorsal and ventral hippocampus contribute to spatial learning in Long-Evans rats. *Neurosci Lett*.

Fernandez SM, Lewis MC, Pechenino AS, Harburger LL, Orr PT, Gresack JE, Schafe GE, Frick KM (2008) Estradiol-Induced Enhancement of Object Memory Consolidation Involves Hippocampal Extracellular Signal-Regulated Kinase Activation and Membrane-Bound Estrogen Receptors. *J Neurosci*.

Ferrari AJ, Charlson FJ, Norman RE, Patten SB, Freedman G, Murray CJ, Vos T, Whiteford HA (2013) Burden of depressive disorders by country, sex, age, and year: findings from the global burden of disease study 2010. *PLoS Med* 10:e1001547 Available at: <http://www.ncbi.nlm.nih.gov/pubmed/24223526>.

Feyissa AM, Chandran A, Stockmeier CA, Karolewicz B (2009) Reduced levels of NR2A and NR2B subunits of NMDA receptor and PSD-95 in the prefrontal cortex in major depression. *Prog Neuro-Psychopharmacology Biol Psychiatry* 33:70–75.

Figueiredo HF, Ulrich-Lai YM, Choi DC, Herman JP (2007) Estrogen potentiates adrenocortical responses to stress in female rats. *Am J Physiol Metab*.

Finnell JE, Muniz BL, Padi AR, Lombard CM, Moffitt CM, Wood CS, Wilson LB, Reagan LP, Wilson MA, Wood SK (2018) Essential Role of Ovarian Hormones in Susceptibility to the Consequences of Witnessing Social Defeat in Female Rats. *Biol Psychiatry*.

Fogaça M V., Duman RS (2019) Cortical GABAergic dysfunction in stress and depression: New insights for therapeutic interventions. *Front Cell Neurosci*.

Frank MG, Baratta M V., Sprunger DB, Watkins LR, Maier SF (2007) Microglia serve as a neuroimmune substrate for stress-induced potentiation of CNS pro-inflammatory cytokine responses. *Brain Behav Immun.*

Franscina Pinto E, Andrade C (2016) Interferon-Related Depression: A Primer on Mechanisms, Treatment, and Prevention of a Common Clinical Problem. *Curr Neuropharmacol.*

Freeman EW, Sammel MD, Boorman DW, Zhang R (2014) Longitudinal pattern of depressive symptoms around natural menopause. *JAMA Psychiatry.*

Freeman EW, Sammel MD, Lin H, Nelson DB (2006) Associations of hormones and menopausal status with depressed mood in women with no history of depression. *Arch Gen Psychiatry.*

Freeman EW, Sammel MD, Liu L, Gracia CR, Nelson DB, Hollander L (2004) Hormones and Menopausal Status as Predictors of Depression in Women in Transition to Menopause. *Arch Gen Psychiatry* 61:62 Available at:

<http://archpsyc.jamanetwork.com/article.aspx?doi=10.1001/archpsyc.61.1.62> [Accessed July 10, 2019].

Friedrich MJ (2017) Depression Is the Leading Cause of Disability Around the World. *JAMA.* Frodl T, Meisenzahl EM, Zetsche T, Born C, Groll C, Jäger M, Leinsinger G, Bottlender R, Hahn K, Möller HJ (2002) Hippocampal changes in patients with a first episode of major depression. *Am J Psychiatry* 159:1112–1118.

Frokjaer VG, Pinborg A, Holst KK, Overgaard A, Henningsson S, Heede M, Larsen EC, Jensen PS, Agn M, Nielsen AP, Stenbæk DS, da Cunha-Bang S, Lehle S, Siebner HR, Mikkelsen JD, Svarer C, Knudsen GM (2015) Role of Serotonin Transporter Changes in Depressive Responses to Sex-Steroid Hormone Manipulation: A Positron Emission Tomography Study.

Biol Psychiatry 78:534–543.

Frye CA, Petralia SM, Rhodes ME (2000) Estrous cycle and sex differences in performance on anxiety tasks coincide with increases in hippocampal progesterone and 3 α ,5 α -THP. Pharmacol Biochem Behav.

Galea LAM, McEwen BS, Tanapat P, Deak T, Spencer RL, Dhabhar FS (1997) Sex differences in dendritic atrophy of CA3 pyramidal neurons in response to chronic restraint stress. Neuroscience 81:689–697.

Galea LAM, Roes MM, Dimech CJ, Chow C, Mahmoud R, Lieblich SE, Duarte-Guterman P (2018) Premarin has opposing effects on spatial learning, neural activation, and serum cytokine levels in middle-aged female rats depending on reproductive history. Neurobiol Aging.

Galea LAM, Wainwright SR, Roes MM, Duarte-Guterman P, Chow C, Hamson DK (2013) Sex, hormones and neurogenesis in the hippocampus: Hormonal modulation of neurogenesis and potential functional implications. J Neuroendocrinol 25:1039–1061.

Galea LAM, Wide JK, Barr AM (2001) Estradiol alleviates depressive-like symptoms in a novel animal model of post-partum depression. Behav Brain Res.

Ganter S, Northoff H, Männel D, Gebicke-Härter PJ (1992) Growth control of cultured microglia. J Neurosci Res.

Garcia AN, Bezner K, Depena C, Yin W, Gore AC (2017) The effects of long-term estradiol treatment on social behavior and gene expression in adult female rats. Horm Behav.

Garrett JE, Wellman CL (2009) Chronic stress effects on dendritic morphology in medial prefrontal cortex: sex differences and estrogen dependence. Neuroscience 162:195–207.

Gaskins AJ, Wilchesky M, Mumford SL, Whitcomb BW, Browne RW, Wactawski-Wende J,

- Perkins NJ, Schisterman EF (2012) Endogenous reproductive hormones and C-reactive protein across the menstrual cycle: The BioCycle Study. *Am J Epidemiol* 175:423–431.
- Georgakis MK, Thomopoulos TP, Diamantaras AA, Kalogirou EI, Skalkidou A, Daskalopoulou SS, Petridou ET (2016) Association of age at menopause and duration of reproductive period with depression after menopause: A systematic review and meta-analysis. *JAMA Psychiatry*.
- Giebing-Kröll C, Berger P, Lepperdinger G, Grubeck-Loebenstein B (2015) How sex and age affect immune responses, susceptibility to infections, and response to vaccination. *Aging Cell*.
- Gobinath AR, Mahmoud R, Galea LAM (2015) Influence of sex and stress exposure across the lifespan on endophenotypes of depression: Focus on behavior, glucocorticoids, and hippocampus. *Front Neurosci* 9.
- Gobinath AR, Workman JL, Chow C, Lieblich SE, Galea LAM (2016) Maternal postpartum corticosterone and fluoxetine differentially affect adult male and female offspring on anxiety-like behavior, stress reactivity, and hippocampal neurogenesis. *Neuropharmacology*.
- Godbout JP, Moreau M, Lestage J, Chen J, Sparkman NL, O'Connor J, Castanon N, Kelley KW, Dantzer R, Johnson RW (2008) Aging exacerbates depressive-like behavior in mice in response to activation of the peripheral innate immune system. *Neuropsychopharmacology*.
- Goel N, Workman JL, Lee TT, Innala L, Viau V (2014) Sex differences in the HPA axis. *Compr Physiol* 4:1121–1155.
- Gogos A, McCarthy M, Walker AJ, Udwawela M, Gibbons A, Dean B, Kuslje S (2018) Differential effects of chronic 17 β -oestradiol treatment on rat behaviours relevant to

depression. *J Neuroendocrinol.*

Gold PW (2015) The organization of the stress system and its dysregulation in depressive illness. *Mol Psychiatry* 20:32–47.

Gonçalves JT, Schafer ST, Gage FH (2016) Adult Neurogenesis in the Hippocampus: From Stem Cells to Behavior. *Cell* 167:897–914.

Goodman SH (2007) Depression in mothers. *Annu Rev Clin Psychol* 3:107–135 Available at: <http://www.ncbi.nlm.nih.gov/pubmed/17716050> [Accessed August 21, 2019].

Goshen I, Kreisel T, Ben-Menachem-Zidon O, Licht T, Weidenfeld J, Ben-Hur T, Yirmiya R (2008) Brain interleukin-1 mediates chronic stress-induced depression in mice via adrenocortical activation and hippocampal neurogenesis suppression. *Mol Psychiatry*.

Goshen I, Yirmiya R (2009) Interleukin-1 (IL-1): A central regulator of stress responses. *Front Neuroendocrinol.*

Gouin JP, Glaser R, Malarkey WB, Beversdorf D, Kiecolt-Glaser J (2012) Chronic stress, daily stressors, and circulating inflammatory markers. *Heal Psychol*.

Gould E, Reeves AJ, Fallah M, Tanapat P, Gross CG, Fuchs E (1999) Hippocampal neurogenesis in adult Old World primates. *Proc Natl Acad Sci* 96:5263–5267 Available at: <http://eutils.ncbi.nlm.nih.gov/entrez/eutils/elink.fcgi?dbfrom=pubmed&id=10220454&retmode=ref&cmd=prlinks%5Cnpapers3://publication/uuid/9AA65B74-22D9-4E29-B4B7-A144BB0B5E6F>.

Gould E, Tanapat P, McEwen BS, Flugge G, Fuchs E (1998) Proliferation of granule cell precursors in the dentate gyrus of adult monkeys is diminished by stress. *Proc Natl Acad Sci* 95:3168–3171 Available at: <http://www.pnas.org/cgi/doi/10.1073/pnas.95.6.3168>.

Green AD, Barr AM, Galea LAM (2009) Role of estradiol withdrawal in “anhedonic” sucrose

- consumption: A model of postpartum depression. *Physiol Behav*.
- Green AD, Galea LAM (2008) Adult hippocampal cell proliferation is suppressed with estrogen withdrawal after a hormone-simulated pregnancy. *Horm Behav* 54:203–211.
- Greicius MD, Flores BH, Menon V, Glover GH, Solvason HB, Kenna H, Reiss AL, Schatzberg AF (2007) Resting-State Functional Connectivity in Major Depression: Abnormally Increased Contributions from Subgenual Cingulate Cortex and Thalamus. *Biol Psychiatry* 62:429–437.
- Griffin MG, Resick PA, Yehuda R (2005) Enhanced cortisol suppression following dexamethasone administration in domestic violence survivors. *Am J Psychiatry*.
- Grippo AJ, Sullivan NR, Damjanoska KJ, Crane JW, Carrasco GA, Shi J, Chen Z, Garcia F, Muma NA, Van De Kar LD (2005) Chronic mild stress induces behavioral and physiological changes, and may alter serotonin 1A receptor function, in male and cycling female rats. *Psychopharmacology (Berl)*.
- Grossman CJ (1985) Interactions between the gonadal steroids and the immune system. *Science* (80-).
- Guechot J, Lepine JP, Cohen C, Fiet J, Lemperiere T, Dreux C (1988) Simple laboratory test of neuroendocrine disturbance in depression: 11 p.m. saliva cortisol. *Neuropsychobiology*.
- Guirado R, Sanchez-Matarredona D, Varea E, Crespo C, Blasco-Ibáñez JM, Nacher J (2012) Chronic fluoxetine treatment in middle-aged rats induces changes in the expression of plasticity-related molecules and in neurogenesis. *BMC Neurosci*.
- Gundlah C, Lu NZ, Bethea CL (2002) Ovarian steroid regulation of monoamine oxidase-A and B mRNAs in the macaque dorsal raphe and hypothalamic nuclei. *Psychopharmacology (Berl)*.

