The Role Of Estrogen In Emotional And Cognitive Processes Integral To Major Depressive Disorder

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ABSTRACT

Women have greater incidence and prevalence of Major Depressive Disorder (MDD) than men during the reproductive life phase when ovarian hormones fluctuate, suggesting that ovarian hormones have a significant role in MDD etiology in women. As the core symptoms of MDD are indicative of alterations in stress responding, emotional processing, and mood regulation, examining the effects of the estrogen on these processes in women may provide a better understanding of the role of estrogen in the sex difference in MDD rates. The general aim of this dissertation was to examine neural, emotional, and attentional processes related to stress response alterations and cognitive bias in MDD in women.

To examine menstrual phase and estradiol level effects on the neural and mood response to psychosocial stress, healthy, normally cycling women were examined at either the high or low estradiol phase of the menstrual cycle. Participants were exposed to the Montreal Imaging Stress Task (MIST), with brain activity measured through functional magnetic resonance imaging (fMRI), and behavioral response assessed with subjective mood and stress measures. We found that women during the high estradiol phase showed significantly less hippocampal deactivation during psychosocial stress compared to women during the low estradiol phase. Additionally, women with higher estradiol levels also had less subjective distress in response to the MIST than women with lower estradiol levels. These results suggest that high estradiol may be protective against the shifts in brain system activity and negative mood responses associated with psychosocial stress. Periods of low estradiol may enhance the negative impact of psychosocial stress on neural activity and mood and thus contribute to MDD risk in vulnerable women.

The relation of cognitive bias to depression history in women was examined in postmenopausal women with and without a history of major depression using an emotion dot probe task during fMRI. Women with remitted MDD showed greater attentional facilitation for negative images than women with no history of MDD that was directly correlated with amygdala activity for negative images and amygdala-hippocampal connectivity in a resting scan. These findings provide evidence that differences in activity and functional connectivity in emotional processing networks may provide a neurobiological basis for continued cognitive bias in remitted MDD. Preliminary data indicate that estradiol treatment reduces amygdala-hippocampal connectivity specifically in women with a history of MDD and has interactive effects with MDD history on the mood response to psychosocial stress following the MIST such that women with a history of MDD appear to benefit from estradiol treatment while women without such history do not. Women with a history of or vulnerability to MDD may be particularly sensitive to the positive effects of estradiol on brain systems important to regulating emotional responses to psychosocial stress. The findings presented in this dissertation suggest that estrogen fluctuations across the menstrual cycle and at other reproductive events may contribute to depression risk through effects on brain systems integral to emotional evaluation and response with potential cognitive consequences.
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Chapter 1: Comprehensive Literature review

1.1. Introduction

Major depressive disorder (MDD) is a complex disorder that impacts multiple systems in the brain and periphery and alters emotional and cognitive processes that are integral to healthy daily functioning and quality of life. The incidence and prevalence of MDD in women is 2-3 times higher than in men (Kessler et al., 2003, 2005). In men new onset rates and 12 month prevalence of MDD remain fairly constant from puberty to old age, but increase in women at puberty and remain higher than men until menopause (Kessler et al., 2003, 2005a). Depression risk for women changes across the lifespan with higher risk corresponding to life stages in which ovarian hormones fluctuate across the monthly menstrual cycle or with reproductive events such as parturition and the menopause transition (Gonda et al., 2008; Schiller et al., 2015; Schmidt and Rubinow, 2010). Ovarian hormones have varied effects in the brain including modulation of emotional perception, mood regulation, and stress response, as well as effects on cognition. The concurrence of increased depression risk with the reproductive life phase indicates that ovarian hormone fluctuations may contribute to mood disruption in women. Naturally occurring periods of low estrogen (premenstrually and during late perimenopause) may introduce windows of increased vulnerability for depression through the withdrawal of beneficial modulation of emotional processing and mood regulation. The research included in this dissertation examined the role of estrogen in emotional and cognitive processes integral to MDD.
1.2. Major Depressive Disorder

1.2.1 MDD Diagnosis and Epidemiology

According to the Diagnostic and Statistical Manual of Mental Disorders 5, MDD is defined as experiencing one or more major depressive episodes (lasting at least 2 weeks), that are not better explained by another medical condition or other psychiatric disorder (American Psychiatric Association, 2013). Major depressive episodes must include at least 5 of 9 symptoms within the same 2 week period: depressed/ sad mood, anhedonia, change in appetite, insomnia or hypersomnia, psychomotor agitation or retardation, fatigue, negative feelings of self (worthlessness, guilt), impaired concentration or decision making, thoughts of death or suicidal ideation/attempt. These symptoms must cause significant distress and represent a change from previous functioning (American Psychiatric Association, 2013). The twelve month prevalence of MDD in the United States is just under 7% (Substance Abuse and Mental Health Services Administration, 2013) and is predicted to increase in the future (Brundtland, 2001). MDD is the largest contributor to disability due to mental and behavioral disorders (3.7% of US disability-adjusted life years and 8.3% of US years lived with disability) (Murray et al., 2013). Although mood and anxiety disorders are the most prevalent psychiatric disorders in the US, current strategies for treating MDD fail to induce lasting remission or prevent recurrence in a significant portion of sufferers.

Current first-line antidepressant medications are ineffective in inducing remission in almost a third of patients (Adli et al., 2006). If patients do not respond to the first antidepressant treatment subsequent attempts with other medications are less effective
(Oestergaard and Møldrup, 2011). The response rate for the most commonly prescribed anti-depressants, selective serotonin reuptake inhibitors (SSRIs) is 30% (Trivedi et al., 2006), and depressive symptoms often return with SSRI discontinuation (Glue et al., 2010). Because many individuals with MDD do not experience successful treatment with standard pharmacological approaches, or have returning depressive symptoms after discontinuing antidepressants, augmenting current treatments and preventing depression recurrence remains a significant goal for treatment strategies. Better understanding the role of ovarian hormones in MDD in women may provide novel targets or better focus prevention strategies.

1.2.2 MDD Recurrence

MDD is often recurrent, with recurrence rates as high as 85% in 15 years in individuals with past MDD episodes either in specialized mental health or primary care for MDD, and up to 35% in 15 years in the general population (Hardeveld et al., 2010). Most individuals with MDD have more than one depressive episode, with the response to antidepressant treatments decreasing with subsequent episodes (Lee et al., 2012). While there are a number of risk factors that contribute to first onset depression, the primary predictors of recurrence are the number of previous episodes and residual depressive symptoms that remain during remission (Hardeveld et al., 2010). The “kindling” hypotheses of depression recurrence suggests that with successive depressive episodes the threshold for response to stressful life events or depression triggers is lowered and vulnerability for future depressive episodes increases (Monroe and Harkness, 2005). Depressive symptoms during remission may indicate trait vulnerability factors that predispose emotional or cognitive processes towards depressive responding.
Hammen and colleagues have proposed a “stress generation” hypothesis of depression recurrence; individuals who experience recurrent depression are more likely than people with no medical illness to experience subsequent stressful events (Hammen, 1991), particularly “dependent” stressful events, meaning generally interpersonal conflict to which the depressed individual contributes. According to this stress generation hypothesis, recurrence results from a cycle of psychosocial stress and depression in which stress both contributes to and is a consequence of depressive episodes (Hammen, 1991). Depressive episodes may leave a “scar”, such as cognitive bias for negative information which results in maladaptive behavior and dependent stressful events (Raedt and Koster, 2010). Thus, with each depressive episode the association between negative cognitive patterns and negative mood may be reinforced and make the individual more vulnerable to future depressive episodes (Dent and Teasdale, 1988).

1.3 Cognitive Models of MDD

1.3.1 Ventral and Dorsal Systems of Emotion Perception and Regulation

Theories of the neurobiology of MDD have evolved into complex network models that include cognitive and emotion regulation mechanisms. One useful model that incorporates emotion processing and cognitive function is the division of stimuli evaluation and emotion regulation into ventral and dorsal brain systems (Figure 1) (Phillips et al., 2008). Structures in the ventral system include the amygdala and ventral and orbital prefrontal areas (Drevets et al., 2008; Mayberg, 1997; Phillips et al., 2008). The dorsal division includes the hippocampus, dorsal anterior cingulate cortex (dACC), subgenual prefrontal cortex (subgenual PFC) and dorsal lateral prefrontal cortex (dLPFC) (Drevets et al., 2008; Mayberg, 1997; Phillips et al., 2008). The
ventral system allows for the rapid appraisal of emotionally valenced stimuli, while the dorsal system provides the capacity to modulate the affective, physiological, and cognitive consequences of ventral output (Phillips et al., 2003).

The overlap in circuits for mood regulation and cognitive function in the ventral-dorsal model is indicative of an intimate connection between cognition and mood (Fink et al., 1995). Becks’ cognitive model of depression posits that individuals with MDD experience information according to mood congruent schemas, and are apt to interpret information as reflecting a negative self-image, a negative view of external information, and pessimism about the future (Beck and Haigh, 2014). This predisposition for experiencing and interpreting information such that negative schemas are reinforced may reflect dysregulated ventral-dorsal interactions in MDD. Cognitive bias is the predisposition to attend to or remember certain types of information over others. The cognitive model of MDD posits that emotional processing circuits in the brain

**Figure 1: Ventral and dorsal brain systems for emotional appraisal and regulation.**
are altered in depression such that there is a bias toward negative information, and attenuated processing of positive information (Disner et al., 2011).