- Gutiérrez-Lobos K, Scherer M, Anderer P, Katschnig H (2002) The influence of age on the female/male ratio of treated incidence rates in depression. *BMC Psychiatry*.
- Haapakoski R, Mathieu J, Ebmeier KP, Alenius H, Kivimäki M (2015) Cumulative meta-analysis of interleukins 6 and 1 β , tumour necrosis factor α and C-reactive protein in patients with major depressive disorder. *Brain Behav Immun* 49:206–215.
- Habib P, Beyer C (2015) Regulation of brain microglia by female gonadal steroids. *J Steroid Biochem Mol Biol* 146:3–14 Available at: <http://dx.doi.org/10.1016/j.jsbmb.2014.02.018>.
- Hamson DK, Roes MM, Galea LAM (2016) Sex Hormones and Cognition: Neuroendocrine Influences on Memory and Learning. *Compr Physiol*.
- Hamson DK, Wainwright SR, Taylor JR, Jones BA, Watson N V., Galea LAM (2013) Androgens increase survival of adult-born neurons in the dentate gyrus by an androgen receptor-dependent mechanism in male rats. *Endocrinology* 154:3294–3304.
- Hantsoo L, Epperson CN (2015) Premenstrual Dysphoric Disorder: Epidemiology and Treatment. *Curr Psychiatry Rep*.
- Harburger LL, Bennett JC, Frick KM (2007) Effects of estrogen and progesterone on spatial memory consolidation in aged females. *Neurobiol Aging*.
- Haroon E, Miller AH (2017) Inflammation effects on brain glutamate in depression: Mechanistic considerations and treatment implications. In: *Current Topics in Behavioral Neurosciences*.
- Hasin DS, Sarvet AL, Meyers JL, Saha TD, Ruan WJ, Stohl M, Grant BF (2018) Epidemiology of adult DSM-5 major depressive disorder and its specifiers in the United States. *JAMA Psychiatry* 75:336–346.
- He J, Evans CO, Hoffman SW, Oyesiku NM, Stein DG (2004) Progesterone and allopregnanolone reduce inflammatory cytokines after traumatic brain injury. *Exp Neurol*.

Hendrick V, Altshuler LL, Suri R (1998) Hormonal changes in the postpartum and implications for postpartum depression. *Psychosomatics* 39:93–101.

Herman JP, McKlveen JM, Ghosal S, Kopp B, Wulsin A, Makinson R, Scheimann J, Myers B (2016) Regulation of the hypothalamic-pituitary- adrenocortical stress response. *Compr Physiol* 6:603–621.

Heshmati M, Russo SJ (2015) Anhedonia and the Brain Reward Circuitry in Depression. *Curr Behav Neurosci Reports*.

Hewitt SC, Korach KS (2018) Estrogen Receptors: New Directions in the New Millennium. Available at: <https://academic.oup.com/edrv>.

Hildebrandt MG, Stage KB, Kragh-Soerensen P (2003) Gender differences in severity, symptomatology and distribution of melancholia in major depression. *Psychopathology* 36:204–212.

Hillerer KM, Neumann ID, Couillard-Despres S, Aigner L, Slattery DA (2013) Sex-dependent regulation of hippocampal neurogenesis under basal and chronic stress conditions in rats. *Hippocampus* 23:476–487.

Hodes GE et al. (2014) Individual differences in the peripheral immune system promote resilience versus susceptibility to social stress. *Proc Natl Acad Sci U S A*.

Hodes GE et al. (2015a) Sex Differences in Nucleus Accumbens Transcriptome Profiles Associated with Susceptibility versus Resilience to Subchronic Variable Stress. *J Neurosci* 35:16362–16376 Available at:

<http://www.jneurosci.org/cgi/doi/10.1523/JNEUROSCI.1392-15.2015>.

Hodes GE, Kana V, Menard C, Merad M, Russo SJ (2015b) Neuroimmune mechanisms of depression. *Nat Neurosci* 18:1386–1393 Available at:

<http://www.nature.com/doifinder/10.1038/nn.4113>.

Hodes GE, Ménard C, Russo SJ (2016) Integrating Interleukin-6 into depression diagnosis and treatment. *Neurobiol Stress.*

Hollis F, Kabbaj M (2014) Social defeat as an animal model for depression. *ILAR J.*

Hollos P, Marchisella F, Coffey ET (2018) JNK Regulation of Depression and Anxiety. *Brain Plast* 3:145–155.

Hong S, Beja-Glasser VF, Nfonoyim BM, Frouin A, Li S, Ramakrishnan S, Merry KM, Shi Q, Rosenthal A, Barres BA, Lemere CA, Selkoe DJ, Stevens B (2016) Complement and microglia mediate early synapse loss in Alzheimer mouse models. *Science* (80-).

Howard DM et al. (2019) Genome-wide meta-analysis of depression identifies 102 independent variants and highlights the importance of the prefrontal brain regions. *bioRxiv*:433367 Available at: <https://www.biorxiv.org/content/early/2019/01/08/433367>.

Hsieh JT, Lei B, Sheng H, Venkatraman T, Lascola CD, Warner DS, James ML (2016) Sex-specific effects of progesterone on early outcome of intracerebral hemorrhage. *Neuroendocrinology*.

Hsiung SC, Adlersberg M, Arango V, Mann JJ, Tamir H, Liu KP (2003) Attenuated 5-HT1A receptor signaling in brains of suicide victims: Involvement of adenylyl cyclase, phosphatidylinositol 3-kinase, Akt and mitogen-activated protein kinase. *J Neurochem.*

Hudson SP, Jacobson-Pick S, Anisman H (2014) Sex differences in behavior and pro-inflammatory cytokine mRNA expression following stressor exposure and re-exposure. *Neuroscience* 277:239–249.

Ishihara Y, Itoh K, Ishida A, Yamazaki T (2015) Selective estrogen-receptor modulators suppress microglial activation and neuronal cell death via an estrogen receptor-dependent

pathway. *J Steroid Biochem Mol Biol.*

Ising M, Horstmann S, Kloiber S, Lucae S, Binder EB, Kern N, Künzel HE, Pfennig A, Uhr M, Holsboer F (2007) Combined dexamethasone/corticotropin releasing hormone test predicts treatment response in major depression - a potential biomarker? *Biol Psychiatry* 62:47–54 Available at: <http://www.ncbi.nlm.nih.gov/pubmed/17123470>.

Isingrini E, Camus V, Le Guisquet AM, 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.*

Iwasaki-Sekino A, Mano-Otagiri A, Ohata H, Yamauchi N, Shibasaki T (2009) Gender differences in corticotropin and corticosterone secretion and corticotropin-releasing factor mRNA expression in the paraventricular nucleus of the hypothalamus and the central nucleus of the amygdala in response to footshock stress or psychological. *Psychoneuroendocrinology.*

James SL et al. (2018) Global, regional, and national incidence, prevalence, and years lived with disability for 354 Diseases and Injuries for 195 countries and territories, 1990-2017: A systematic analysis for the Global Burden of Disease Study 2017. *Lancet.*

Johnson AB, Sohrabji F (2005) Estrogen's effects on central and circulating immune cells vary with reproductive age. *Neurobiol Aging.*

Johnson GL, Lapadat R (2002) Mitogen-activated protein kinase pathways mediated by ERK, JNK, and p38 protein kinases. *Science* (80-).

Kaiser RH, Andrews-Hanna JR, Wager TD, Pizzagalli DA (2015) Large-scale network dysfunction in major depressive disorder: A meta-analysis of resting-state functional connectivity. *JAMA Psychiatry.*

- Kalantaridou SN, Makrigiannakis A, Zoumakis E, Chrousos GP (2004) Stress and the female reproductive system. In: *Journal of Reproductive Immunology*.
- Kallarackal AJ, Kvarta MD, Cammarata E, Jaber L, Cai X, Bailey AM, Thompson SM (2013) Chronic Stress Induces a Selective Decrease in AMPA Receptor-Mediated Synaptic Excitation at Hippocampal Temporoammonic-CA1 Synapses. *J Neurosci*.
- Kalynchuk LE, Gregus A, Boudreau D, Perrot-Sinal TS (2004) Corticosterone increases depression-like behavior, with some effects on predator odor-induced defensive behavior, in male and female rats. *Behav Neurosci*.
- Kamper EF, Chatzigeorgiou A, Tsimpoukidi O, Kamper M, Dalla C, Pitychoutis P, Papadopoulou-Daifoti Z (2009) Sex differences in oxidant/antioxidant balance under a chronic mild stress regime. *Physiol Behav*.
- Kanes S, Colquhoun H, Gunduz-Bruce H, Raines S, Arnold R, Schacterle A, Doherty J, Epperson CN, Deligiannidis KM, Riesenber R, Hoffmann E, Rubinow D, Jonas J, Paul S, Meltzer-Brody S (2017) Brexanolone (SAGE-547 injection) in post-partum depression: a randomised controlled trial. *Lancet* 390:480–489.
- Kang HJ, Voleti B, Hajszan T, Rajkowska G, Stockmeier CA, Licznerski P, Lepack A, Majik MS, Jeong LS, Banasr M, Son H, Duman RS (2012) Decreased expression of synapse-related genes and loss of synapses in major depressive disorder. *Nat Med*.
- Karperien A, Ahammer H, Jelinek HF (2013) Quantitating the subtleties of microglial morphology with fractal analysis. *Front Cell Neurosci*.
- Kastenberger I, Lutsch C, Schwarzer C (2012) Activation of the G-protein-coupled receptor GPR30 induces anxiogenic effects in mice, similar to oestradiol. *Psychopharmacol*:527–535

Available at: <http://empress.har.mrc.ac.uk>.

- Kato A, Hojo Y, Higo S, Komatsuzaki Y, Murakami G, Yoshino H, Uebayashi M, Kawato S (2013) Female hippocampal estrogens have a significant correlation with cyclic fluctuation of hippocampal spines. *Front Neural Circuits*.
- Katz RJ (1981) Animal model of depression: Effects of electroconvulsive shock therapy. *Neurosci Biobehav Rev*.
- Katz RJ (1982) Animal model of depression: Pharmacological sensitivity of a hedonic deficit. *Pharmacol Biochem Behav*.
- Keilhoff G, Becker A, Grecksch G, Bernstein HG, Wolf G (2006) Cell proliferation is influenced by bulbectomy and normalized by imipramine treatment in a region-specific manner. *Neuropsychopharmacology* 31:1165–1176.
- Kempermann G, Gage FH, Aigner L, Song H, Curtis MA, Thuret S, Kuhn HG, Jessberger S, Frankland PW, Cameron HA, Gould E, Hen R, Abrous DN, Toni N, Schinder AF, Zhao X, Lucassen PJ, Frisén J (2018) Human Adult Neurogenesis: Evidence and Remaining Questions. *Cell Stem Cell* 23:25–30 Available at: <http://www.ncbi.nlm.nih.gov/pubmed/29681514> [Accessed August 22, 2019].
- Kempermann G, Song H, Gage FH (2015) Neurogenesis in the Adult Hippocampus. *Cold Spring Harb Perspect Biol* 7:a018812 Available at: <http://cshperspectives.cshlp.org/lookup/doi/10.1101/cshperspect.a018812> [Accessed August 23, 2019].
- Kendler KS, Karkowski LM, Prescott CA (1999) Causal Relationship Between Stressful Life Events and the Onset of Major Depression. *Psychiatry Interpers Biol Process* 156:837–841 Available at: <http://ajp.psychiatryonline.org/cgi/content/abstract/156/6/837>.
- Kesner RP, Hunsaker MR, Ziegler W (2011) The role of the dorsal and ventral hippocampus in

- olfactory working memory. *Neurobiol Learn Mem.*
- Kessler RC (1997) the Effects of Stressful Life Events on Depression. *Annu Rev Psychol* 48:191–214.
- Kessler RC, Bromet EJ (2013) The epidemiology of depression across cultures. *Annu Rev Public Health* 34:119–138 Available at: <http://www.ncbi.nlm.nih.gov/pubmed/23514317> [Accessed February 23, 2014].
- Kettenmann H, Kirchhoff F, Verkhratsky A (2013) Microglia: New Roles for the Synaptic Stripper. *Neuron* 77:10–18 Available at: <http://dx.doi.org/10.1016/j.neuron.2012.12.023>.
- Khan A, Brodhead AE, Schwartz KA, Kolts RL, Brown WA (2005) Sex differences in antidepressant response in recent antidepressant clinical trials. *J Clin Psychopharmacol.*
- Khandaker GM, Pearson RM, Zammit S, Lewis G, Jones PB (2014) Association of serum interleukin 6 and C-reactive protein in childhood with depression and psychosis in young adult life a population-based longitudinal study. *JAMA Psychiatry*.
- Kim DM, Leem YH (2016) Chronic stress-induced memory deficits are reversed by regular exercise via AMPK-mediated BDNF induction. *Neuroscience*.
- Kim E, Sheng M (2004) PDZ DOMAIN PROTEINS OF SYNAPSES. *Nat Rev | Neurosci* 5:1 Available at: www.nature.com/reviews/neuro.
- Kinsey SG, Bailey MT, Sheridan JF, Padgett DA (2008) The inflammatory response to social defeat is increased in older mice. *Physiol Behav*.
- KITAY JI (1961) Sex differences in adrenal cortical secretion in the rat. *Endocrinology*.
- Klein SL, Flanagan KL (2016) Sex differences in immune responses. *Nat Rev Immunol* 16:626–638 Available at: <http://www.nature.com/doifinder/10.1038/nri.2016.90>.
- Koebele S V., Bimonte-Nelson HA (2016) Modeling menopause: The utility of rodents in

- translational behavioral endocrinology research. *Maturitas*.
- Koenigs M, Grafman J (2009) The functional neuroanatomy of depression: Distinct roles for ventromedial and dorsolateral prefrontal cortex. *Behav Brain Res* 201:239–243.
- Köhler-Forsberg O, Buttenschøn HN, Tansey KE, Maier W, Hauser J, Dernovsek MZ, Henigsberg N, Souery D, Farmer A, Rietschel M, McGuffin P, Aitchison KJ, Uher R, Mors O (2017) Association between C-reactive protein (CRP) with depression symptom severity and specific depressive symptoms in major depression. *Brain Behav Immun* 62:344–350.
- Köhler CA, Freitas TH, Maes M, de Andrade NQ, Liu CS, Fernandes BS, Stubbs B, Solmi M, Veronese N, Herrmann N, Raison CL, Miller BJ, Lanctôt KL, Carvalho AF (2017) Peripheral cytokine and chemokine alterations in depression: a meta-analysis of 82 studies. *Acta Psychiatr Scand*.
- Koolschijn PCMP, Van Haren NEM, Lensvelt-Mulders GJLM, Hulshoff Pol HE, Kahn RS (2009) Brain volume abnormalities in major depressive disorder: A meta-analysis of magnetic resonance imaging studies. *Hum Brain Mapp* 30:3719–3735.
- Kornstein SG, Harvey AT, Rush AJ, Wisniewski SR, Trivedi MH, Svikis DS, McKenzie ND, Bryan C, Harley R (2005) Self-reported premenstrual exacerbation of depressive symptoms in patients seeking treatment for major depression. *Psychol Med*.
- Kornstein SG, Pedersen RD, Holland PJ, Nemeroff CB, Rothschild AJ, Thase ME, Trivedi MH, Ninan PT, Keller MB (2014) Influence of Sex and Menopausal Status on Response, Remission, and Recurrence in Patients With Recurrent Major Depressive Disorder Treated With Venlafaxine Extended Release or Fluoxetine. *J Clin Psychiatry*.
- Kornstein SG, Schatzberg AF, Thase ME, Yonkers KA, McCullough JP, Keitner GI, Gelenberg AJ, Davis SM, Harrison WM, Keller MB (2000) Gender differences in treatment response

- to sertraline versus imipramine in chronic depression. *Am J Psychiatry* 157:1445–1452.
- Kornstein SG, Toups M, Rush AJ, Wisniewski SR, Thase ME, Luther J, Warden D, Fava M, Trivedi MH (2013) Do menopausal status and use of hormone therapy affect antidepressant treatment response? Findings from the sequenced treatment alternatives to relieve depression (STAR*D) study. *J Women's Heal.*
- Korzhevskii DE, Kirik O V. (2016) Brain Microglia and Microglial Markers. *Neurosci Behav Physiol* 46:284–290.
- Kovats S (2015) Estrogen receptors regulate innate immune cells and signaling pathways. *Cell Immunol.*
- Kreisel T, Frank MG, Licht T, Reshef R, Ben-Menachem-Zidon O, Baratta M V., Maier SF, Yirmiya R (2014) Dynamic microglial alterations underlie stress-induced depressive-like behavior and suppressed neurogenesis. *Mol Psychiatry* 19:699–709.
- Kreutzberg GW (1996) Microglia: A sensor for pathological events in the CNS. *Trends Neurosci.*
- Kringelbach ML, Rolls ET (2004) The functional neuroanatomy of the human orbitofrontal cortex: Evidence from neuroimaging and neuropsychology. *Prog Neurobiol.*
- Krishnan V, Nestler EJ (2008) The molecular neurobiology of depression. *Nature.*
- Krishnan V, Nestler EJ (2011) Animal models of depression: Molecular perspectives. *Curr Top Behav Neurosci.*
- Kronmüller KT, Schröder J, Köhler S, Götz B, Victor D, Unger J, Giesel F, Magnotta V, Mundt C, Essig M, Pantel J (2009) Hippocampal volume in first episode and recurrent depression. *Psychiatry Res - Neuroimaging* 174:62–66.
- Kubera M, Obuchowicz E, Goehler L, Brzeszcz J, Maes M (2011) In animal models,

psychosocial stress-induced (neuro)inflammation, apoptosis and reduced neurogenesis are associated to the onset of depression. *Prog Neuro-Psychopharmacology Biol Psychiatry*.

Kuhn HG, Dickinson-Anson H, Gage FH (1996) Neurogenesis in the dentate gyrus of the adult rat: age-related decrease of neuronal progenitor proliferation. *J Neurosci* 16:2027–2033 Available at: <http://www.ncbi.nlm.nih.gov/pubmed/8604047>.

Labonté B et al. (2017) Sex-specific transcriptional signatures in human depression. *Nat Med* 23:1102–1111 Available at: <http://www.nature.com/doifinder/10.1038/nm.4386>.

Laflamme N, Nappi RE, Drolet G, Labrie C, Rivest S (1998) Expression and neuropeptidergic characterization of estrogen receptors (ER α and ER β) throughout the rat brain: Anatomical evidence of distinct roles of each subtype. *J Neurobiol*.

Lagunas N, Calmarza-Font I, Diz-Chaves Y, Garcia-Segura LM (2010) Long-term ovariectomy enhances anxiety and depressive-like behaviors in mice submitted to chronic unpredictable stress. *Horm Behav* 58:786–791.

LaPlant Q, Chakravarty S, Vialou V, Mukherjee S, Koo JW, Kalahasti G, Bradbury KR, Taylor S V., Maze I, Kumar A, Graham A, Birnbaum SG, Krishnan V, Truong HT, Neve RL, Nestler EJ, Russo SJ (2009) Role of Nuclear Factor κ B in Ovarian Hormone-Mediated Stress Hypersensitivity in Female Mice. *Biol Psychiatry* 65:874–880 Available at: <https://www-sciencedirect-com.ezproxy.library.ubc.ca/science/article/pii/S0006322309001073?via%3Dihub#fig1> [Accessed July 11, 2019].

Laubach M, Amarante LM, Swanson K, White SR (2018) What, if anything, is rodent prefrontal cortex? *eNeuro*.

Lee EE, Nieman LK, Martinez PE, Harsh VL, Rubinow DR, Schmidt PJ (2012) ACTH and

cortisol response to Dex/CRH testing in women with and without premenstrual dysphoria during GnRH agonist-induced hypogonadism and ovarian steroid replacement. *J Clin Endocrinol Metab.*

Lei B, Mace B, Dawson HN, Warner DS, Laskowitz DT, James ML (2014) Anti-inflammatory effects of progesterone in lipopolysaccharide-stimulated BV-2 microglia. *PLoS One* 9.

Leuner B, Mendolia-Loffredo S, Shors TJ (2004) Males and females respond differently to controllability and antidepressant treatment. *Biol Psychiatry*.

Levin ER, Hammes SR (2016) Nuclear receptors outside the nucleus: extranuclear signalling by steroid receptors. *Nat Rev Mol Cell Biol.*

Levitian RD, Vaccarino FJ, Brown GM, Kennedy SH (2002) Low-dose dexamethasone challenge in women with atypical major depression: Pilot study. *J Psychiatry Neurosci.*

Lewis DK, Johnson AB, Stohlgren S, Harms A, Sohrabji F (2008) Effects of estrogen receptor agonists on regulation of the inflammatory response in astrocytes from young adult and middle-aged female rats. *J Neuroimmunol.*

Li C, Brake WG, Romeo RD, Dunlop JC, Gordon M, Buzescu R, Magarinos AM, Allen PB, Greengard P, Luine V, McEwen BS (2004) Estrogen alters hippocampal dendritic spine shape and enhances synaptic protein immunoreactivity and spatial memory in female mice. *Proc Natl Acad Sci.*

Li LH, Wang ZC, Yu J, Zhang YQ (2014) Ovariectomy results in variable changes in nociception, mood and depression in adult female rats. *PLoS One.*

Li Y, Pehrson AL, Budac DP, Sánchez C, Gulinello M (2012) A rodent model of premenstrual dysphoria: Progesterone withdrawal induces depression-like behavior that is differentially sensitive to classes of antidepressants. *Behav Brain Res.*

Liu MY, Yin CY, Zhu LJ, Zhu XH, Xu C, Luo CX, Chen H, Zhu DY, Zhou QG (2018) Sucrose preference test for measurement of stress-induced anhedonia in mice. *Nat Protoc* 13:1686–1698 Available at: <https://doi.org/10.1038/s41596-018-0011-z>.

Liu Y, Chun-Man Ho R, Mak A (2012) Interleukin (IL)-6, tumour necrosis factor alpha (TNF- α) and soluble interleukin-2 receptors (sIL-2R) are elevated in patients with major depressive disorder: A meta-analysis and meta-regression.

Lopez-Duran NL, Kovacs M, George CJ (2009) Hypothalamic-pituitary-adrenal axis dysregulation in depressed children and adolescents: A meta-analysis. *Psychoneuroendocrinology*.

Lorenzetti V, Allen NB, Fornito A, Yücel M (2009) Structural brain abnormalities in major depressive disorder: A selective review of recent MRI studies. *J Affect Disord* 117:1–17.

Lorsch ZS et al. (2018) Estrogen receptor α drives pro-resilient transcription in mouse models of depression. *Nat Commun*.

Lotan A, Lifschytz T, Wolf G, Keller S, Ben-Ari H, Tatarsky P, Pillar N, Oved K, Sharabany J, Merzel TK, Matsumoto T, Yamawaki Y, Mernick B, Avidan E, Yamawaki S, Weller A, Shomron N, Lerer B (2018) Differential effects of chronic stress in young-adult and old female mice: Cognitive-behavioral manifestations and neurobiological correlates. *Mol Psychiatry*.

Lu NZ, Bethea CL (2002) Ovarian steroid regulation of 5-HT1A receptor binding and G protein activation in female monkeys. *Neuropsychopharmacology*.

Lucassen PJ, Oomen CA, Naninck EFG, Fitzsimons CP, Van Dam AM, Czech B, Korosi A (2015) Regulation of adult neurogenesis and plasticity by (Early) stress, glucocorticoids, and inflammation. *Cold Spring Harb Perspect Biol*.

- Luine VN (2014) Estradiol and cognitive function: Past, present and future. *Horm Behav.*
- Lund TD, Rovis T, Chung WCJ, Handa RJ (2005) Novel actions of estrogen receptor- β on anxiety-related behaviors. *Endocrinology.*
- MacQueen GM, Campbell S, McEwen BS, Macdonald K, Amano S, Joffe RT, Nahmias C, Young LT (2003) Course of illness, hippocampal function, and hippocampal volume in major depression. *Proc Natl Acad Sci U S A* 100:1387–1392 Available at: <http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=298782&tool=pmcentrez&rendertype=abstract>.
- Maes M, Berk M, Goehler L, Song C, Anderson G, Gałecki P, Leonard B (2012) Depression and sickness behavior are Janus-faced responses to shared inflammatory pathways. *BMC Med* 10.
- Maes M, Claes M, Schotte C, Delbeke L, Jacquemyn Y, Verkerk R, De Meester I, Scharpé S (1992) Disturbances in dexamethasone suppression test and lower availability of L-tryptophan and tyrosine in early puerperium and in women under contraceptive therapy. *J Psychosom Res.*
- Mahajan GJ, Vallender EJ, Garrett MR, Challagundla L, Overholser JC, Jurus G, Dieter L, Syed M, Romero DG, Benguzzi H, Stockmeier CA (2018) Altered neuro-inflammatory gene expression in hippocampus in major depressive disorder. *Prog Neuro-Psychopharmacology Biol Psychiatry.*
- Mahmoud R, Wainwright SR, Chatton JA, Lieblich SE, Galea LAM (2016a) Ovarian hormones, but not fluoxetine, impart resilience within a chronic unpredictable stress model in middle-aged female rats. *Neuropharmacology* 107:278–293 Available at: <http://dx.doi.org/10.1016/j.neuropharm.2016.01.033>.

Mahmoud R, Wainwright SR, Galea LAM (2016b) Sex hormones and adult hippocampal neurogenesis: Regulation, implications, and potential mechanisms. Available at: <http://dx.doi.org/10.1016/j.yfrne.2016.03.002>.