### 1.3.2 Attention Bias

Attention is the process by which cognitive resources are allocated to processing information and selecting which information is blocked from further processing (Chun and Turk-Browne, 2007). The focus of attention is determined by interactions between bottom-up (stimulus-driven) processes in the ventral system and top-down processes in the dorsal system (Egeth and Yantis, 1997). The interplay between attention and memory is complex and the pathways and mechanisms involved are not well understood (Chun and Turk-Browne, 2007). Because memory capacity is limited, attention has a significant role in determining what information is encoded into memory (Chun and Turk-Browne, 2007). Selective attention, the process of choosing which stimuli receive attention and which are ignored, is crucial when there are competing stimuli present (Chun and Turk-Browne, 2007). Memory deficits and bias for negative information have commonly been found in MDD (Sears et al., 2011; Sheline, 2000). It may be that altered memory in MDD is largely due to the effects of attention bias on sensory processing of emotional information and subsequent effects on memory encoding (Raedt and Koster, 2010). Emotionally-valenced stimuli drive bottom-up attention more than emotionally neutral stimuli (Holtmann et al., 2013; McHugo et al., 2013; Peers et al., 2013; Stollstorff et al., 2013). Vuilleumier and colleagues propose a model of attention by which emotional information is “amplified” and preferentially processed. In this model the amygdala has a significant role in generating “emotional bias signals”; ventral system processes initiated by the amygdala direct
attention to emotionally-relevant stimuli and modulate sensory systems such that the processing of such stimuli is enhanced and maintained (Pourtois et al., 2013).

Ventral and dorsal mechanisms interact to guide attention; the ventral system facilitates the evaluation of stimuli and directs top-down processes towards emotionally-relevant information (Chun and Turk-Browne, 2007), while dorsal system processes maintain attention congruent to endogenous goals and motivational states (Mohanty and Sussman, 2013). Conflict in top-up and bottom-down attentional processes occur when ventral system evaluation of stimuli and subsequent emotional responses do not accord with the motivations and goals impacting dorsal-system activity (Chun and Turk-Browne, 2007; Okon-Singer et al., 2015). Dorsal system regulation of attention is influenced by endogenous motivational and emotional states. Depending on these states the dorsal system may respond to attentional conflict by counteracting amygdala-driven activity and reducing attention to and sensory processing of emotional stimuli, directing attention to competing stimuli, or directing top-down attention to the stimuli driving bottom-up attention (Pourtois et al., 2013). Studies demonstrating that acute stress and anxiety enhances both amygdala response and subsequent sensory systems activity to negative emotional stimuli (Bishop et al., 2004; Cornwell et al., 2011; van Marle et al., 2009; Shackman et al., 2011; Vogel and Luck, 2000) provide evidence that negative mood states bias attention and sensory processes toward negative information.
1.3.3 Cognitive Bias in Depression

Mood congruent cognitive bias is a common finding in currently depressed individuals (Gaddy and Ingram, 2014). A recent meta-analysis of eye tracking in attention tasks found that individuals with current MDD show blunted orienting responses and reduced maintenance of attention to pleasant stimuli but maintain attention for negatively valenced stimuli (Armstrong and Olatunji, 2013). Negative cognitive bias not only affects processing of emotional information but appears to be related to emotional responding to psychosocial stress. Individuals with current MDD fixate longer and more frequently on sad faces than healthy controls, and also take longer to recover from to negative mood following a laboratory stressor (Sanchez et al., 2013). Negative cognitive bias in current MDD may result from mood-congruent cognitive processing during depressive episodes; negative information is processed more readily because it accords with internal emotional processes during depressive episodes. However, according to Beck’s cognitive model of depression (Beck and Haigh, 2014; Beck, 2005), cognitive bias for negative information remains during remission and presents a trait vulnerability for depression rather than a consequence of negative mood. A causative link between cognitive bias and mood is also suggested by the finding that experimental methods that modulate attention bias also affect anxiety and depression symptoms. Cognitive bias modification (CBM) is an experimental technique in which attention is trained away from or towards negative stimuli and has been found to be effective in modulating depressive symptoms as well as attentional performance (Hallion and Ruscio, 2011). The mood effects of CBM training demonstrate that cognitive bias may be a causative factor in mood disruption rather than a consequence (Hallion and Ruscio, 2011).
Cognitive models of MDD accord with neural system dysregulation models in that cognitive bias is associated with altered activity in dorsal and ventral systems (Raedt and Koster, 2010). Greater activity in ventral system structures and less activity in dorsal structures to negative stimuli is a consistent finding in MDD (Drevets et al., 2008; Savitz and Drevets, 2009). Enhanced amygdala activity in currently depressed and at risk individuals (Arnone et al., 2012; Zhong et al., 2012) may indicate greater signal driven automatic evaluation or reduced regulation by dorsal system structures resulting in biased processing of negative information (Sears et al., 2011). Neuroimaging studies show that in MDD additional regions of the prefrontal cortex are recruited during automatic control of emotional responses, suggesting that compensatory activity in dorsal regions may be activated to control enhanced bottom-up activity, driven by amygdala hyperactivity in MDD (Drevets, 2003; Drevets et al., 2002; Zhong et al., 2012).

Individuals with MDD appear to have diminished dorsal system capacity to regulate ventral-driven sensory and emotional processing of negatively valenced information. Establishing dorsal system regulation of emotional processing may be an important component of successful MDD remission (Erk et al., 2010; Gotlib and Joormann, 2010). However, even during remission, automatic ventral system processes may be prioritized when cognitive resources need to be allocated efficiently (such as during stress) resulting in negative mood and reinforcing negative cognitive bias. Cognitive bias in remitted MDD may reflect continued dysregulation in emotional processes and a neurobiological basis for vulnerability to MDD recurrence, especially following stress.
1.4 Stress and Major Depression

1.4.1 The Stress Response System

Stressors are perceived or actual threats to homeostasis that may be physical or psychosocial and the healthy stress response is a coordinated neuroendocrine reaction that maintains or restores homeostasis (Gold, 2015). The system that is involved in the response to stress includes the autonomic nervous system, the hypothalamic pituitary adrenal (HPA) axis, and the brain regions that orchestrate the autonomic, hormonal, and emotional response to stress (Chrousos and Gold, 1992; Johnson et al., 1992; Sternberg et al., 1992). This system normally serves to coordinate optimal neuroendocrine, immune, and autonomic responses to stress (for review see Stokes, 1995). During stress, the peptides cortisol releasing factor and arginine vasopressin are secreted from the hypothalamus and induce the release of adrenocorticotropic hormone from the anterior pituitary. In response to adrenocorticotropic hormone, glucocorticoids are released from the cortex of the adrenal glands. In humans the primary glucocorticoid is cortisol. Cortisol acts throughout the body to increase the energy available to manage the demands of the stressor and maintain homeostasis. The endocrine response to stress is regulated through negative feedback mechanisms of glucocorticoids at the pituitary, hypothalamus, hippocampus, and limbic regions (Gillies and McArthur, 2010) (Figure 2).
The optimal HPA axis response to stress is dynamic, both quickly releasing cortisol and quickly stopping the action of cortisol. An efficient HPA response prevents peripheral and central nervous system damage due to prolonged cortisol exposure (Lupien et al., 2005). Inefficient regulation of the HPA axis and chronic high cortisol exposure is associated with increased blood pressure, increased risk for diabetes, hypertension, arterial diseases, impaired growth and tissue repair, and suppressed immune function (Derijk and Sternberg, 1994; Lupien et al., 2009; Moulton et al., 2015; Munck and Guyre, 1991). Thus, HPA dysregulation is a
common factor in co-morbid diseases such as depression and cardiovascular disease (Carney and Freedland, 2003; Chen et al., 2007; Jiang et al., 2002; Joynt et al., 2003; Miller et al., 2002; Nikkheslat et al., 2015). Chronic cortisol dysregulation may negatively impact a variety of body systems and may present a general vulnerability factor that contributes to the risk for a number of diseases including MDD.

1.4.2 Ventral and Dorsal Systems in the Stress Response

The hypothesis that stress system dysfunction is integral to the etiology of MDD is consistent with neuroanatomical models of MDD that posit mood dysregulation as a result of an imbalance in functional activity in the ventral and dorsal systems, which are sensitive to cortisol and show reciprocal activity changes during stress responding (Drevets et al., 2008; Mayberg, 1997; Phillips et al., 2008).

During the healthy stress response activity in the brain shifts from dorsal system mood regulation to ventral system threat evaluation and management as cognitive resources are preferentially allocated to automatic processes for responding to threat (Arnsten, 2009). Enhanced ventral activity results in acute dysphoria which is a normal and necessary component of the stress response as negative mood motivates managing stressors (Gold, 2015). There are two regulatory components of the dorsal system; hippocampal activity is automatically enhanced in response to amygdala activity while frontal activity is recruited through later stage or voluntary processes (Phillips et al., 2008). In the unstressed state the subgenual PFC inhibits amygdala activity and consequently attenuates amygdala-driven attention (Gold, 2015). During stress dorsal system function is down-regulated in favor of automatic, rapid emotional responses.
in the ventral system. Once dorsal activity is decreased through automatic or voluntary processes, the amygdala is released from inhibition (Drevets et al., 1997; Simpson et al., 2001).

The amygdala has a central role in both the mood and cognitive response to stress, through reciprocal projections with dorsal system structures, and in orchestrating endocrine and autonomic responses through projections to the hypothalamus and central autonomic centers (Gold, 2015). The release of amygdala inhibition promotes negative mood and cognitive response to stress and both directly and indirectly (through down-regulation of dorsal region activity) drives HPA axis and autonomic responses. In a recent expert review Gold posits that an unregulated feed-forward system decreased dorsal system activity and increased amygdala activation causes a prolonged dysphoric state and biases cognitive processes towards negative emotionally valenced information (Gold, 2015).

1.4.3 Stress and Cognition

The changes in ventral and dorsal activity that occur during that normal stress response result in altered cognitive processing of emotional information including memory (McGaugh, 2004) and attention bias for threatening or negatively valenced stimuli (Arnsten, 2009). Normally short-term cognitive bias towards negative information serves to efficiently process and manage stressors, however in a dysregulated stress system persistent cognitive bias may become established and contribute to cognitive vulnerability for depression.