Malberg JE, Eisch a J, Nestler EJ, Duman RS (2000) Chronic antidepressant treatment increases neurogenesis in adult rat hippocampus. *J Neurosci* 20:9104–9110.

Malendowicz LK (1980) Sex differences in adrenocortical structure and function. VI. Long-term effect of gonadectomy and testosterone or estradiol replacement on rat adrenal cortex. *Endokrinologie* 75:311–323 Available at: <http://www.ncbi.nlm.nih.gov/pubmed/6775939> [Accessed September 6, 2019].

Malki K, Mineur YS, Tosto MG, Campbell J, Karia P, Jumabhoy I, Sluyter F, Crusio WE, Schalkwyk LC (2015) Pervasive and opposing effects of Unpredictable Chronic Mild Stress (UCMS) on hippocampal gene expression in BALB/cJ and C57BL/6J mouse strains. *BMC Genomics*.

Marcus SM, Kerber KB, Rush AJ, Wisniewski SR, Nierenberg A, Balasubramani GK, Ritz L, Kornstein S, Young EA, Trivedi MH (2008) Sex differences in depression symptoms in treatment-seeking adults: confirmatory analyses from the Sequenced Treatment Alternatives to Relieve Depression study. *Compr Psychiatry* 49:238–246.

Marcus SM, Young EA, Kerber KB, Kornstein S, Farabaugh AH, Mitchell J, Wisniewski SR, Balasubramani GK, Trivedi MH, Rush AJ (2005) Gender differences in depression: Findings from the STAR*D study. *J Affect Disord* 87:141–150.

Marino M, Galluzzo P, Ascenzi P (2006) Estrogen Signaling Multiple Pathways to Impact Gene Transcription. *Curr Genomics*.

Marrocco J, Einhorn NR, Petty GH, Li H, Dubey N, Hoffman J, Berman KF, Goldman D, Lee

FS, Schmidt PJ, McEwen BS (2018) Epigenetic intersection of BDNF Val66Met genotype with premenstrual dysphoric disorder transcriptome in a cross-species model of estradiol add-back. *Mol Psychiatry*.

Marsh WK, Bromberger JT, Crawford SL, Leung K, Kravitz HM, Randolph JF, Joffe H, Soares CN (2017) Lifelong estradiol exposure and risk of depressive symptoms during the transition to menopause and postmenopause. *Menopause*.

Mazzucco CA, Lieblich SE, Bingham BI, Williamson MA, Viau V, Galea LAM (2006) Both estrogen receptor α and estrogen receptor β agonists enhance cell proliferation in the dentate gyrus of adult female rats. *Neuroscience* 141:1793–1800.

McClure RES, Barha CK, Galea LAM (2013) 17- β Estradiol, but not estrone, increases the survival and activation of new neurons in the hippocampus in response to spatial memory in adult female rats. *Horm Behav* 63:144–157.

McCormick CM, Linkroum W, Sallinen BJ, Miller NW (2002) Peripheral and central sex steroids have differential effects on the HPA axis of male and female rats. *Stress*.

McEwen B, Akama K, Alves S, Brake WG, Bulloch K, Lee S, Li C, Yuen G, Milner TA (2001) Tracking the estrogen receptor in neurons: Implications for estrogen-induced synapse formation. *Proc Natl Acad Sci U S A*.

McEwen BS (2013) The Brain on Stress: Toward an Integrative Approach to Brain, Body, and Behavior. *Perspect Psychol Sci*.

McEwen BS (2014) Sex, stress and the brain: interactive actions of hormones on the developing and adult brain. *Climacteric* 17 Suppl 2:18–25 Available at:
<http://www.ncbi.nlm.nih.gov/pubmed/25225752>.

McEwen BS, Bowles NP, Gray JD, Hill MN, Hunter RG, Karatsoreos IN, Nasca C (2015)

Mechanisms of stress in the brain. *Nat Neurosci* 18:1353–1363 Available at:
<http://www.nature.com/doifinder/10.1038/nn.4086>.

McEwen BS, Nasca C, Gray JD (2016) Stress Effects on Neuronal Structure: Hippocampus, Amygdala, and Prefrontal Cortex. *Neuropsychopharmacology*.

McEwen BS, Weiss JM, Schwartz LS (1968) Selective retention of corticosterone by limbic structures in rat brain [18]. *Nature*.

McGinn LK, Asnis GM, Rubinson E (1996) Biological and clinical validation of atypical depression. *Psychiatry Res.*

McGuirk J, Muscat R, Willner P (1992) Effects of chronically administered fluoxetine and fenfluramine on food intake, body weight and the behavioural satiety sequence. *Psychopharmacology (Berl)*.

McIntyre RS, Mancini D, Eisfeld BS, Soczynska JK, Grupp L, Konarski JZ, Kennedy SH (2006) Calculated bioavailable testosterone levels and depression in middle-aged men. *Psychoneuroendocrinology*.

McKim DB, Weber MD, Niraula A, Sawicki CM, Liu X, Jarrett BL, Ramirez-Chan K, Wang Y, Roeth RM, Sucaldito AD, Sobol CG, Quan N, Sheridan JF, Godbout JP (2018) Microglial recruitment of IL-1 β -producing monocytes to brain endothelium causes stress-induced anxiety. *Mol Psychiatry*.

McKinnon MC, Yucel K, Nazarov A, MacQueen GM (2009) A meta-analysis examining clinical predictors of hippocampal volume in patients with major depressive disorder. *J Psychiatry Neurosci* 34:41–54 Available at:

<http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2612082/> &tool=pmcentrez&rendertype=abstract%5Cn[http://www.ncbi.nlm.nih.gov/pubmed/19125212%5Cn](http://www.ncbi.nlm.nih.gov/pubmed/19125212)<http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2612082/>

bmedcentral.nih.gov/articlerender.fcgi?artid=PMC2612082.

- McKlveen JM, Myers B, Herman JP (2015) The Medial Prefrontal Cortex: Coordinator of Autonomic, Neuroendocrine and Behavioural Responses to Stress. *J Neuroendocrinol*.
- McLaughlin KJ, Wilson JO, Harman J, Wright RL, Wieczorek L, Gomez J, Korol DL, Conrad CD (2010) Chronic 17 β -estradiol or cholesterol prevents stress-induced hippocampal CA3 dendritic retraction in ovariectomized female rats: Possible correspondence between CA1 spine properties and spatial acquisition. *Hippocampus* 20:768–786.
- Mehta D, Newport DJ, Frishman G, Kraus L, Rex-Haffner M, Ritchie JC, Lori A, Knight BT, Stagnaro E, Ruepp A, Stowe ZN, Binder EB (2014) Early predictive biomarkers for postpartum depression point to a role for estrogen receptor signaling. Available at: <http://www.r-project.org>.
- Meltzer-Brody S, Colquhoun H, Riesenber R, Epperson CN, Deligiannidis KM, Rubinow DR, Li H, Sankoh AJ, Clemson C, Schacterle A, Jonas J, Kanes S (2018) Brexanolone injection in post-partum depression: two multicentre, double-blind, randomised, placebo-controlled, phase 3 trials. *Lancet*.
- Menke A (2019) Is the HPA axis as target for depression outdated, or is there a new hope? *Front Psychiatry*.
- Meyers MJ, Sun J, Carlson KE, Marriner GA, Katzenellenbogen BS, Katzenellenbogen JA (2001) Estrogen receptor- β potency-selective ligands: Structure-activity relationship studies of diarylpropionitriles and their acetylene and polar analogues. *J Med Chem* 44:4230–4251.
- Meziane H, Ouagazzal AM, Aubert L, Wietrzych M, Krezel W (2007) Estrous cycle effects on behavior of C57BL/6J and BALB/cByJ female mice: Implications for phenotyping strategies. *Genes, Brain Behav*.

Miller AH, Haroon E, Raison CL, Felger JC (2014) Cytokine Targets in the Brain: Impact on Neurotransmitters and Neurocircuits Andrew. *Depress Anxiety* 30:297–306.

Miller AH, Maletic V, Raison CL (2009) Inflammation and Its Discontents: The Role of Cytokines in the Pathophysiology of Major Depression. *Biol Psychiatry* 65:732–741 Available at: <http://dx.doi.org/10.1016/j.biopsych.2008.11.029>.

Miller AH, Raison CL (2006) Cytokines, p38 MAP Kinase and the Pathophysiology of Depression. *Neuropsychopharmacology*.

Miller AH, Raison CL (2016) The role of inflammation in depression: From evolutionary imperative to modern treatment target. *Nat Rev Immunol* 16:22–34.

Miller AH, Spencer RL, Pulera M, Kang S, McEwen BS, Stein M (1992) Adrenal steroid receptor activation in rat brain and pituitary following dexamethasone: Implications for the dexamethasone suppression test. *Biol Psychiatry*.

Miller MW, Nowakowski RS (1988) Use of bromodeoxyuridine-immunohistochemistry to examine the proliferation, migration and time of origin of cells in the central nervous system. *Brain Res.*

Mishra A, Brinton RD (2018) Inflammation: Bridging age, menopause and APOE ϵ 4 genotype to Alzheimer's disease. *Front Aging Neurosci.*

Mitra SW, Hoskin E, Yudkovitz J, Pear L, Wilkinson HA, Hayashi S, Pfaff DW, Ogawa S, Rohrer SP, Schaeffer JM, McEwen BS, Alves SE (2003) Immunolocalization of estrogen receptor β in the mouse brain: Comparison with estrogen receptor α . *Endocrinology*.

Modrego PJ, Ferrández J (2004) Depression in patients with mild cognitive impairment increases the risk of developing dementia of Alzheimer type: A prospective cohort study. *Arch Neurol.*

Moieni M, Irwin MR, Jevtic I, Olmstead R, Breen EC, Eisenberger NI (2015) Sex Differences in Depressive and Socioemotional Responses to an Inflammatory Challenge: Implications for Sex Differences in Depression. *Neuropsychopharmacology* 40:1709–1716 Available at: <http://www.nature.com/doifinder/10.1038/npp.2015.17>.

Monje ML, Toda H, Palmer TD (2003) Inflammatory Blockade Restores Adult Hippocampal Neurogenesis. *Science* (80-).

Monteiro S, Roque S, de Sá-Calçada D, Sousa N, Correia-Neves M, Cerqueira JJ (2015) An efficient chronic unpredictable stress protocol to induce stress-related responses in C57BL/6 mice. *Front Psychiatry*.

Moreno-Jiménez EP, Flor-García M, Terreros-Roncal J, Rábano A, Cafini F, Pallas-Bazarrá N, Ávila J, Llorens-Martín M (2019) Adult hippocampal neurogenesis is abundant in neurologically healthy subjects and drops sharply in patients with Alzheimer's disease. *Nat Med.*

Moretti M, de Souza AG, de Chaves G, de Andrade VM, Romao PRT, Gavioli EC, Boeck CR (2011) Emotional behavior in middle-aged rats: Implications for geriatric psychopathologies. *Physiol Behav*.

Morgan MA, Pfaff DW (2001) Effects of estrogen on activity and fear-related behaviors in mice. *Horm Behav*.

Morgan MA, Pfaff DW (2002) Estrogen's effects on activity, anxiety, and fear in two mouse strains. *Behav Brain Res*.

Moses-Kolko EL, Berga SL, Kalro B, Sit DKY, Wisner KL (2009) Transdermal estradiol for postpartum depression: A promising treatment option. *Clin Obstet Gynecol*.

Naito S, Sato K, Yoshida K, Higuchi H, Takahashi H, Kamata M, Ito K, Ohkubo T, Shimizu T

(2007) Gender differences in the clinical effects of fluvoxamine and milnacipran in Japanese major depressive patients. *Psychiatry Clin Neurosci*.

Nakagawasaki O, Oba A, Sato A, Arai Y, Mitazaki S, Onogi H, Wakui K, Niijima F, Tan-No K, Tadano T (2009) Subchronic stress-induced depressive behavior in ovariectomized mice. *Life Sci*.

Nemeth CL, Reddy R, Bekhbat M, Bailey J, Neigh GN (2014) Microglial activation occurs in the absence of anxiety-like behavior following microembolic stroke in female, but not male, rats. *J Neuroinflammation*.

Niciu MJ, Luckenbaugh DA, Ionescu DF, Guevara S, Machado-Vieira R, Richards EM, Brutsche NE, Nolan NM, Zarate CA (2014) Clinical predictors of ketamine response in treatment-resistant major depression. *J Clin Psychiatry*.

Nikolich-Žugich J (2018) The twilight of immunity: Emerging concepts in aging of the immune system review-article. *Nat Immunol*.