The organization of the stress system in the brain allows for cognitive alterations in response to acute cortisol changes. There are two types of receptors for glucocorticoids, mineralocorticoid receptors (MRs) and glucocorticoid receptors (GRs) (Reul and de Kloet, 2015).
MRs are expressed exclusively in limbic regions and have much higher affinity for glucocorticoids (6-10 times) than GRs which are expressed throughout the brain with high density in frontal regions (Fuxe et al., 1985; Patel et al., 2000; Reul and de Kloet, 1985). Because of the difference in affinity, MRs are occupied at low glucocorticoid levels while GRs only become significantly occupied at high glucocorticoid levels. This dynamic ratio of activation between receptors allows for the effects of acute stress to be mediated mainly through GRs (Lupien et al., 2005). Basal and acutely elevated glucocorticoid levels have different effects on brain function via action at MRs or GRs respectively.

In human and rodent studies acute glucocorticoids appear to have a biphasic effect on cognition with both significantly decreased and increased glucocorticoids impairing memory. Low to moderate GR activity may benefit memory and cognitive performance while high GR activity under acute stress has detrimental effects on those domains (Lupien et al., 2002; Newcomer et al., 1994, 1998, 1999; de Quervain et al., 2000). In both young and older adults hippocampal function is impaired under high endogenous cortisol paralleling reduced long term potentiation related to high GR occupation in animal models (Lupien et al., 2005, 2009). These findings in healthy adults, as well as memory impairment, and hippocampal volume loss in patients with Cushing’s disease or on cortisol treatments support the “Glucocorticoid cascade hypothesis” which posits a direct effect of chronic high cortisol on hippocampal integrity (impaired function and decreased volume) (Sapolsky et al., 1986).

The hippocampus is an important negative feedback site for cortisol and regulator of HPA axis activity (Herman and Cullinan, 1997; Jankord and Herman, 2008). Glucocorticoid receptors occupation results in increased hippocampal activity and inhibits HPA axis function; however this regulatory loop is suppressed during acute stress to maintain the endocrine response.
Mild acute stress promotes neurogenesis in the dentate gyrus of the hippocampus and appears to support adaptive responding to stress and the efficient return to basal HPA axis activity and cortisol levels (Duman and Li, 2012; Ming and Song, 2011). Blocking hippocampal neurogenesis during acute stress in mice produces depression-like behavior (Snyder et al., 2011), suggesting that hippocampal adaptation may be an integral component of the healthy stress response that prevents depressive responding. Additionally, animal studies suggest a beneficial effect of acute glucocorticoids on hippocampal function, removing corticosterone impairs hippocampally-mediated memory, and replacing corticosterone rescues performance (Lupien et al., 2005). However, under chronic stress in rodent models hippocampal volume and plasticity is reduced (Magariños and McEwen, 1995; Watanabe et al., 1992), suggesting that chronically elevated glucocorticoids negatively impact the hippocampus. These alterations in hippocampal structure and function may result in stress system dysregulation as hippocampal regulation of the HPA axis is reduced. Reduced ability of the hippocampus to regulate stress responding and modify its activity in response to acute stress may result in stress system dysregulation.

Prolonged HPA dysregulation may alter the dynamics of MR/GR activity and modify the cognitive effects of acute stress. Chronic high cortisol is reduces MR expression in animal studies and thus results in high GR activation (Herman et al., 1995; Kitraki et al., 1999; Sapolsky et al., 1986). Lupien’s longitudinal studies in older adults provide evidence that chronically high cortisol similarly alters the cognitive effects of stress in humans. Older adults with normal, moderate basal cortisol show a beneficial effect of acute cortisol increase on memory, however in older adults with high basal cortisol, acutely increased cortisol impairs memory performance, suggesting that chronic high cortisol reduces the effect of acute cortisol changes in humans.
(Lupien et al., 2005). As MR expression decreases under prolonged HPA dysregulation and GRs become occupied at lower cortisol levels the cognitive impact of acute stress and chronic high cortisol may become more pronounced (Lupien et al., 2005).

The acute stress response in the brain is characterized by a shift towards ventral system activity and away from dorsal system activity. This shift results in dysphoric mood which motivates behavioral responses to stress and cognitive bias in attention and memory that focus cognitive resources on managing the stressor. In healthy systems these responses only last as long as necessary to cope with the stressor. However, dysregulation of the stress response may result in prolonged dysphoric state and a cognitive bias for negative information followed by depression. Cognitive and stress-exposure models of MDD are complimentary as altered cognitive processing of negative emotional information may result from and be maintained by stress system dysregulation.

1.4.4 Stress Dysregulation in MDD

Altered HPA axis function is common in MDD (Burke et al., 2005; Lupien et al., 2009) and the onset of depressive episodes is often attributed to stressful life events (Frank et al., 1994; Kendler et al., 2000, 1999). The association of stress with MDD supports a stress-exposure model of MDD in which depressive episodes are triggered by stressful events and maladaptive responding (Hankin et al., 2007; Liu and Alloy, 2010). Individuals with MDD show chronically elevated cortisol, however, cortisol feedback at the pituitary appears to be intact indicating that HPA axis dysregulation originates from blunted feedback at the hypothalamus or in higher level brain systems that regulate HPA axis activity (Holsboer et al., 1984). Evidence that frontal dorsal
system regions may be less sensitive to HPA axis negative feedback in depression is provided by reduced cortisol releasing hormone receptor densities in frontal regions in post mortem studies following suicide (Nemeroff et al., 1988) indicating that. Cortisol releasing hormone levels are elevated in depressed patients and decrease with successful treatment (Nemeroff et al., 1985; Widerlöv et al., 1988) again suggesting that MDD is characterized by HPA axis dysregulation and remission is associated with restored regulation. Chronically altered sensitivity for cortisol releasing hormone and cortisol may have long-term effects on brain regions that respond to these hormones.

The alterations in brain structure and function that are found under chronic stress parallel changes in MDD; patients with MDD show structural changes in brain regions that are integral to stress responding including decreased subgenual PFC and hippocampal volume and increased amygdala volume (McKinnon et al., 2009). Dorsal system volume reductions are likely due to the loss of neuronal and glial cells and diminished dendritic arborization (Drevets et al., 1997, 2008; Jaako-Movits et al., 2006), while in the amygdala dendritic arborization and spine density are increased (Drevets, 2003; Price and Drevets, 2012). These changes suggest that plasticity is reduced in dorsal regions and increased in amygdala which accords with disrupted dorsal system-amygdala interactions as a mechanism in MDD etiology (Gold, 2015).

Cognitive alterations in MDD also parallel shifts in cognitive resource allocation during stress responding (Gotlib et al., 2004; Isaac et al., 2014; Suslow et al., 2001), indicating that dysregulated stress system function may contribute to behavioral changes in MDD. In addition to the effects of amygdala hyperactivity, altered HPA axis function and reduced negative feedback by glucocorticoids may contribute to hippocampal atrophy seen in MDD. MDD is associated with bilateral hippocampal atrophy (8-19%) that appears to have functional significance, as
individuals with past MDD (currently in remission) continue to show deficits in hippocampally-mediated tasks (declarative and verbal memory) (Sheline, 2000). The origin of HPA axis dysregulation in MDD remains unknown; however the organization of peripheral and brain stress systems make these systems vulnerable to dysregulation (Gold, 2015). MDD may result from an initial alteration in stress system function (perhaps through genetic traits or environmental factors) that is maintained and amplified through the numerous feedback loops and reciprocal interactions that constitute the stress system.

In a recent expert review, Gold defines MDD as a disease that contributes to “multiple system pathologies” (Gold, 2015). The organization of the stress system with multiple feed forward loops to assure successful management of stressors and the mood and cognitive effects of even acute, well-managed, stress create a system that is vulnerable to dysregulation when confronted with genetic vulnerability or extreme life stressors. The symptoms that characterize MDD resemble prolonged unregulated stress responses; once the stress system becomes dysregulated, feed-forward mechanisms sustain hypercortisolism and enhanced amygdala function, reinforcing the mood and cognitive consequences of stress system activity (Gold, 2015). Successful MDD treatment likely requires approaches that target multiple mechanisms that are impacted by stress system activity including promoting neuroplasticity and cognitive strategies to support adaptive cognitive processes.

Altered HPA function may be especially important in the etiology of MDD in women. Although there is no difference in the number of stressful life events (Young and Korszun, 2010), or the perception of these events (Burt et al., 1988) between men and women, the cortisol response to stress does show sex differences, and decreases during high estrogen phases of the menstrual cycle (Kirschbaum et al., 1992, 1999) and during pregnancy (Altemus, 2006). Also,
women appear to remain more sensitive than men to lower levels of cortisol following repeated stressors (Vamvakopoulos, 1995; Wang et al., 2007). These findings provide evidence that ovarian hormone levels may modulate stress system functioning in women. Estrogen may support efficient and dynamic stress responding and prevent disrupted ventral-dorsal system interactions through supporting neuroplasticity in prefrontal areas and hippocampus. Maintaining function in dorsal system structures preserves regulatory control over ventral activity (particularly the amygdala) and allows for the efficient return to a non-stressed state. Loss of dorsal system function may predispose the brain to rely on ventral system processes which bias attention and memory towards negative information and prolongs emotional, endocrine, and autonomic stress responding aberrantly. Periods of increased stress sensitivity, due to ovarian hormone changes, may present windows of vulnerability to mood dysregulation in women who are at risk for MDD.

1.5 Hypothalamic-Pituitary-Gonadal Axis

Depressive symptoms and depressive episodes in women appear to be linked to ovarian hormone changes. Women with a history of depression are more likely to experience increased depressive symptoms during periods of ovarian hormone fluctuation than at other times (Young and Korszun, 2002). Also a history of MDD increases a woman’s risk for mood disorders that are directly associated with ovarian hormone changes including premenstrual mood disorder (Halbreich et al., 1986, 1984), postpartum (Reich and Winokur, 1970) and perimenopausal depression (Freeman et al., 2014). Even in healthy women, low estrogen phases of the menstrual cycle are associated with increased symptoms of negative mood (Gonda et al., 2008). Women
with MDD and reproductive phase-related mood disorders generally have normal ovarian hormone levels (O’Toole and Rubin, 1995; Schmidt and Rubinow, 2010; Schmidt et al., 1998), indicating that these disorders aren’t directly contributable to abnormally low estrogen (Young et al., 2000). While most women do not develop mood disorders as a result of ovarian hormone changes, the increased risk for first onset depression as estrogen declines during the late perimenopause (Bromberger et al., 2010; Harsh et al., 2009; Schmidt and Rubinow, 2010), and the relation of premenstrual dysphoric disorder to the low estrogen phase of the menstrual cycle (Steiner et al., 2003), suggests that some women are susceptible to mood dysregulation as a result of normally low estrogen levels. It may be that the role of estrogen in MDD is characterized by altered brain response to circulating levels rather than differences in estrogen levels.

The hypothalamic-pituitary-gonadal axis is a multilevel hormonal system that regulates the secretion of ovarian hormones in females (estrogen and progesterone) through multiple feedback mechanisms at the ovary, pituitary, and brain (for review see Vadakkadath Meethal and Atwood, 2005). Gonadotropin releasing hormone (GnRH) from the hypothalamus induces the release of luteinizing hormone (LH) and follicle stimulating hormone (FSH) from the anterior pituitary. LH and FSH modulate the ovarian cycle, with FSH stimulating follicle maturation and the release of estrogen during the follicular phase and LH triggering ovulation as well as progesterone release from the corpus luteum (ruptured follicle) during the luteal phase. Estrogen feedback to the pituitary and hypothalamus controls the release of GnRH, FSH, and LH and this system of feed forward and feedback mechanisms orchestrates activity within the HPG system that establishes the menstrual cycle (Figure 3).
Figure 2: Ovarian Hormones

(A) Hypothalamic-pituitary-gonadal axis in women. (B) The menstrual cycle
Women experience dramatic changes in cyclic ovarian hormones across the lifespan. During reproductive life estrogen and progesterone levels fluctuate across the monthly menstrual cycle. During the early follicular phase and menstruation both estradiol (the main circulating estrogen in non-pregnant reproductive aged women) and progesterone levels are low. Estradiol increases in the mid to late follicular phase prior to ovulation. Estradiol levels decrease immediately following ovulation, and both estradiol and progesterone levels increase during the luteal phase until menstruation. Outside of the periovulatory phase, progesterone fluctuations parallel those of estradiol, thus it is difficult to ascertain that separate effects of progesterone in studies of endogenous ovarian hormones. Progesterone generally appears to antagonize that effect of estradiol on mood and cognition (Greendale et al., 1998; Resnick et al., 1998; Toren et al., 1996), however progesterone effect have been much less studied.

The reproductive life phase is both preceded and followed by hypogonadal states prior to puberty and during menopause. The menopause transition is characterized by irregular ovarian hormone fluctuations; both estradiol and progesterone levels can vary dramatically during the early transition period (which may last years), and generally settles into a stable low estradiol state proximal (within 6 months) to the final menstrual period. This variability in ovarian hormones during the reproductive years and menopausal transition creates a dynamic environment for the functioning of mood regulatory and cognitive systems that are influenced by these hormones.
1.6 Estrogen in the Brain

Estrogen has a variety of effects at the cellular level including genomic actions, interaction with second messenger and G-protein systems, effects on calcium signaling, and neuroprotection (McEwen et al., 2012). Classical nuclear receptors (ERα and ERβ) are ligand-dependent transcription factors that interact at estrogen response elements on DNA and result in cascades of intra-cellular reactions which alter protein synthesis (Greene et al., 1986; Kuiper et al., 1996). These actions are relatively slow, but result in long-lasting changes at the synapse or in neural function (Luine, 2014). Recently, membrane-bound estrogen receptors have been discovered that may activate signaling pathways and have rapid effects on the order of seconds and minutes (Kelly and Levin, 2001; Kelly et al., 2003). These membrane-bound receptors are found in the prefrontal cortex and hippocampus, and may be responsible the acute effects of estrogen on cognition (Luine, 2014).

Estrogen modulation of overlapping and interacting systems for stress responding and emotional and cognitive processing of information provides a number of pathways through which estrogen fluctuations may contribute to MDD risk. In women with MDD vulnerability, estrogen may support healthy function of these systems, while having little or opposite effects for women in whom these systems are already functioning optimally. Periods of low estrogen may represent windows of risk to depressive episodes in women with a previous history of MDD because of differential effects of estrogen on mood regulation, stress, and cognitive systems (Newhouse and Albert, 2015). Understanding the interactions of vulnerability to MDD, stress, and estrogen will be important for developing more effective prevention and treatments for women with MDD.
1.6.1 Estrogen and Emotion Processing

Sex differences in evaluating and responding to emotional information provides a basis for understanding the effects of ovarian hormones on emotional processing in women. It is important to note that androgens are aromatized to estrogen in the brain so that men generally experience constant high estrogen activity in the brain, while women experience fluctuations in estrogen including periods of low estrogen (Fink et al., 1998). Thus, emotional processing in men may represent the effects of high estrogen (although interactions with organizational sex differences must be considered). Accordingly, sex differences in emotional processing are most apparent when men are compared to women during low estrogen. Neuroimaging studies of emotion detection or recognition show sex differences in brain regions used during these tasks, with women showing greater limbic, inferior frontal, and temporal activity and men showing greater prefrontal and parietal activity (Whittle et al., 2011). It has been suggested that this difference in activation patterns during emotion recognition may indicate that men and women perceive emotion at different levels of visual processing (Hall et al., 2004). Greater limbic activity in women suggests that emotion perception occurs at a primary level, while emotion perception in men occurs at a secondary level involving the prefrontal areas. This sex difference in emotion perception may contribute to the speed and accuracy advantage in women (especially for identifying negative emotions such as fear and anger) (Hampson et al., 2006; Merten, 2005; Montagne et al., 2005; Thompson and Voyer, 2014), as emotion recognition occurs early in visual processing, while in men emotion detection requires additional information about the learned significance of emotional facial expressions (Whittle et al., 2011).

Earlier recognition of negative emotional information in women may influence women’s ability to regulate emotional responses to such stimuli. Enhanced emotional detection at lower
levels of processing may represent greater signal driven automatic activity in the ventral system in women that requires greater top-down modulation to maintain adaptive mood responses. Earlier detection of emotional information and greater amygdala activity to negative information in women suggest that emotional evaluative processes in women are predominated by ventral system activity. Regulation of ventral system activity in women may be particularly important for healthy mood and supported by estrogen’s action in the dorsal system, while loss of estrogen may predispose emotional responding through the ventral system.

Generally studies of passive viewing of emotional images or words have found that women are more reactive to emotional stimuli whether measured through self-report, behavioral response, physiological response, or neuroimaging (Kret and De Gelder, 2012; Whittle et al., 2011). The sex difference appears to be specific for negative emotional information, with consistent findings of greater amygdala activity during negative images or words in women (regardless of valence or arousal ratings) (Whittle et al., 2011). Sex differences in activation patterns during encoding and recalling emotional information indicate greater overlap in activity in brain areas that are involved in processing current emotional experiences and encoding emotional information into memory in women including limbic, insular and prefrontal regions (Damasio et al., 2000; George et al., 1996; Piefke et al., 2005). In women this overlap in activity correlates with subsequent successful memory recall (Canli et al., 2002), suggesting that differences in memory encoding for emotional information may contribute to better memory for emotional experiences in women than men. More integrated brain circuits for emotional and cognitive processes may enhance attention and memory for emotional information and contribute to a cognitive vulnerability for depression in women.
Sex differences in emotion processing and responding are modulated by ovarian hormone fluctuations, indicating that these processes and vulnerability to mood dysregulation may be influenced by circulating ovarian hormones. Sex differences in emotion recognition (Hampson et al., 2006) are reduced during high estrogen in women (Derntl et al., 2008), suggesting that estrogen enhances emotional processing through higher-level association pathways and may support healthy mood responding to emotional information. High estrogen levels reduce ventral activity (Derntl et al., 2008; Goldstein et al., 2005; Pearson and Lewis, 2005) Fluctuating ovarian hormones in women alter the perception of emotionally valenced information and the mood and cognitive responses to such information. In healthy women these changes may be experienced as the “normal” mood changes that accompany periods of low estrogen, however in a subset of vulnerable women they may contribute to increased depression risk. Although studies demonstrate that estrogen attenuates emotional processing and supports mood regulation in healthy women, the effects of estrogen in women with current or remitted MDD, in whom ventral-dorsal interactions are likely altered, remain unclear.

1.6.2 Estrogen and Cognition

In addition to the putative indirect effects through emotional processing, estrogen may directly modulate cognitive processes that are altered in MDD (Halbreich et al., 1995). Cognitive declines in a number of cognitive domains including attention and processing speed following menopause are greater than would be expected from the effects of age alone (Halbreich et al., 1995 b), indicating that estrogen has positive effects on cognition that diminish as estradiol declines in menopause. Consistent findings of cognitive decline and increased risk for dementia in women who undergo early menopause, and thus experience a greater period of life with low ovarian hormone levels, further supports a negative impact of estrogen loss beyond the effects of
age in older women (Farrag et al., 2002; Nappi et al., 1999; Rocca et al., 2008). In meta-analyses estrogen replacement generally benefits cognition in young postmenopausal women (Hogervorst and Bandelow, 2010; Maki, 2005) and following oophorectomy (Bove et al., 2014; Phillips and Sherwin, 1992; Rocca et al., 2008; Sherwin, 1988; Sherwin and Gelfand, 1985), while combined estrogen and progestin treatments have little or negative effect (Hogervorst and Bandelow, 2010) indicating that estrogen withdraw at menopause is a significant factor in cognitive decline with age in women (for review see: Maki and Dumas, 2009; Newhouse and Dumas, 2015; Sherwin and Henry, 2008).