Nollet M, Gaillard P, Tanti A, Girault V, Belzung C, Leman S (2012) Neurogenesis-Independent Antidepressant-Like Effects on Behavior and Stress Axis Response of a Dual Orexin Receptor Antagonist in a Rodent Model of Depression. *Neuropsychopharmacology* 37:2210–2221 Available at: <http://www.nature.com/doifinder/10.1038/npp.2012.70>.

Nordell VL, Scarborough MM, Buchanan AK, Sohrabji F (2003) Differential effects of estrogen in the injured forebrain of young adult and reproductive senescent animals. *Neurobiol Aging*.

Oizumi H, Kuriyama N, Imamura S, Tabuchi M, Omiya Y, Mizoguchi K, Kobayashi H (2019) Influence of aging on the behavioral phenotypes of C57BL/6J mice after social defeat Ryabinin AE, ed. *PLoS One* 14:e0222076 Available at:

<http://dx.plos.org/10.1371/journal.pone.0222076> [Accessed September 21, 2019].

Okada M, Hayashi N, Kometani M, Nakao K, Inukai T (1997) Influences of ovariectomy and continuous replacement of 17 β -estradiol on the tail skin temperature and behavior in the forced swimming test in rats. *Jpn J Pharmacol.*

Olde B, Leeb-Lundberg LMF (2009) GPR30/GPER1: searching for a role in estrogen physiology. *Trends Endocrinol Metab.*

Olsen NJ, Kovacs WJ (1996) Gonadal steroids and immunity. *Endocr Rev.*

Ongur D (2000) The Organization of Networks within the Orbital and Medial Prefrontal Cortex of Rats, Monkeys and Humans. *Cereb Cortex.*

Opendak M, Gould E (2015) Adult neurogenesis: A substrate for experience-dependent change. *Trends Cogn Sci* 19:151–161 Available at: <http://dx.doi.org/10.1016/j.tics.2015.01.001>.

Orlowski D, Elfving B, Müller HK, Wegener G, Bjarkam CR (2012) Wistar rats subjected to chronic restraint stress display increased hippocampal spine density paralleled by increased expression levels of synaptic scaffolding proteins. *Stress.*

Ormerod BK, Galea LAM (2001) Reproductive status influences cell proliferation and cell survival in the dentate gyrus of adult female meadow voles: A possible regulatory role for estradiol. *Neuroscience.*

Österlund M, G.j.m. Kuiper G, Gustafsson JÅ, Hurd YL (1998) Differential distribution and regulation of estrogen receptor- α and - β mRNA within the female rat brain. *Mol Brain Res.*

Otte C, Hart S, Neylan TC, Marmar CR, Yaffe K, Mohr DC (2005) A meta-analysis of cortisol response to challenge in human aging: Importance of gender. *Psychoneuroendocrinology.*

Pacheco A, Aguayo FI, Aliaga E, Muñoz M, García-Rojo G, Olave FA, Parra-Fiedler NA, García-Pérez A, Tejos-Bravo M, Rojas PS, Parra CS, Fiedler JL (2017) Chronic Stress

Triggers Expression of Immediate Early Genes and Differentially Affects the Expression of AMPA and NMDA Subunits in Dorsal and Ventral Hippocampus of Rats. *Front Mol Neurosci.*

Padilla-Coreano N, Bolkan SS, Pierce GM, Blackman DR, Hardin WD, Garcia-Garcia AL, Spellman TJ, Gordon JA (2016) Direct Ventral Hippocampal-Prefrontal Input Is Required for Anxiety-Related Neural Activity and Behavior. *Neuron.*

Pak TR, Chung WCJ, Lund TD, Hinds LR, Clay CM, Handa RJ (2005) The androgen metabolite, 5 α -androstane-3 β , 17 β -diol, is a potent modulator of estrogen receptor- β 1-mediated gene transcription in neuronal cells. *Endocrinology.*

Pan Y-W, Chan GCK, Kuo CT, Storm DR, Xia Z (2012) Inhibition of Adult Neurogenesis by Inducible and Targeted Deletion of ERK5 Mitogen-Activated Protein Kinase Specifically in Adult Neurogenic Regions Impairs Contextual Fear Extinction and Remote Fear Memory. *J Neurosci.*

Paolicelli RC, Bolasco G, Pagani F, Maggi L, Scianni M, Panzanelli P, Giustetto M, Ferreira TA, Guiducci E, Dumas L, Raguzzino D, Gross CT (2011) Synaptic pruning by microglia is necessary for normal brain development. *Science* (80-).

Parker G, Parker K, Austin MP, Mitchell P, Brotchie H (2003) Gender differences in response to differing antidepressant drug classes: Two negative studies. *Psychol Med* 33:1473–1477.

Parkhurst CN, Yang G, Ninan I, Savas JN, Yates JR, Lafaille JJ, Hempstead BL, Littman DR, Gan WB (2013) Microglia promote learning-dependent synapse formation through brain-derived neurotrophic factor. *Cell* 155:1596–1609.

Paslakis G, Krumm B, Gilles M, Schweiger U, Heuser I, Richter I, Deuschele M (2011) Discrimination between patients with melancholic depression and healthy controls:

Comparison between 24-h cortisol profiles, the DST and the Dex/CRH test.

Psychoneuroendocrinology.

Penninx BWJH, Milaneschi Y, Lamers F, Vogelzangs N (2013) Understanding the somatic consequences of depression: Biological mechanisms and the role of depression symptom profile. *BMC Med.*

Petit-Demouliere Franck Chenu Michel Bourin B (2005) Forced swimming test in mice: a review of antidepressant activity. *Psychopharmacology (Berl)* 177:245–255 Available at: <http://dx.doi.org/10.1007/s00213-004->.

Petrides M (2005) Lateral prefrontal cortex: Architectonic and functional organization. *Philos Trans R Soc B Biol Sci.*

Picazo O, Estrada-Camarena E, Hernandez-Aragon A (2006) Influence of the post-ovariectomy time frame on the experimental anxiety and the behavioural actions of some anxiolytic agents. *Eur J Pharmacol.*

Pitychoutis PM, Nakamura K, Tsonis PA, Papadopoulou-Daifoti Z (2009) Neurochemical and behavioral alterations in an inflammatory model of depression: Sex differences exposed. *Neuroscience* 159:1216–1232 Available at: <http://linkinghub.elsevier.com/retrieve/pii/S0306452209001705> [Accessed April 22, 2018].

Plant TM (2015) The hypothalamo-pituitary-gonadal axis. *J Endocrinol* 226:T41–T54.
Porsolt RD, Bertin A, Jalfre M (1978) “Behavioural despair” in rats and mice: Strain differences and the effects of imipramine. *Eur J Pharmacol.*

Porsolt RD, Le Pichon M, Jalfre M (1977) Depression: A new animal model sensitive to antidepressant treatments [27]. *Nature.*

Porter RJ, Gallagher P (2006) Abnormalities of the HPA axis in affective disorders: Clinical

- subtypes and potential treatments. *Acta Neuropsychiatr.*
- Pothion S, Bizot JC, Trovero F, Belzung C (2004) Strain differences in sucrose preference and in the consequences of unpredictable chronic mild stress. *Behav Brain Res.*
- Pozzi S, Benedusi V, Maggi A, Vegeto E (2006) Estrogen action in neuroprotection and brain inflammation. In: *Annals of the New York Academy of Sciences*, pp 302–323.
- Rachman IM, Unnerstall JR, Pfaff DW, Cohen RS (1998) Estrogen alters behavior and forebrain c-fos expression in ovariectomized rats subjected to the forced swim test. *Proc Natl Acad Sci U S A.*
- Ramsey JM, Cooper JD, Bot M, Guest PC, Lamers F, Weickert CS, Penninx BWJH, Bahn S (2016) Sex differences in serum markers of major depressive disorder in the Netherlands Study of Depression and Anxiety (NESDA). *PLoS One* 11.
- Rannevik G, Carlström K, Jeppsson S, Bjerre B, Svanberg L (1986) A prospective long-term study in women from pre-menopause to post-menopause: changing profiles of gonadotrophins, oestrogens and androgens. *Maturitas.*
- Rao MS, Hattiangady B, Abdel-Rahman A, Stanley DP, Shetty AK (2005) Newly born cells in the ageing dentate gyrus display normal migration, survival and neuronal fate choice but endure retarded early maturation. *Eur J Neurosci.*
- Rapaport MH, Clary C, Fayyad R, Endicott J (2005) Quality-of-life impairment in depressive and anxiety disorders. *Am J Psychiatry.*
- Rasgon NL, Dunkin J, Fairbanks L, Altshuler LL, Troung C, Elman S, Wroolie TE, Brunhuber M V., Rapkin A (2007) Estrogen and response to sertraline in postmenopausal women with major depressive disorder: A pilot study. *J Psychiatr Res.*
- Récamier-Carballo S, Estrada-Camarena E, Reyes R, Fernández-Guasti A (2012) Synergistic

- effect of estradiol and fluoxetine in young adult and middle-aged female rats in two models of experimental depression. *Behav Brain Res.*
- Reed BG, Carr BR (2000) The Normal Menstrual Cycle and the Control of Ovulation.
- Remus JL, Dantzer R (2016) Inflammation models of depression in rodents: Relevance to psychotropic drug discovery. *Int J Neuropsychopharmacol* 19:1–13.
- Reul JMHM, De Kloet ER (1985) Two receptor systems for corticosterone in rat brain: Microdistribution and differential occupation. *Endocrinology*.
- Revankar CM, Cimino DF, Sklar LA, Arterburn JB, Prossnitz ER (2005) A transmembrane intracellular estrogen receptor mediates rapid cell signaling. *Science* (80-).
- Rocha BA, Fleischer R, Schaeffer JM, Rohrer SP, Hickey GJ (2005) 17 β -Estradiol-induced antidepressant-like effect in the Forced Swim Test is absent in estrogen receptor- β knockout (BERKO) mice. *Psychopharmacology (Berl)*.
- Roy A, Linnoila M, Jimerson DC, Gold P, Pickar D (1986) Pre-menopausal and post-menopausal depressed women. *Australas Psychiatry*.
- Rudy JW, Matus-Amat P (2005) The ventral hippocampus supports a memory representation of context and contextual fear conditioning: Implications for a unitary function of the hippocampus. *Behav Neurosci.*
- Rupprecht R, Papadopoulos V, Rammes G, Baghai TC, Fan J, Akula N, Groyer G, Adams D, Schumacher M (2010) Translocator protein (18 kDa) (TSPO) as a therapeutic target for neurological and psychiatric disorders. *Nat Rev Drug Discov.*
- Russo SJ, Nestler EJ (2013) The brain reward circuitry in mood disorders. *Nat Rev Neurosci.*
- Saijo K, Collier JG, Li AC, Katzenellenbogen JA, Glass CK (2011) An ADIOL-ER β -CtBP transrepression pathway negatively regulates microglia-mediated inflammation. *Cell.*

Salk RH, Hyde JS, Abramson LY (2017) Gender differences in depression in representative national samples: Meta-analyses of diagnoses and symptoms. *Psychol Bull* 143:783–822 Available at: <http://doi.apa.org/getdoi.cfm?doi=10.1037/bul0000102>.

Samuels BA, Hen R (2011) Novelty-suppressed feeding in the mouse. *Neuromethods*. Santarelli L, Saxe M, Gross C, Surget A, Battaglia F, Dulawa S, Weisstaub N, Lee J, Duman R, Arancio O, Belzung C, Hen R (2003) Requirement of hippocampal neurogenesis for the behavioral effects of antidepressants. *Science* 301:805–809 Available at: <http://www.ncbi.nlm.nih.gov/pubmed/12907793> [Accessed December 12, 2013].

Sárvári M, Kalló I, Hrabovszky E, Solymosi N, Liposits Z (2014) Ovariectomy and subsequent treatment with estrogen receptor agonists tune the innate immune system of the hippocampus in middle-aged female rats. *PLoS One* 9.

Sayeed I, Stein DG (2009) Progesterone as a neuroprotective factor in traumatic and ischemic brain injury. *Prog Brain Res*.

Schafer DP, Lehrman EK, Stevens B (2013) The “quad-partite” synapse: Microglia-synapse interactions in the developing and mature CNS. *Glia*.

Schatzberg AF (2015) Anna-Monika Award Lecture, DGPPN kongress, 2013: The role of the hypothalamic-pituitary-adrenal (HPA) axis in the pathogenesis of psychotic major depression. *World J Biol Psychiatry*.