Studies examining the effects of estrogen fluctuation across the menstrual cycle indicate that performance on tasks that rely on the hippocampus and prefrontal cortex are enhanced during high estradiol phases of the menstrual cycle (Sundström, Poromaa, and Gingnell, 2014) including cognitive control, response inhibition (Colzato et al., 2010, 2012), and verbal (Rosenberg and Park, 2002) and spatial memory (Hampson and Morley, 2013). Suppression of estradiol in young women causes reductions in blood flow and metabolism to frontal regions which are restored with estradiol administration (Berman et al., 1997), demonstrating that even acute and short-term changes in estrogen may impact dorsal region function (Craig and Murphy, 2007).

Estrogen’s effects at the amygdala and hippocampus may alter processing and subsequent memory for emotionally valenced information. Memory for emotional information is enhanced during menstrual phases when estradiol is low and progesterone is high (Ertman et al., 2012). Of note is that memory is not only enhanced for the emotional content, but also for peripheral information (Nielsen et al., 2013), indicating that low estradiol may enhance attentional vigilance for emotional information and more strongly associate previously neutral
information with emotional information. This linking of neutral and emotional information in memory may underlie spontaneous intrusive recollections in PTSD or rumination in depression. Intrusive recollections are more common for traumatic events that occur during low estradiol/high progesterone phases of the menstrual cycle (Bryant et al., 2011; Ferree and Cahill, 2009; Ferree et al., 2011), suggesting that ovarian hormone effects on memory may contribute to maladaptive cognitive processes following psychosocial stress. Further evidence that estradiol levels and menstrual phase impact memory processes for emotional information is provided by a series of studies by Milad and colleagues which demonstrated that extinction of fear conditioning is greater during high estradiol phases of the menstrual cycle and even after acute exogenous estradiol administration (Milad et al., 2006, 2010; Zeidan et al., 2011). Whereas low estrogen or high progesterone appear to enhance emotional processing and memory for negative information, high estradiol supports top-down modulation of cognitive processes and may restrain ventral participation in attention and memory.

1.7 Potential Mechanisms of Estrogen’s Role in MDD

Acting at nuclear and membrane-associated receptors allows estrogen to have both rapid and long-term effects on neuronal function, as well as varied modes of action in brain regions that are important for emotional and cognitive processes. There are a number of mechanisms through which ovarian hormones may affect brain function; including modulating neurotransmitter system functions and neurotrophic effects that promote neuronal plasticity (Rubinow and Schmidt, 1995, 2006; Schmidt and Rubinow, 2010). Estrogen interactions with and modulation of serotoninergic and cholinergic neurotransmitter systems have particular
relevance as putative mechanisms that intersect with estrogen’s support of dorsal regulatory system function.

1.7.1 Neurotransmitter Systems

Commonly used antidepressant medications alter synaptic levels of the monoamines serotonin and norepinephrine or alter receptors for these neurotransmitters (Stahl, 1998). The effectiveness of these treatments accord with the monoamine hypothesis of MDD that altered levels of serotonin or norepinephrine underlay the etiology of depression (Schildkraut, 1973; Schildkraut et al., 1969). Norepinephrine from the locus ceruleus has projections to frontal and limbic regions (Moore and Bloom, 1979) and appears to participate in a number of stress system feedback loops with effect on arousal and sympathetic activity (Gold, 2015). There is some evidence that estrogen increases central norepinephrine levels (Etgen and Karkanias, 1994) and that the reduction of hot flashes following hormone replacement in menopausal women may be due to estrogen’s effect on norepinephrine (Archer et al., 2011). However, estrogen-norepinephrine interactions and the role of these effects in depression has been little studied (Borrow and Cameron, 2014; Genazzani et al., 2005). The effects of estrogen on the serotonergic system has been more studied and is one of the strongest candidate mechanisms by which estrogen influences depression (Borrow and Cameron, 2014)

Studies using tryptophan depletion (which decreases central serotonin) demonstrate that lower serotonin levels are more likely to precipitate depressive symptoms in women than in men (Booij et al., 2002; Ellenbogen et al., 1996; Moreno et al., 2006), however this sex difference was not seen following norepinephrine depletion (Moreno et al., 2006), suggesting that serotonin
dysfunction may be of particular importance to MDD in women. The antidepressant and SSRI augmentative effects of estrogen in women also suggest that estrogen may have beneficial mood effects through action on the serotonergic system (Halbreich et al., 1995a). In animal models, ovariectomy reduces and estrogen add-back increases serotonin receptors in the dorsal raphe nucleus (Sumner and Fink, 1993), anterior frontal, and cingulate regions (Sumner and Fink, 1995). This experimental finding is paralleled by an increased density of serotonin receptors in the forebrains of female rats during natural pro-estrus when endogenous estrogen levels are high (Sumner and Fink, 1997). Further evidence that the presence of estrogen alters serotonin function in the brain arises from studies of androgens in male animals. Testosterone, but not non-aromatizable androgens, in male animals increase serotonin receptor density (Sumner and Fink, 1998) suggesting that the effects of testosterone on serotonin function are mediated through aromatization of testosterone to estrogen.

In women, serotonin responsivity is reduced after menopause and restored following estrogen treatment (Halbreich et al., 1995b). While studies of estrogen alone as an antidepressant agent in peri and postmenopausal depression have had inconsistent findings (Cohen et al., 2003; Morrison et al., 2004; Rasgon et al., 2001; Schmidt et al., 2000; Soares et al., 2001; Stahl, 2001), estrogen-serotonin interactions may impact depressive symptoms or vulnerability to MDD through the cognitive and mood regulatory functions of the serotonergic system. Tryptophan depletion impairs the consolidation of episodic memory (Mendelsohn et al., 2009), reduces activity in brain areas important to the encoding of memory (hippocampus) (van der Veen et al., 2006), and induces negative memory and attentional bias (Delgado et al., 1989; Roiser et al., 2008) and difficulty with autobiographical memory (Haddad et al., 2009; Hayward et al., 2005) in remitted depressed individuals. These cognitive changes under reduced serotonin support the
hypothesis that diminished serotonin function may contribute to cognitive mechanisms of
depression, such as preferential processing of and memory for negative information. Epperson
and colleagues have shown that estrogen treatment reduces the effects on tryptophan depletion
on brain activity during both verbal working memory and emotion recognition tasks, suggesting
that estrogen supports serotonergic cognitive function (Epperson et al., 2012).

Tryptophan depletion generally has little mood effect in healthy individuals (Young,
2013) however, mood effects are common in individuals with remitted MDD or family histories
of MDD (Booij et al., 2003; Ruhé et al., 2007; Young and Leyton, 2002). Increased risk for
mood dysregulation during periods of low estrogen may be due to the loss of estrogen support of
the serotonergic system (Halbreich et al., 1995b) or indirect effects on shared pathways (Schmidt
and Rubinow, 2010).

Estrogen’s pro-cognitive effects may be mediated though interactions with the
cholinergic system which is critical for global cognitive function, including primary processes
such as attention and memory which underlie higher order cognition. Loss of cholinergic
function appears to be one of the primary mechanisms of cognitive change in pathological aging,
particularly the memory detriments seen in Alzheimer’s disease (AD) (Grothe et al., 2015;
Iraizoz et al., 1999; Schliebs and Arendt, 2006; Whitehouse et al., 1982). Cholinergic function
modulates both bottom-up and top-down attentional processes, and is thus involved in cognitive
processes that require the differentiation of relevant and irrelevant information and the efficient
allocation of attentional resources (Gibbs, 2010; Sarter and Bruno, 1997; Sarter et al., 2003).

In animal models ovariectomy produces poor performance on learning and memory
tasks paralleled by a decline in cholinergic activity and choline acetyltransferase levels in several
brain regions including basal forebrain neurons projecting to the hippocampus (Gibbs, 2010; Luine and Rodriguez, 1994; O’Malley et al., 1987) and treatment with estrogen replacement restores these markers (Gibbs, 2000; Luine, 1985). Estrogen replacement in ovariectomized animals counteracts the negative effects of cholinergic antagonists on spatial learning and memory (Gibbs, 2000a; Luine, 1985). Studies in postmenopausal women similarly demonstrate that estrogen replacement protects against impaired verbal working memory, episodic memory, learning, and attention following cholinergic antagonism (Dumas et al., 2006, 2008, 2012). However the beneficial effect of estrogen is only seen in animals with relatively intact cholinergic systems (Gibbs, 2002, 2007), suggesting that estrogen modulates cholinergic function rather than acting through a separate parallel mechanism. Estrogen’s support of cholinergic function may essentially increase cognitive resources and maintain dorsal regulation of emotional cognition.

1.7.2 Estrogen Effects in the Dorsal Regulatory System

The amygdala has reciprocal interactions with the prefrontal cortex and hippocampus, such that amygdala activity suppresses activity in dorsal regions. During the healthy stress response these reciprocal interactions allow for quick evaluation and response to stressors, however these feed-forward systems may become dysregulated; reduced dorsal activity releases amygdala inhibition and may have long-term negative effects on dorsal region structure and function (Gold, 2015). Loss of estrogen support for dorsal system functioning may contribute to this dysregulation and thus increase depression risk in vulnerable women.
The hippocampus is uniquely situated at the intersection of cognitive, emotional, and endocrine circuits and thus may have a particularly central role in vulnerability to MDD. Hippocampal structure appears to be sensitive to depression history; a meta-analysis of imaging studies in depression found that reduced hippocampal volume is a consistent finding in individuals with MDD (Videbech and Ravnkilde, 2004). Furthermore, hippocampal volume reduction is associated with depression recurrence with smaller volume in individuals with more past episodes (Sheline et al., 1996) and longer illness duration (Colla et al., 2007). Whether smaller hippocampal volume is the result of depression or rather a marker of vulnerability due to developmental differences (Lupien et al., 2007) remains unclear, however the association between depression history and hippocampal structure accords with the hippocampus as a central component of overlapping networks for endocrine regulation, emotion, and cognitive processes that are integral to MDD etiology.