Scheggi S, De Montis MG, Gambarana C (2018) Making Sense of Rodent Models of Anhedonia. *Int J Neuropsychopharmacol* 21:1049–1065 Available at: <https://academic.oup.com/ijnp/article-abstract/21/11/1049/5104314>.

Scheller J, Chalaris A, Schmidt-Arras D, Rose-John S (2011) The pro- and anti-inflammatory properties of the cytokine interleukin-6. *Biochim Biophys Acta - Mol Cell Res*.

- Schiller CE, Meltzer-Brody S, Rubinow DR (2015) The role of reproductive hormones in postpartum depression. *CNS Spectr.*
- Schmaal L et al. (2016) Subcortical brain alterations in major depressive disorder: findings from the ENIGMA Major Depressive Disorder working group. *Mol Psychiatry* 21:806–812 Available at: <http://www.nature.com/doifinder/10.1038/mp.2015.69>.
- Schmidt PJ, Ben Dor R, Martinez PE, Guerrieri GM, Harsh VL, Thompson K, Koziol DE, Nieman LK, Rubinow DR (2015) Effects of Estradiol Withdrawal on Mood in Women With Past Perimenopausal Depression. *JAMA Psychiatry* 72:714 Available at: <http://archpsyc.jamanetwork.com/article.aspx?doi=10.1001/jamapsychiatry.2015.0111> [Accessed July 16, 2019].
- Schoenfeld TJ, Gould E (2012) Stress, stress hormones, and adult neurogenesis. *Exp Neurol* 233:12–21.
- Schoenrock SA, Oreper D, Young N, Ervin RB, Bogue MA, Valdar W, Tarantino LM (2016) Ovariectomy results in inbred strain-specific increases in anxiety-like behavior in mice. *Physiol Behav*.
- Scholzen T, Gerdes J (2000) The Ki-67 protein: From the known and the unknown. *J Cell Physiol* 182:311–322.
- Schuch JJ, Roest AM, Nolen WA, Penninx BWJH, De Jonge P (2014) Gender differences in major depressive disorder: Results from the Netherlands study of depression and anxiety. *J Affect Disord* 156:156–163.
- Schüle C, Nothdurfter C, Rupprecht R (2014) The role of allopregnanolone in depression and anxiety. *Prog Neurobiol*.
- Scott KM, Lim C, Al-Hamzawi A, Alonso J, Bruffaerts R, Caldas-De-Almeida JM, Florescu S,

- De Girolamo G, Hu C, De Jonge P, Kawakami N, Medina-Mora ME, Moskalewicz J, Navarro-Mateu F, O'Neill S, Piazza M, Posada-Villa J, Torres Y, Kessler RC (2016) Association of mental disorders with subsequent chronic physical conditions: World mental health surveys from 17 countries. *JAMA Psychiatry*.
- Seale J V., Wood SA, Atkinson HC, Bate E, Lightman SL, Ingram CD, Jessop DS, Harbuz MS (2004) Gonadectomy reverses the sexually diergic patterns of circadian and stress-induced hypothalamic-pituitary-adrenal axis activity in male and female rats. *J Neuroendocrinol*.
- Seidman SN, Rabkin JG (1998) Testosterone replacement therapy for hypogonadal men with SSRI-refractory depression. *J Affect Disord*.
- Sell SL, Craft RM, Seitz PK, Stutz SJ, Cunningham KA, Thomas ML (2008) Estradiol-sertraline synergy in ovariectomized rats. *Psychoneuroendocrinology*.
- Sellers K, Raval P, Srivastava DP (2015) Molecular signature of rapid estrogen regulation of synaptic connectivity and cognition. *Front Neuroendocrinol*.
- Selvamani A, Sohrabji F (2010a) The neurotoxic effects of estrogen on ischemic stroke in older female rats is associated with age-dependent loss of insulin-like growth factor-1. *J Neurosci* 30:6852–6861.
- Selvamani A, Sohrabji F (2010b) Reproductive age modulates the impact of focal ischemia on the forebrain as well as the effects of estrogen treatment in female rats. *Neurobiol Aging*.
- Seney ML, Huo Z, Cahill K, French L, Puralewski R, Zhang J, Logan RW, Tseng G, Lewis DA, Sibille E (2018) Opposite molecular signatures of depression in men and women. *Biol Psychiatry* Available at: <http://dx.doi.org/10.1016/j.biopsych.2018.01.017>.
- Sens J, Schneider E, Mauch J, Schaffstein A, Mohamed S, Fasoli K, Saurine J, Britzolaki A, Thelen C, Pitychoutis PM (2017) Lipopolysaccharide administration induces sex-dependent

behavioural and serotonergic neurochemical signatures in mice. *Pharmacol Biochem Behav* 153:168–181.

Serova LI, Harris HA, Maharjan S, Sabban EL (2010) Modulation of responses to stress by estradiol benzoate and selective estrogen receptor agonists. *J Endocrinol.*

Setiawan E, Attwells S, Wilson AA, Mizrahi R, Rusjan PM, Miler L, Xu C, Sharma S, Kish S, Houle S, Meyer JH (2018) Association of translocator protein total distribution volume with duration of untreated major depressive disorder: a cross-sectional study. :339 Available at: <http://dx.doi.org/10.1016/> [Accessed June 5, 2019].

Setiawan E, Wilson AA, Mizrahi R, Rusjan PM, Miler L, Rajkowska G, Suridjan I, Kennedy JL, Rekkas PV, Houle S, Meyer JH (2015) Role of Translocator Protein Density, a Marker of Neuroinflammation, in the Brain During Major Depressive Episodes. *JAMA Psychiatry* 72:268 Available at: <http://archpsyc.jamanetwork.com/article.aspx?doi=10.1001/jamapsychiatry.2014.2427>.

Shansky RM, Hamo C, Hof PR, Lou W, McEwen BS, Morrison JH (2010) Estrogen promotes stress sensitivity in a prefrontal cortex-amygala pathway. *Cereb Cortex* 20:2560–2567.

Shansky RM, Hamo C, Hof PR, McEwen BS, Morrison JH (2009) Stress-induced dendritic remodeling in the prefrontal cortex is circuit specific. *Cereb Cortex* 19:2479–2484.

Shappell NW, Hyndman KM, Bartell SE, Schoenfuss HL (2010) Comparative biological effects and potency of 17 α - and 17 β -estradiol in fathead minnows. *Aquat Toxicol.*

Sheppard PAS, Choleris E, Galea LAM (2019) Structural plasticity of the hippocampus in response to estrogens in female rodents. *Mol Brain.*

Sher L, Oquendo MA, Burke AK, Cooper TB, John Mann J (2013) Combined dexamethasone suppression-corticotrophin-releasing hormone stimulation test in medication-free major

- depression and healthy volunteers. *J Affect Disord.*
- Shoji H, Takao K, Hattori S, Miyakawa T (2016) Age-related changes in behavior in C57BL/6J mice from young adulthood to middle age. *Mol Brain.*
- Shores MM, Kivlahan DR, Sadak TI, Li EJ, Matsumoto AM (2009) A randomized, double-blind, placebo-controlled study of testosterone treatment in hypogonadal older men with subthreshold depression (dysthymia or minor depression). *J Clin Psychiatry.*
- Shores MM, Moceri VM, Sloan KL, Matsumoto AM, Kivlahan DR (2005) Low testosterone levels predict incident depressive illness in older men: effects of age and medical morbidity. *J Clin Psychiatry.*
- Shors TJ, Mathew J, Sisti HM, Edgecomb C, Beckoff S, Dalla C (2007) Neurogenesis and Helplessness Are Mediated by Controllability in Males But Not in Females. *Biol Psychiatry* 62:487–495.
- Shughrue PJ, Lane M V., Merchenthaler I (1997) Comparative distribution of estrogen receptor- α and - β mRNA in the rat central nervous system. *J Comp Neurol.*
- Shughrue PJ, Merchenthaler I (2001) Distribution of estrogen receptor beta immunoreactivity in the rat central nervous system. *J Comp Neurol.*
- Sibille E, Wang Y, Joeyen-Waldorf J, Gaiteri C, Surget A, Oh S, Belzung C, Tseng GC, Lewis DA (2009) A molecular signature of depression in the amygdala. *Am J Psychiatry.*
- Sierra A, Gottfried-Blackmore A, Milner TA, McEwen BS, Bulloch K (2008) Steroid hormone receptor expression and function in microglia. *Glia* 56:659–674.
- Silva MTA, Alves CRR, Santarem EMM (1999) Anxiogenic-like effect of acute and chronic fluoxetine on rats tested on the elevated plus-maze. *Brazilian J Med Biol Res.*
- Silverstein B (2002) Gender differences in the prevalence of somatic versus pure depression: A

- replication. *Am J Psychiatry* 159:1051–1052.
- Sipe GO, Lowery RL, Tremblay M, Kelly EA, Lamantia CE, Majewska AK (2016) Microglial P2Y12 is necessary for synaptic plasticity in mouse visual cortex. *Nat Commun*.
- Slattery DA, Cryan JF (2012) Using the rat forced swim test to assess antidepressant-like activity in rodents. *Nat Protoc* 7.
- Smith JA, Das A, Butler JT, Ray SK, Banik NL (2011) Estrogen or estrogen receptor agonist inhibits lipopolysaccharide induced microglial activation and death. *Neurochem Res*.
- Smith SS, Ruderman Y, Frye C, Homanics G, Yuan M (2006) Steroid withdrawal in the mouse results in anxiogenic effects of 3 α ,5 β -THP: A possible model of premenstrual dysphoric disorder. *Psychopharmacology (Berl)*.
- Snyder JS, Soumier A, Brewer M, Pickel J, Cameron HA (2011) Adult hippocampal neurogenesis buffers stress responses and depressive behaviour. *Nature* 476:458–461
Available at: <http://www.nature.com/doifinder/10.1038/nature10287>.
- Soares CN (2013) Depression in peri- and postmenopausal women: Prevalence, pathophysiology and pharmacological management. *Drugs and Aging*.
- Soares CN (2014) Mood disorders in midlife women: Understanding the critical window and its clinical implications. *Menopause*.
- Sohrabji F, Lewis DK (2006) Estrogen-BDNF interactions: Implications for neurodegenerative diseases. *Front Neuroendocrinol*.
- Spencer-Segal JL, Tsuda MC, Mattei L, Waters EM, Romeo RD, Milner TA, McEwen BS, Ogawa S (2012) Estradiol acts via estrogen receptors alpha and beta on pathways important for synaptic plasticity in the mouse hippocampal formation. *Neuroscience*.
- Spencer JL, Waters EM, Milner TA, Lee FS, McEwen BS (2010) BDNF variant Val66Met

- interacts with estrous cycle in the control of hippocampal function. *Proc Natl Acad Sci.*
- Spierling SR, Zorrilla EP (2017) Don't stress about CRF: assessing the translational failures of CRF₁ antagonists. *Psychopharmacology (Berl).*
- Spies M, Knudsen GM, Lanzenberger R, Kasper S (2015) The serotonin transporter in psychiatric disorders: Insights from PET imaging. *The Lancet Psychiatry.*
- Sramek JJ, Murphy MF, Cutler NR (2016) Sex differences in the psychopharmacological treatment of depression. *Dialogues Clin Neurosci* 18:447–457.
- Srivastava DP, Penzes P (2011) Rapid estradiol modulation of neuronal connectivity and its implications for disease. *Front Endocrinol (Lausanne).*
- Stauffer SR, Coletta CJ, Tedesco R, Nishiguchi G, Carlson K, Sun J, Katzenellenbogen BS, Katzenellenbogen J a (2000) Pyrazole ligands: structure-affinity/activity relationships and estrogen receptor-alpha-selective agonists. *J Med Chem* 43:4934–4947.
- Stedenfeld KA, Clinton SM, Kerman IA, Akil H, Watson SJ, Sved AF (2011) Novelty-seeking behavior predicts vulnerability in a rodent model of depression. *Physiol Behav.*
- Sterner EY, Kalynchuk LE (2010) Behavioral and neurobiological consequences of prolonged glucocorticoid exposure in rats: Relevance to depression. *Prog Neuro-Psychopharmacology Biol Psychiatry.*
- Sterrenburg L, Gaszner B, Boerrigter J, Santbergen L, Bramini M, Roubos EW, Peeters BWMM, Kozicz T (2012) Sex-dependent and differential responses to acute restraint stress of corticotropin-releasing factor-producing neurons in the rat paraventricular nucleus, central amygdala, and bed nucleus of the stria terminalis. *J Neurosci Res.*
- Steru L, Chermat R, Thierry B, Simon P (1985) The tail suspension test: A new method for screening antidepressants in mice. *Psychopharmacology (Berl).*

Stetler C, Miller GE (2011) Depression and Hypothalamic-Pituitary-Adrenal Activation: A Quantitative Summary of Four Decades of Research. *Psychosom Med* 73:114–126

Available at:

<http://content.wkhealth.com/linkback/openurl?sid=WKPTLP:landingpage&an=00006842-201102000-00002>.