Estrogen may support adaptive mood regulation through effects on hippocampal functioning and neuronal plasticity. Estrogen has been shown to increase the density of dendritic spines on CA1 pyramidal neurons (especially following neuronal damage or estrogen loss (McEwen et al., 1995; Woolley, 1999; Woolley and McEwen, 1992) ), and estrogen receptor antagonists block this effect (McEwen et al., 1999). Estrogen receptor localization to the plasma membrane in dendritic shafts and spines (McEwen et al., 1995; Woolley and McEwen, 1992) suggest that estrogen may have local effects on plasticity in the hippocampus. The presence of estrogen appears to prime the neuron for new synapse creation through an increase in dendritic spines, however these new spines are only maintained following synapse activation (Woolley, 1998, 2000; Woolley and McEwen, 1992; Woolley et al., 1997) ; estrogen does not globally increase hippocampus synapses, but supports the production and maintenance of synapses that
are used. Additionally, estrogen treatment protects hippocampal synapses from the detrimental effects of acute cortisol increases (Ooishi et al., 2012). Paralleling to these structural changes, estrogen replacement in animal models improves performance on cognitive tasks that are hippocampally mediated (Gibbs, 2000b; Inagaki et al., 2010; Luine and Rodriguez, 1994). Similarly, cognitive performances changes across the menstrual cycle and with estrogen replacement in postmenopausal women suggest that ovarian hormones have specific effects on cognitive tasks that rely on hippocampal function.

Estrogen receptor density in the hippocampus responds dynamically to ovarian hormone changes; increasing in dendritic spines during low estrogen phases of the estrous cycle and decreasing during high estrogen phases (McEwen et al., 2012). These changes in ER density may be part of a coping mechanism that maintains stable hippocampal function across estrogen fluctuations. A deficit in this dynamic response to estrogen changes may be associated with increased vulnerability to mood and anxiety disorders in some women.

Estrogen has similar effects on cholinergic and serotonergic functioning that may converge in the dorsal system that are supported by these neurotransmitter systems. In postmenopausal women, estradiol treatment prevents anticholinergic impairment in memory and attention tasks (Dumas et al., 2006, 2008, 2012), indicating that estrogen’s support of cholinergic function benefits prefrontal and hippocampal function. Similarly, estrogen replacement in postmenopausal women increases serotonin tone in the hippocampus (Compton et al., 2008), prefrontal cortex, and anterior cingulate, paralleled by improvements in tasks of executive function and verbal memory (Kugaya et al., 2003). Estrogen’s effects in the prefrontal cortex may modulate executive function thus having global implications for cognition and emotional response regulation. Neuroimaging studies examining the effects of estrogen replacement in
postmenopausal women show that estrogen replacement enhances hippocampal and prefrontal activity during tasks in which estrogen treatment improves performance (episodic memory and working memory encoding) (Joffe et al., 2006; Maki and Resnick, 2000; Resnick et al., 1998; Shaywitz et al., 1999). Additionally, ovarian hormone suppression in young women impairs executive function and cognitive flexibility and decreases metabolism in prefrontal, temporal, and parietal regions (Berman et al., 1997). Enhanced function in these dorsal system regions may support higher order cognitive resources and decrease automatic emotional processing or provide better top-down regulation of emotional responses.

1.8 Introduction to Current work

Although the sex difference in MDD and concurrence of ovarian hormone fluctuation with increased risk for mood disorders in women suggest that estrogen may have a significant role in MDD, how estrogen influences depression vulnerability is not clear. Perhaps estrogen’s role in MDD can be best understood through examining estrogen’s role in emotional and cognitive processes that are integral to healthy mood. MDD is characterized by dysfunction in mood regulation and endocrine responses to stress and negative emotional information. These core symptoms are indicative of altered function in proposed systems for emotional evaluation and response (ventral system) and regulation of emotional and cognitive processes (dorsal system).

The ventral and dorsal systems are interconnected and work to manage acute responses to environmental stimuli and coordinate long-term cognitive consequences through attention and memory. As detailed in Gold’s model, dysphoric mood is a natural and healthy response to
stress, however the ability to efficiently return to normal ventral-dorsal system dynamics and euthymic mood confers resilience (Gold, 2015). Healthy stress responses acutely bias stimuli processing towards automatic ventral evaluation and response pathways. Consistent findings of endocrine dysregulation in the HPA axis and the relation of stressful life event to depressive episodes indicate that in MDD stress system function is dysregulated with possible consequences for cognitive processing of emotional information. When information must be evaluated quickly, such as during stress, altered brain networks in MDD may be prone to ventral activity, resulting in enhanced attention and cognitive processing of negative information along with dysphoric mood. According to cognitive models of MDD, these alterations remain during remission and contribute to continued recurrence risk, especially following psychosocial stress.

Evidence of estrogen’s positive effects on both cognitive and emotional processes in dorsal system structures indicates that estrogen supports healthy functioning in dorsal regions (particularly hippocampus and prefrontal cortex). Estrogen’s effect of enhancing dorsal system function may attenuate ventral system activity or allow dorsal system regions to regain regulatory function more efficiently. Conversely, low estradiol reproductive phases in women and loss of estrogen support of dorsal systems may predispose brain activity to ventral system automatic processing. Sex differences in emotion recognition suggest that women recognize and respond to emotional information at earlier stages in visual processing, before modulation by dorsal system and integration with associative information. Loss of estrogen support for dorsal systems may bias emotional processing to these lower-level automatic systems predominated by amygdala activity and enhance the salience of negative emotional information, thus increasing depression risk in vulnerable women.
The general aim of this dissertation was to investigate the role of estrogen in the cognitive and emotional processes that are associated with depression to better understand the greater prevalence of MDD in women. Previous studies indicate that in healthy young women endocrine response to physical and psychosocial stress is altered across that menstrual cycle and by hormonal contraception. However, the neural processes that are associated with psychosocial stress responding and the effects of estrogen on brain systems that are involved in emotional and cognitive responses to psychosocial stress in women have not been examined. Additionally, whether estrogen’s effects on endocrine response to stress are related to mood response to stress has not been investigated. In Chapter 2 of this dissertation, the effect of menstrual phase and estradiol level on the neural and mood response to psychosocial stress in young, normally cycling women was investigated. We hypothesized that high estradiol around ovulation reduces negative mood response and dorsal system deactivation during psychosocial stress compared to low estradiol during the early follicular phase around menstruation.

Cognitive models of MDD posit that altered cognitive processing of emotional information maintains negative mood in depressive episodes and confers recurrence risk during remission. Attention and memory bias for negative information is a consistent finding in studies of currently depressed individuals. Attention bias and the postulated relation to enhanced amygdala activity that remains in remitted MDD has been less studied. In Chapter 3 the effect of MDD history on neural response and attention to emotionally valenced images was investigated. We hypothesized that women with a history of MDD would show enhanced amygdala activity and attentional bias for negative image compared to women with no history of MDD.
1.9 Magnetic Resonance Imaging Methodology

Magnetic resonance imaging (MRI) produces images of biologic tissue through the use of strong magnetic fields (for review see Huettel, et al. 2009). Because the body has a high water content (50-60% by weight), MRI is commonly implemented to detect and measure signals based on the properties of hydrogen nuclei (protons) in magnetic fields. Hydrogen protons have a positive electric charge that creates a small magnetic field as they spin. These magnetic fields are normally randomly directed and thus the net magnetization is nearly zero. In MRI a strong magnetic field tuned to the frequency of hydrogen protons induces alignment of the magnetic spins and increases the net magnetization of hydrogen protons in the body. Once the magnetic spins of the hydrogen protons are aligned, energy can be applied to the system and the release of that energy as the system returns to a lower energy state can be measured to provide information about biologic tissues.

For both structural and function MRI, radio frequency pulses are applied to the target tissue (for the purposes of the following studies that target will be the brain). Radio frequency applied to the brain perturbs the equilibrium state created by the static field and the energy from these pulses are absorbed by hydrogen protons. Hydrogen protons release the radio frequency energy and realign with the static magnetic field; the release of this energy can be detected and defines the raw MRI signal.

The time that it takes for proton spins to realign with the static magnetic field is unique to each tissue type because of local magnetic field inhomogeneities. The difference in energy released over time between tissues allows for the detection of tissue types in structural MRI. Local magnetic properties are also disturbed by difference in blood oxygenation in and near
tissue. These local disruptions in magnetic field affect the time to return to alignment with the static field and are the basis for blood oxygen level-dependent (BOLD) imaging used in function MRI (fMRI). In areas of increased neural activity blood flow increases to supply oxygen for aerobic metabolism. Blood flow to active areas is increased beyond the need for oxygen so that areas of increased activity have higher levels of oxygenated blood relative to less active areas. Deoxygenated hemoglobin has unbound electrons in the iron within the heme group and is thus diamagnetic. Because of the diamagnetic properties of deoxygenated hemoglobin, realignment of hydrogen protons with the static field occurs faster in less active than more active areas. Measuring radio frequency energy released at a time lag after realignment by less active areas but before realignment by more active areas allows for the identification of areas of increased neural activity.

These imaging techniques are combined with carefully designed tasks for task-related fMRI which identifies areas of the brain that are presumed to be active during particular brain functions or cognitive processes. Resting fMRI is used to identify functional connectivity between brain regions and networks. During resting fMRI BOLD imaging is implemented without the participant explicitly performing a task. Spontaneous activity is measured and the activity in different brain regions is analyzed for correlated time courses which are interpreted as integrated function between regions.
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CHAPTER 2: ESTRADIOL LEVELS MODULATE BRAIN ACTIVITY AND NEGATIVE RESPONSES TO PSYCHOSOCIAL STRESS ACROSS THE MENSTRUAL CYCLE

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Abstract:

Although ovarian hormones are thought to have a potential role in the well-known sex difference in mood and anxiety disorders, the mechanisms through which ovarian hormone changes contribute to stress regulation are not well understood. One mechanism by which ovarian hormones might impact mood regulation is by mediating the effect of psychosocial stress, which often precedes depressive episodes and may have mood consequences that are particularly relevant in women. In the current study, brain activity and mood response to psychosocial stress was examined in healthy, normally cycling women at either the high or low estradiol phase of the menstrual cycle. Twenty eight women were exposed to the Montreal Imaging Stress Task (MIST), with brain activity determined through functional magnetic resonance imaging, and behavioral response assessed with subjective mood and stress measures. Brain activity responses to psychosocial stress differed between women in the low versus high estrogen phase of the menstrual cycle: women with high estradiol levels showed significantly less deactivation in limbic regions during psychosocial stress compared to women with low estradiol levels. Additionally, women with higher estradiol levels also had less subjective distress in response to the MIST than women with lower estradiol levels. The results of this study suggest that, in normally cycling premenopausal women, high estradiol levels attenuate the brain activation changes and negative mood response to psychosocial stress. Normal ovarian hormone fluctuations may alter the impact of psychosocially stressful events by presenting periods of increased vulnerability to psychosocial stress during low estradiol phases of the menstrual cycle. This menstrual cycle – related fluctuation in stress vulnerability may be relevant to the greater risk for affective disorder or post-traumatic stress disorder in women.
**Introduction**

The sex difference in affective and stress-related disease rates (2 to 3 times greater incidence and prevalence of major depression in women compared to men (Bromet et al., 2011; Kessler et al., 2005)) emerges at puberty and remains until menopause (Kessler et al., 1994). The stress exposure model of depression suggests that MDD is the result of a vulnerability to depression, combined with the trigger of stressful life events (Hankin et al., 2007; Liu and Alloy, 2010). Accordingly, psychosocial stressors are among the top reported antecedents to depression episodes (Frank et al., 1994; Kendler et al., 1999, 2000), and dysregulation of the hypothalamic-pituitary-adrenal (HPA) axis response is a consistent finding in major depression (Burke et al., 2005; Lupien et al., 2005). Psychosocial stress may be especially important in the etiology of mood disorders in women as the depressogenic effects of life stressors are reportedly greater in women than men (Mezulis et al., 2010), even when there is no difference in the number of stressful life events or in the subjective perception of these events (Young and Korszun, 2010). Although this finding is consistent and has been replicated by studies across the globe (Bromet et al., 2011), the reasons for the sex difference in incidence and prevalence of mood and anxiety disorders are not well understood.

The cortisol response to stress shows consistent sex differences (Vamvakopoulos, 1995), and changes across the menstrual cycle, decreasing during the late follicular phase when circulating estrogen is high (Kirschbaum et al., 1992, 1999). Women are more likely than men to show variations in HPA function in response to stressors (Weiss et al., 1999) and during depressive episodes (Young and Korszun, 2010). These differences in stress system response likely contribute to mood disorder risk in women (Weiss et al., 1999), and may be modulated by ovarian hormone fluctuations across the menstrual cycle (Kajantie and Phillips, 2006; Roca et
The role of corticosteroids in stress response and regulation is well known, however the effects of gonadal steroids (e.g. estradiol) –which may be specifically important in women– are less well characterized.

The concurrence of increased major depressive disorder (MDD) and post-traumatic stress disorder (PTSD) risk in women during the reproductive period of life suggests that the cyclic fluctuation of ovarian hormones during this period may contribute to the risk for psychopathology. Understanding the role of ovarian hormones in emotional processing and mood regulation in women may provide important insight into the mechanisms underlying the stress response and potentially the increased incidence of MDD and PTSD in women.

Ovarian hormones may modulate the effects of stress on mood. Brain areas that are central to mood regulation (including the amygdala and hippocampus) show some of the largest densities of estrogen receptors in the human brain (Merchenthaler et al., 2004; Ostlund et al., 2003); interestingly, those areas are also very rich in cortisol receptors. Estrogen may modulate the activity of these areas; large community and clinic based studies indicate that negative mood complaints (Davydov et al., 2005; Gonda et al., 2008) (even in healthy women) and suicidal behavior (Baca-Garcia et al., 2004; Saunders and Hawton, 2006) increase in women during low estrogen phases of the menstrual cycle. Brain activity related to processing negative emotional information is also modulated by changing estradiol levels across the menstrual cycle (Goldstein et al., 2005; Merz et al., 2012), suggesting that estrogen may alter the mood response to negative information, making this information more or less salient to cognitive processes and subsequently mood states.

Although there is strong evidence that estrogen modulates the response to negatively valenced stimuli, such as negative images (Andreano and Cahill, 2010; Goldstein et al., 2005),
the effect of estrogen on the response to psychosocial stress is less well understood. Psychosocial stress differs from processing emotional information in a number of ways - stress includes elements of self-esteem, uncontrollability, and personal threat. Further, studies using performance–based stressors provide evidence that there is a sex difference in the endocrine (cortisol) response to this type of stress, and that cycling ovarian hormones may modulate this response in women (Kirschbaum et al., 1992, 1999). Social-evaluative threat is one element of psychosocial stress that may be especially salient for women, and has face validity for the real life stressors women experience and that contribute to mood disorder risk (Kendler et al., 1999, 2000; Stroud et al., 2002). Understanding the effect of ovarian hormones on the response to these types of stressors is thus important in understanding the role of ovarian hormones in the brain mechanisms of emotional processing and mood in women. Investigating the stress response, under controlled laboratory conditions, provides an opportunity to examine the effects of ovarian hormones on emotional processing, stress response, and subsequent mood.

In this study, we examined the effect of estradiol levels across the menstrual cycle on both the brain activity and behavioral response to a laboratory-based model of psychosocial stress. We hypothesized that high circulating estrogen would reduce the effect of acute stress on brain activity and be associated with less subjective distress following the stress-inducing task.

**Methods**

**Overview:**

This cross sectional study included 28 normally cycling women (ages 18-45) who were examined during early follicular phase (day 1-2 of menstrual cycle), or at ovulation (day 12-14), and who participated in a psychological stress fMRI task. Due to the psychosocial stress task
used in this study, and the requirement to debrief participants about the focus of the study once the stress task was completed, we were unable to repeat the stress task for a within subjects comparison, and were limited to between subjects/group comparisons. Approximately half of the participants were studied at the University of Vermont and half were completed at Vanderbilt University. The study coordinator was the same throughout the study and the MRI scanners were the identical make and model, with the same software version in operation at the time of the study. The study procedures were identical at both Universities, and imaging and behavior data were analyzed for study location effects. The study was reviewed and approved by both the University of Vermont and Vanderbilt University Institutional Review Boards, and all participants provided written informed consent.

**Participants:**

Participants were recruited and told that they would be participating in a study to examine the effects of menstrual cycle hormone changes on mathematical ability in women. All participants were healthy, with regular menstrual cycles (21-35 days), no hormonal contraceptive or centrally active medication use, and no history of severe pain or mood changes related to their menstrual cycles. We excluded participants with current or past Axis I psychiatric disorders using the Structured Clinical Interview for DSM Disorders (Spitzer et al., 1992). Beck Depression Index score was required to be less than 7, and Beck Anxiety Index score less than 15 (Beck et al., 1961). Current illicit drug use and pregnancy were exclusions.

**Screening and Characterization:**

To assure all participants were of at least average intelligence, we administered the Wechsler Abbreviated Scale of Intelligence (Wechsler, 1999). The Composite International
Diagnostic Interview for premenstrual dysphoric disorder (Kessler and Ustun, 2004) was used to collect menstrual cycle history and to rule out premenstrual dysphoric disorder, and physical and psychological symptoms associated with the menstrual cycle were assessed using the Moos Menstrual Distress Questionnaire (Moos, 1968). Personality factors were assessed at screening using the NEO Five Factor Inventory (Costa and McCrae, 2000). Following screening, participants tracked their menstrual cycles for three months to determine an average cycle length and to calculate the study day date. At the University of Vermont, participants tracked using a paper calendar; at Vanderbilt University, women filled in daily surveys using the on-line REDCap system.

Women were randomly assigned to return for the study day during the early follicular phase (days 1-2 of the menstrual cycle) or at ovulation (day 12-14); before the study began, participant research ID numbers were assigned a randomly generated number, ranked according to this random number and then alternatingly assigned to the two groups. The study day took approximately 3 hours to complete. Upon arrival at the Clinical Research Center, participants completed the State and Trait Anxiety Inventories (Spielberger, 2010) and the Beck Depression and Anxiety Inventories (Beck et al., 1961).

Stress Task:

We employed the Montreal Imaging Stress Task (MIST) for psychosocial stress induction (Pruessner et al., 2008). The MIST produces moderate psychosocial stress through a combination of motivated performance and social-evaluative threat. We presented the MIST as an arithmetic task in the MRI scanner, and instructed participants that they should achieve an 80-90% correct performance for their data to be usable in the context of this experiment. Unbeknownst to the subjects, the MIST contains an algorithm producing script that
automatically adjusts the difficulty of the math tasks to the aptitude of the participant, this way maintaining a low performance rate (between 40-50%) by changing either the problem difficulty or the allotted time. A “control” condition, in which the participants solve arithmetic problems with no time limit and no performance limit, serves as a contrast to control for brain activity changes induced by arithmetic task demands (visual stimuli, motor response, and mental arithmetic). Social evaluative threat comes from scripted experimenter interaction; the experimenters enter the MRI room between runs and inform the participants that they are not doing well enough and that they need to improve their performance for the experiment to be successful.

In this study, participants completed three runs of the MIST; the MRI sessions were scheduled during the afternoon when baseline cortisol levels are low. After the first run, experimenter 1 entered the scanner room and provided the scripted feedback and asked the participant to complete a second run of the task. After the second run the experimenter 1 entered the scanner room and told the participant that the experimenter 2 (“doctor”) would like to speak with them about their performance, at which point the experimenter 2 entered the scanner room and provided the scripted feedback and asked the participant to complete a third run of the task. This protocol was designed to maintain both performance and social evaluative threat throughout the MIST and to prevent participants from habituating to the performance challenge or giving up on completing the task. Specifically the interaction of the participants and the experimenters was structured to generate psychosocial stress. To habituate the participants to the scanning environment and decrease scanner-related stress at the study day, participants entered an MRI simulator during the screening visit; in the MRI simulator, participants watched a nature video
while being exposed to the MRI environment (simulated MRI sounds, head coil, and being placed in the MRI bore) and the stimulus presentation system.