Stewart JW, Quitkin FM, McGrath PJ, Klein DF (2005) Defining the boundaries of atypical depression: Evidence from the HPA axis supports course of illness distinctions. *J Affect Disord.*

Stockmeier CA, Mahajan GJ, Konick LC, Overholser JC, Jurjus GJ, Meltzer HY, Uylings HBM, Friedman L, Rajkowska G (2004) Cellular changes in the postmortem hippocampus in major depression. *Biol Psychiatry*.

Strange BA, Witter MP, Lein ES, Moser EI (2014) Functional organization of the hippocampal longitudinal axis. *Nat Rev Neurosci*.

Straub RH (2007) The complex role of estrogens in inflammation. *Endocr Rev*.

Strawbridge R, Young AH, Cleare AJ (2017) Biomarkers for depression: Recent insights, current challenges and future prospects. *Neuropsychiatr Dis Treat*.

Surget A, Tanti A, Leonardo ED, Laugery A, Rainer Q, Touma C, Palme R, Griebel G, Ibarguen-Vargas Y, Hen R, Belzung C (2011) Antidepressants recruit new neurons to improve stress response regulation. *Mol Psychiatry* 16:1177–1188 Available at: <http://www.nature.com/doifinder/10.1038/mp.2011.48>.

Suuronen T, Nuutinen T, Huuskonen J, Ojala J, Thornell A, Salminen A (2005) Anti-inflammatory effect of selective estrogen receptor modulators (SERMs) in microglial cells. *Inflamm Res* 54:194–203 Available at: <http://www.ncbi.nlm.nih.gov/pubmed/15953991>

[Accessed July 9, 2019].

Suzuki S, Brown CM, Wise PM (2009) Neuroprotective effects of estrogens following ischemic stroke. *Front Neuroendocrinol* 30:201–211.

Syed SA, Beurel E, Loewenstein DA, Lowell JA, Craighead WE, Dunlop BW, Mayberg HS, Dhabhar F, Dietrich WD, Keane RW, de Rivero Vaccari JP, Nemeroff CB (2018) Defective Inflammatory Pathways in Never-Treated Depressed Patients Are Associated with Poor Treatment Response. *Neuron*.

Tanapat P, Hastings NB, Gould E (2005) Ovarian steroids influence cell proliferation in the dentate gyrus of the adult female rat in a dose- and time-dependent manner. *J Comp Neurol*.

Tanapat P, Hastings NB, Reeves AJ, Gould E (1999) Estrogen stimulates a transient increase in the number of new neurons in the dentate gyrus of the adult female rat. *J Neurosci* 19:5792–5801 Available at: <http://www.ncbi.nlm.nih.gov/pubmed/10407020>.

Tang AC, Nakazawa M, Romeo RD, Reeb BC, Sisti H, McEwen BS (2005) Effects of long-term estrogen replacement on social investigation and social memory in ovariectomized C57BL/6 mice. *Horm Behav*.

Tanti A, Belzung C (2013) Neurogenesis along the septo-temporal axis of the hippocampus: Are depression and the action of antidepressants region-specific? *Neuroscience* 252:234–252.

Tapia-Gonzalez S, Carrero P, Pernia O, Garcia-Segura LM, Diz-Chaves Y (2008) Selective oestrogen receptor (ER) modulators reduce microglia reactivity in vivo after peripheral inflammation: potential role of microglial ERs. *J Endocrinol*.

Taupin P (2007) BrdU immunohistochemistry for studying adult neurogenesis: paradigms, pitfalls, limitations, and validation. *Brain Res Rev* 53:198–214 Available at: <http://www.ncbi.nlm.nih.gov/pubmed/17020783> [Accessed December 16, 2013].

Thase ME, Entsuah R, Cantillon M, Kornstein SG (2005) Relative Antidepressant Efficacy of Venlafaxine and SSRIs: Sex-Age Interactions. *J Women's Heal* 14:609–616 Available at: <http://www.liebertonline.com/doi/abs/10.1089/jwh.2005.14.609>.

Tian Z, Wang Y, Zhang N, Guo Y yan, Feng B, Liu S bing, Zhao M gao (2013) Estrogen receptor GPR30 exerts anxiolytic effects by maintaining the balance between GABAergic and glutamatergic transmission in the basolateral amygdala of ovariectomized mice after stress. *Psychoneuroendocrinology*.

Tobin MK, Musaraca K, Disouky A, Shetti A, Bheri A, Honer WG, Kim N, Dawe RJ, Bennett DA, Arfanakis K, Lazarov O (2019) Human Hippocampal Neurogenesis Persists in Aged Adults and Alzheimer's Disease Patients. *Cell Stem Cell*.

Toda T, Gage FH (2018) Review: adult neurogenesis contributes to hippocampal plasticity. *Cell Tissue Res.*

Toda T, Parylak SL, Linker SB, Gage FH (2019) The role of adult hippocampal neurogenesis in brain health and disease. *Mol Psychiatry*.

Trigunaite A, Dimo J, Jørgensen TN (2015) Suppressive effects of androgens on the immune system. *Cell Immunol* 294:87–94.

Trivedi MH, Rush AJ, Wisniewski SR, Nierenberg AA, Warden D, Ritz L, Norquist G, Howland RH, Lebowitz B, McGrath PJ, Shores-Wilson K, Biggs MM, Balasubramani GK, Fava M (2006) Evaluation of outcomes with citalopram for depression using measurement-based care in STAR*D: Implications for clinical practice. *Am J Psychiatry* 163:28–40.

Tuglu C, Kara SH, Caliyurt O, Vardar E, Abay E (2003) Increased serum tumor necrosis factor-alpha levels and treatment response in major depressive disorder. *Psychopharmacology (Berl)*.

Tunc-Ozcan E, Peng C-Y, Zhu Y, Dunlop SR, Contractor A, Kessler JA (2019) Activating newborn neurons suppresses depression and anxiety-like behaviors. *Nat Commun* 10:3768 Available at: <http://www.nature.com/articles/s41467-019-11641-8> [Accessed September 5, 2019].

Turner BB (1990) Sex difference in glucocorticoid binding in rat pituitary is estrogen dependent. *Life Sci.*

Twenge JM, Nolen-Hoeksema S (2002) Age, gender, race, socioeconomic status, and birth cohort difference on the children's depression inventory: A meta-analysis. *J Abnorm Psychol* 111:578–588 Available at: <http://doi.apa.org/getdoi.cfm?doi=10.1037/0021-843X.111.4.578>.

Ulrich-Lai YM, Herman JP (2009) Neural regulation of endocrine and autonomic stress responses. *Nat Rev Neurosci* 10:397–409 Available at: <http://www.nature.com/doifinder/10.1038/nrn2647>.

Vakili K, Pillay SS, Lafer B, Fava M, Renshaw PF, Bonello-Cintron CM, Yurgelun-Todd DA (2000) Hippocampal volume in primary unipolar major depression: a magnetic resonance imaging study. *Biol Psychiatry* 47:1087–1090.

Van Lunzen J, Altfeld M (2014) Sex differences in infectious diseases-common but neglected. *J Infect Dis.*

van Praag H, Kempermann G, Gage FH (1999) Running increases cell proliferation and neurogenesis in the adult mouse dentate gyrus. *Nat Neurosci* 2:266–270 Available at: http://www.ncbi.nlm.nih.gov/pubmed/10195220%255Cnhttp://www.nature.com/neuro/journal/v2/n3/pdf/nn0399_266.pdf.

Vega-Rivera NM, Fernández-Guasti A, Ramírez-Rodríguez G, Estrada-Camarena E (2015)

Effect of sub-optimal doses of fluoxetine plus estradiol on antidepressant-like behavior and hippocampal neurogenesis in ovariectomized rats. *Psychoneuroendocrinology*.

Vegeto E, Belcredito S, Etteri S, Ghisletti S, Brusadelli A, Meda C, Krust A, Dupont S, Ciana P, Chambon P, Maggi A (2003) Estrogen receptor- α mediates the brain antiinflammatory activity of estradiol. *Proc Natl Acad Sci.*

Vegeto E, Benedusi V, Maggi A (2008) Estrogen anti-inflammatory activity in brain: A therapeutic opportunity for menopause and neurodegenerative diseases. *Front Neuroendocrinol* 29:507–519.

Vegeto E, Bonincontro C, Pollio G, Sala a, Viappiani S, Nardi F, Brusadelli a, Viviani B, Ciana P, Maggi a (2001) Estrogen prevents the lipopolysaccharide-induced inflammatory response in microglia. *J Neurosci* 21:1809–1818.

Vetter ML, Wadden TA, Vinnard C, Moore RH, Khan Z, Volger S, Sarwer DB, Faulconbridge LF (2013) Gender differences in the relationship between symptoms of depression and high-sensitivity CRP. *Int J Obes* 37:S38–S43.

Viau V, Bingham B, Davis J, Lee P, Wong M (2005) Gender and puberty interact on the stress-induced activation of parvocellular neurosecretory neurons and corticotropin-releasing hormone messenger ribonucleic acid expression in the rat. *Endocrinology*.

Viau V, Meaney MJ (1991) Variations in the hypothalamic-pituitary-adrenal response to stress during the estrous cycle in the rat. *Endocrinology*.

Villa A, Della Torre S, Maggi A (2019) Sexual differentiation of microglia. *Front Neuroendocrinol* 52:156–164 Available at:

<https://www.sciencedirect.com/science/article/pii/S009130221830092X?via%3Dihub>

[Accessed July 2, 2019].

Villa A, Gelosa P, Castiglioni L, Sironi L, Vegeto E, Correspondence AM (2018) Sex-Specific Features of Microglia from Adult Mice. *Cell Rep* 23:3501–3511 Available at: <https://doi.org/10.1016/j.celrep.2018.05.048> [Accessed July 2, 2019].

Villa A, Vegeto E, Poletti A, Maggi A (2016) Estrogens, Neuroinflammation, and Neurodegeneration. Available at: <https://academic.oup.com/edrv/article-abstract/37/4/372/2567099> [Accessed July 2, 2019].

Vogelzangs N, Duivis HE, Beekman ATF, Kluft C, Neuteboom J, Hoogendoijk W, Smit JH, de Jonge P, Penninx BWJH (2012) Association of depressive disorders, depression characteristics and antidepressant medication with inflammation. *Transl Psychiatry* 2:e79 Available at: <http://www.nature.com/doifinder/10.1038/tp.2012.8>.

Vollmayr B, Henn FA (2001) Learned helplessness in the rat: Improvements in validity and reliability. *Brain Res Protoc.*

Vollmayr B, Mahlstedt MM, Henn F a (2007) Neurogenesis and depression: what animal models tell us about the link. *Eur Arch Psychiatry Clin Neurosci* 257:300–303 Available at: <http://www.ncbi.nlm.nih.gov/pubmed/17401725> [Accessed December 28, 2013].

Wainwright SR, Barha CK, Hamson DK, Epp JR, Chow C, Lieblich SE, Rutishauser U, Galea LAM (2016a) Enzymatic depletion of the polysialic acid moiety associated with the neural cell adhesion molecule inhibits antidepressant efficacy. *Neuropsychopharmacology* 41:1670–1680.

Wainwright SR, Galea L a M (2013) The neural plasticity theory of depression: assessing the roles of adult neurogenesis and PSA-NCAM within the hippocampus. *Neural Plast* 2013:805497 Available at: <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3749690/>

dertype=abstract.

Wainwright SR, Lieblich SE, Galea LAM (2011) Hypogonadism predisposes males to the development of behavioural and neuroplastic depressive phenotypes.

Psychoneuroendocrinology 36:1327–1341.

Wainwright SR, Workman JL, Tehrani A, Hamson DK, Chow C, Lieblich SE, Galea LAM (2016b) Testosterone has antidepressant-like efficacy and facilitates imipramine-induced neuroplasticity in male rats exposed to chronic unpredictable stress. Horm Behav.

Walf AA, Frye CA (2005) ER β -selective estrogen receptor modulators produce antianxiety behavior when administered systemically to ovariectomized rats.

Neuropsychopharmacology.

Walf AA, Frye CA (2010) Estradiol reduces anxiety- and depression-like behavior of aged female mice. Physiol Behav.

Walf AA, Koonce C, Manley K, Frye CA (2009a) Proestrous compared to diestrous wildtype, but not estrogen receptor beta knockout, mice have better performance in the spontaneous alternation and object recognition tasks and reduced anxiety-like behavior in the elevated plus and mirror maze. Behav Brain Res 196:254–260.

Walf AA, Koonce CJ, Frye CA (2008) Estradiol or diarylpropionitrile administration to wild type, but not estrogen receptor beta knockout, mice enhances performance in the object recognition and object placement tasks. Neurobiol Learn Mem 89:513–521.

Walf AA, Paris JJ, Frye CA (2009b) Chronic estradiol replacement to aged female rats reduces anxiety-like and depression-like behavior and enhances cognitive performance. Psychoneuroendocrinology.

Walf AA, Rhodes ME, Frye CA (2004) Antidepressant effects of ER β -selective estrogen

- receptor modulators in the forced swim test. In: Pharmacology Biochemistry and Behavior.
- Walker F, Nilsson M, Jones K (2013) Acute and Chronic Stress-Induced Disturbances of Microglial Plasticity, Phenotype and Function. *Curr Drug Targets* 14:1262–1276 Available at: <http://www.eurekaselect.com/openurl/content.php?genre=article&issn=1389-4501&volume=14&issue=11&spage=1262>.
- Walmer DK, Wrona MA, Hughes CL, Nelson KG (1992) Lactoferrin expression in the mouse reproductive traci during the natural estrous cycle: Correlation with circulating estradiol and progesterone. *Endocrinology*.
- Wang AK, Miller BJ (2018) Meta-analysis of Cerebrospinal Fluid Cytokine and Tryptophan Catabolite Alterations in Psychiatric Patients: Comparisons between Schizophrenia, Bipolar Disorder, and Depression. *Schizophr Bull*.
- Wang JQ, Mao L (2019) The ERK Pathway: Molecular Mechanisms and Treatment of Depression. *Mol Neurobiol*.
- Waters EM, Mitterling K, Spencer JL, Mazid S, McEwen BS, Milner TA (2009) Estrogen receptor alpha and beta specific agonists regulate expression of synaptic proteins in rat hippocampus. *Brain Res*.
- Waters EM, Thompson LI, Patel P, Gonzales AD, Ye H, Filardo EJ, Clegg DJ, Gorecka J, Akama KT, McEwen BS, Milner TA (2015a) G-Protein-Coupled Estrogen Receptor 1 Is Anatomically Positioned to Modulate Synaptic Plasticity in the Mouse Hippocampus. *J Neurosci*.
- Waters RP, Rivalan M, Bangasser DA, Deussing JM, Ising M, Wood SK, Holsboer F, Summers CH (2015b) Evidence for the role of corticotropin-releasing factor in major depressive disorder. *Neurosci Biobehav Rev*.

Weber MD, Godbout JP, Sheridan JF (2017) Repeated Social Defeat, Neuroinflammation, and Behavior: Monocytes Carry the Signal. *Neuropsychopharmacology* 42:46–61 Available at: <http://www.nature.com/doifinder/10.1038/npp.2016.102>.

Weeden CSS, Hu NJ, Ho LUN, Kesner RP (2014) The role of the ventral dentate gyrus in olfactory pattern separation. *Hippocampus*.

Wei J, Yuen EY, Liu W, Li X, Zhong P, Karatsoreos IN, McEwen BS, Yan Z (2014) Estrogen protects against the detrimental effects of repeated stress on glutamatergic transmission and cognition. *Mol Psychiatry*.

Weiser MJ, Foradori CD, Handa RJ (2010) Estrogen receptor beta activation prevents glucocorticoid receptor-dependent effects of the central nucleus of the amygdala on behavior and neuroendocrine function. *Brain Res.*

Weiser MJ, Handa RJ (2009) Estrogen impairs glucocorticoid dependent negative feedback on the hypothalamic-pituitary-adrenal axis via estrogen receptor alpha within the hypothalamus. *Neuroscience*.

Weiser MJ, Wu TJ, Handa RJ (2009) Estrogen receptor- β agonist diarylpropionitrile: Biological activities of R- And S-enantiomers on behavior and hormonal response to stress. *Endocrinology*.

Westenbroek C, Den Boer JA, Ter Horst GJ (2003) Gender-specific effects of social housing on chronic stress-induced limbic FOS expression. *Neuroscience*.

Westenbroek C, Den Boer JA, Veenhuis M, Ter Horst GJ (2004) Chronic stress and social housing differentially affect neurogenesis in male and female rats. *Brain Res Bull* 64:303–308.

Westley CJ, Amdur RL, Irwig MS (2015) High Rates of Depression and Depressive Symptoms

- among Men Referred for Borderline Testosterone Levels. *J Sex Med.*
- Willner P (1997) Validity, reliability and utility of the chronic mild stress model of depression: A 10-year review and evaluation. *Psychopharmacology (Berl).*
- Willner P (2017) The chronic mild stress (CMS) model of depression: History, evaluation and usage. *Neurobiol Stress.*
- 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 (Berl).*
- Wilson L, Pandeya N, Byles J, Mishra G (2018) Hysterectomy and incidence of depressive symptoms in midlife women: The Australian Longitudinal Study on Women's Health. *Epidemiol Psychiatr Sci.*
- Wisner KL, Sit DKY, McShea MC, Rizzo DM, Zoretich RA, Hughes CL, Eng HF, Luther JF, Wisniewski SR, Costantino ML, Confer AL, Moses-Kolko EL, Famy CS, Hanusa BH (2013) Onset timing, thoughts of self-harm, and diagnoses in postpartum women with screen-positive depression findings. *JAMA psychiatry* 70:490–498.
- Wohleb ES, Patterson JM, Sharma V, Quan N, Godbout JP, Sheridan JF (2014) Knockdown of Interleukin-1 Receptor Type-1 on Endothelial Cells Attenuated Stress-Induced Neuroinflammation and Prevented Anxiety-Like Behavior. *J Neurosci.*
- Wohleb ES, Powell ND, Godbout JP, Sheridan JF (2013) Stress-induced recruitment of bone marrow-derived monocytes to the brain promotes anxiety-like behavior. *J Neurosci.*
- Wohlfarth T, Storosum JG, Elferink AJA, Van Zwieten BJ, Fouwels A, Van Den Brink W (2004) Response to Tricyclic Antidepressants: Independent of Gender? *Am J Psychiatry* 161:370–372.

Wolf SA, Boddeke HWGM, Kettenmann H (2017) Microglia in Physiology and Disease. *Annu Rev Physiol* 79:619–643.

Wong ML, Kling MA, Munson PJ, Listwak S, Licinio J, Prolo P, Karp B, McCutcheon IE, Geraciotti TD, DeBellis MD, Rice KC, Goldstein DS, Veldhuis JD, Chrousos GP, Oldfield EH, McCann SM, Gold PW (2000) Pronounced and sustained central hypernoradrenergic function in major depression with melancholic features: Relation to hypercortisolism and corticotropin-releasing hormone. *Proc Natl Acad Sci U S A*.

Wood SK, Wood CS, Lombard CM, Lee CS, Zhang XY, Finnell JE, Valentino RJ (2015) Inflammatory factors mediate vulnerability to a social stress-induced depressive-like phenotype in passive Coping Rats. *Biol Psychiatry*.

Woody CA, Ferrari AJ, Siskind DJ, Whiteford HA, Harris MG (2017) A systematic review and meta-regression of the prevalence and incidence of perinatal depression. *J Affect Disord*.

Woolley CS, Gould E, Frankfurt M, McEwen BS (1990) Naturally occurring fluctuation in dendritic spine density on adult hippocampal pyramidal neurons. *J Neurosci*.

Workman JL, Brummelte S, Galea LAM (2013) Postpartum Corticosterone Administration Reduces Dendritic Complexity and Increases the Density of Mushroom Spines of Hippocampal CA3 Arbours in Dams. *J Neuroendocrinol* 25:119–130.

Workman JL, Chan MYT, Galea LAM (2015) Prior high corticosterone exposure reduces activation of immature neurons in the ventral hippocampus in response to spatial and nonspatial memory. *Hippocampus* 25:329–344.

World Health Organization (2017) Depression and other common mental disorders: global health estimates. World Heal Organ:1–24 Available at:
<http://apps.who.int/iris/bitstream/10665/254610/1/WHO-MSD-MER-2017.2-eng.pdf>.

Wu Y, Dissing-Olesen L, MacVicar BA, Stevens B (2015) Microglia: Dynamic Mediators of Synapse Development and Plasticity. *Trends Immunol* 36:605–613 Available at: <http://dx.doi.org/10.1016/j.it.2015.08.008>.

Yagi S, Chow C, Lieblich SE, Galea LAM (2016) Sex and strategy use matters for pattern separation, adult neurogenesis, and immediate early gene expression in the hippocampus. *Hippocampus*.

Yang F, Tao J, Xu L, Zhao N, Chen J, Chen W, Zhu Y, Qiu J (2014) Estradiol decreases rat depressive behavior by estrogen receptor beta but not alpha: no correlation with plasma corticosterone. *Neuroreport* 25:100–104 Available at: <http://www.ncbi.nlm.nih.gov/pubmed/24128867>.

Yang SJ, Kim SY, Stewart R, Kim JM, Shin IS, Jung SW, Lee MS, Jeong SH, Jun TY (2011) Gender differences in 12-week antidepressant treatment outcomes for a naturalistic secondary care cohort: The CRESCEND study. *Psychiatry Res.*

Yehuda R, Southwick SM, Krystal JH, Bremner D, Charney DS, Mason JW (1993) Enhanced suppression of cortisol following dexamethasone administration in posttraumatic stress disorder. *Am J Psychiatry*.

Yin W, Gallagher NR, Sawicki CM, McKim DB, Godbout JP, Sheridan JF (2019) Repeated social defeat in female mice induces anxiety-like behavior associated with enhanced myelopoiesis and increased monocyte accumulation in the brain. *Brain Behav Immun.*

York EM, Ledue JM, Bernier LP, Macvicar BA (2018) 3dmorph automatic analysis of microglial morphology in three dimensions from ex vivo and in vivo imaging. *eNeuro*.

Young EA (1996) Sex differences in response to exogenous corticosterone: A rat model of hypercortisolism. *Mol Psychiatry*.

- Young EA, Kornstein SG, Marcus SM, Harvey AT, Warden D, Wisniewski SR, Balasubramani GK, Fava M, Trivedi MH, John Rush A (2009) Sex differences in response to citalopram: A STAR*D report. *J Psychiatr Res* 43:503–511.
- Young EA, Kotun J, Haskett RF, Grunhaus L, Greden JF, Watson SJ, Akil H (1993) Dissociation between Pituitary and Adrenal Suppression to Dexamethasone in Depression. *Arch Gen Psychiatry*.
- Zarrouf FA, Artz S, Griffith J, Sirbu C, Kommor M (2009) Testosterone and depression: Systematic review and meta-analysis. *J Psychiatr Pract*.
- Zeidan MA, Igoe SA, Linnman C, Vitalo A, Levine JB, Klibanski A, Goldstein JM, Milad MR (2011) Estradiol modulates medial prefrontal cortex and amygdala activity during fear extinction in women and female rats. *Biol Psychiatry*.
- Zhang D, Zhang J, Bian C, Deng Q (2010) Postnatal and ovariectomy regulation of postsynaptic density protein-95 in the hippocampus of female Sprague-Dawley rats. *Synapse*.
- Zhu C Bin, Lindler KM, Owens AW, Daws LC, Blakely RD, Hewlett WA (2010) Interleukin-1 receptor activation by systemic lipopolysaccharide induces behavioral despair linked to MAPK regulation of CNS serotonin transporters. *Neuropsychopharmacology*.
- Zobel AW, Nickel T, Künzel HE, Ackl N, Sonntag A, Ising M, Holsboer F (2000) Effects of the high-affinity corticotropin-releasing hormone receptor 1 antagonist R121919 in major depression: the first 20 patients treated. *J Psychiatr Res*.
- Zunszain PA, Anacker C, Cattaneo A, Choudhury S, Musaelyan K, Myint AM, Thuret S, Price J, Pariante CM (2012) Interleukin-1 β : A new regulator of the kynurenone pathway affecting human hippocampal neurogenesis. *Neuropsychopharmacology*.