The MIST was presented in a block design; each condition was presented twice per 3 runs (“stress”: 100 seconds, “control”: 50 seconds, ‘rest”: 30 seconds). Participants practiced the MIST task (control trials only) in the MRI simulator, before the MRI session.

**Subjective Measures**

Participants completed subjective measures: the Stress and Arousal Checklist (King et al., 1983) (before and after the MIST), the Profile of Mood States (McNair et al., 1989), and a Visual Analogue Scale for mood and task experience (both after the MIST).

**Endocrine Measurements:**

Saliva samples were collected, using Salimetrics salivettes placed under the tongue, at seven times during the study day to assess the cortisol response to the stress task (Fig.1). Salivettes remained under the tongue for two minutes, and then were stored at -80°C freezer until analysis. Cortisol levels were assessed using the Salimetrics High Sensitivity Salivary Cortisol Enzyme Immunoassay Kit for quantitative determination of salivary cortisol with a sensitivity of 0.007 ug/dL for 25µl sample volume (Salimetrics LLC, Philadelphia, PA). All cortisol measurements were assayed in duplicate, with mean intra-assay coefficient of variation of 7.5% and inter-assay coefficient of variation of 14.3%. Cortisol area under the curve measurements for each participant (over the study day) were calculated using Graphpad Prism.

Blood samples were collected from each participant at the end of the study day to determine the estradiol level and to verify menstrual phase. Estradiol was assessed from sera using the Immulite 1000 endocrine panel immunoassay system with mean intra-assay coefficient
of variation of 5.9%. For analyses by menstrual phase, we required that both the participant’s menstrual cycle tracking and estradiol level indicated that they were in the assigned menstrual phase. Participants’ whose estradiol levels did not concord with their randomly assigned menstrual phase at the study day; women whose estradiol levels were higher or lower than the reference values for their assigned phase, were not included in the low or high estradiol groups, as they could not be accurately assigned to either group. These data were included in all other analyses.

Progesterone levels were assessed using the Salimetrics Salivary Progesterone (P4) Enzyme Immunoassay Kit with a sensitivity of 5pg/mL for 25µl sample volume (Salimetrics LLC, Philadelphia, PA). The first saliva sample of the day was used for progesterone measurements. All progesterone measurements were assayed in duplicate, with mean intra-assay coefficient of variation of < 1%.

**MRI Scan parameters:**

Participants were scanned on a Philips 3.0 Tesla Achieva scanner, with an 8 channel head coil. All participants received the following MR sequences: 1) A sagittal T1-weighted 3D Turbo Field Echo Sensitivity Encoding (TFE SENSE) sequence perpendicular to the anterior commissure (AC) -posterior commissure (PC) line, repetition time (TR) of 9.9 ms, echo time (TE) of 4.6 ms, a flip angle of 8°, number signal averages (NSA) 1.0, a field of view (FOV) of 256 mm, a 256 × 256 matrix, and 1.0 mm slice thickness with no gap for 140 contiguous slices. 2) A T2- weighted Gradient and Spin Echo (GRASE) sequence was run parallel to the AC-PC line, TR 2470 ms, TE 80 ms, NSA 3.0, FOV of 230 mm, 0.7 mm slice thickness with 5.0 mm gap for 28 slices. 3) Three Echoplanar Blood Oxygenation Level Dependent (EpiBOLD) functional sequences, transverse orientation, TR 2500 ms, TE 35 ms, flip angle 90°, 1 NSA for
FOV 240, 240X128 matrix, and 4.0 mm slice thickness with no gap, with ascending interleaved acquisition, for 35 contiguous slices.

**fMRI Analysis:**

fMRI data was processed using Statistical Parametric Mapping (SPM8) (Wellcome Department of Cognitive Neurology, 2008). Preprocessing included: realignment of the three functional runs and correction for bulk-head motion, coregistration of functional and anatomical images for each participant, segmentation of the anatomical image, and normalization of the anatomical and functional images to the standard MNI template, and spatial smoothing with a Gaussian filter (8 mm at full width, half maximum).

Artifact detection was performed on functional images using the ART toolbox in SPM, and outliers for signal intensity (z>3) and motion (movement >3 mm in either translation or rotation) were entered as nuisance regressors at the first level, single-participant analysis. At first level analysis, T-maps were created from linear contrasts for task conditions and between condition comparisons; these T-maps were used in the second level whole-brain random effects analysis of participant group effects with two-sample t-tests. The preprocessed functional images had a voxel size of 2X2X2 mm and cluster threshold correction was used to control for multiple comparisons (from voxel wise \( p = 0.005 \) to corrected \( p < 0.001 \) with \( k = 58 \) (corrected voxel-wise \( p = 0.000001 \)) (from alpha simulation in REST(Song et al., 2011) toolbox for SPM, using whole brain mask).

Two sets of second level analyses were conducted, one analysis with groups defined by menstrual phase and estradiol level at the study day, and a second analysis with groups defined by subjective distress to the MIST (change in Stress Arousal Checklist score). Separate
regression analyses were run for estradiol level and subjective distress by hippocampal activity (using a bilateral hippocampus region of interest mask created from the Automated Anatomical Labeling (AAL) in WFU Pickatlas (Maldjian et al., 2003). Percent signal change in right and left hippocampus was calculated with the REX toolbox for SPM; using masks created from the average peak cluster in the hippocampus ROI (using the contrast “control” condition > “stress” condition in the full subject set) with cluster threshold correction (from voxel wise $p = 0.05$) to corrected $p < 0.001$ with $k = 101$ (corrected voxel-wise $p = 0.0001$).

**Results**

**Participants:**

Average participant age was 30.4 years ($SD = 8.2$ years). There was no difference in age between subject groups either by estradiol level ($t (8) = -1.01, p = 0.33$), or distress ($t (26) = -0.83, p = 0.41$). No participants had Menstrual Distress Questionnaire scores that indicated severe premenstrual somatic or affective changes. There were no differences between any of the participant groups at screening, including Beck Depression Inventory scores, Beck Anxiety Inventory scores, NEO Five Factor Inventory personality measures, or any of the Menstrual Distress Questionnaire measures, and none of the participants had elevated scores on these scales (Table 1). For the analysis of the effect of menstrual phase on stress related brain activity, we included only participants whose estradiol level at the study day confirmed their assigned menstrual phase. The reference value for median early follicular phase estradiol levels using the Immulite 1000 endocrine panel immunoassay system is 31 pg/mL. Women were included in the low estradiol phase group if they were assigned to the early follicular group and had an estradiol level on the study day less than 40 pg/mL ($M = 24.91$ pg/mL). Women were included in the high estradiol phase group if they were assigned to the ovulatory group and had an estradiol level on
the study day greater than 50 pg/mL \( (M = 103.52 \text{pg/mL}) \). This resulted in 8 participants (4 from the low estradiol, and 4 from the high estradiol group) not being included in stress-brain imaging analyses for low vs. high estradiol phase groups; these participants’ data were included in all other analyses.

**Brain Activity - Psychosocial Stress Response**

Second level comparison using a one sample t–test of average images of all subjects \((n = 28)\) (using the 1st level contrast of control condition > stress condition) revealed significantly (uncorr \( p = 0.005 \), cluster size = 58, corr \( p < 0.0001 \)) greater activation in cingulate, temporal (including hippocampus), and frontal regions and activation in parietal and precentral gyrus regions during the control condition compared to the stress condition (Fig.2 and Table 2). This activity pattern is consistent with the activity pattern previously shown by studies using the MIST (Pruessner et al., 2008); reduced activation of hippocampus and para-limbic regions during the stress condition. These results confirm that the overall brain activity response to psychosocial stress in a sample including only women is similar to the brain activity seen in studies that include both men and women.

**Menstrual Cycle Phase/ Estradiol Effects**

Estradiol level was significantly higher in the high estradiol phase group \((M = 103.52 \text{pg/mL}, SD = 34.09)\) than the low estradiol phase group \((M = 24.91 \text{pg/mL}, SD = 9.17)\) \((t (18) = 7.04, p < 0.001)\). All women had salivary progesterone levels under 105 pg/mL \((M = 49.72 \text{pg/mL}, SD = 35.54)\), consistent with the follicular phase (Chatterton et al., 2005). Progesterone levels did not differ between the low estradiol phase group \((M = 48.90 \text{pg/mL}, SD = 36.10)\) and high estradiol phase group \((M = 50.54 \text{pg/mL}, SD = 36.91)\) \((t (18) = 0.099, p = 0.92)\) (Fig.3).
The fMRI estradiol phase group analysis for the effect of psychosocial stress, including participants who showed concordance of assigned menstrual phase and estradiol level, (high estradiol n = 10, low estradiol n = 10), showed a significant (uncorr $p = 0.05$, cluster size = 529, corr $p < 0.005$) effect of estradiol level on brain activity during psychosocial stress and perceived distress following the MIST, however these findings did not survive more conservative correction for multiple comparisons. Women with low estradiol levels had significantly less activity in the left hippocampus than women with high estradiol levels during the stress condition (Fig. 4 and Table 3). This finding indicates that women in the high estradiol phase of the menstrual cycle have a greater left hippocampal activity during psychosocial stress than women in the low estradiol phase.

Bilateral hippocampal activity during psychosocial stress was directly associated with estradiol levels in an analysis of the 20 participants included in the estradiol phase group analysis (uncorr $p = 0.005$, cluster size = 58, corr $p < 0.0001$) with greater activity in the bilateral hippocampal ROI being associated with higher estradiol levels. Examining the individual right or left hippocampal ROIs showed that the correlation between estradiol level and percent signal change in either right or left hippocampus did not meet statistical significance (right hippocampus: $r = -0.14$, $n = 28$, $p = 0.45$; left hippocampus: $r = -0.21$, $n = 28$, $p = 0.37$), indicating that the effect of estradiol was not lateralized.

In an analysis that included all participants grouped according to their assigned menstrual phase (without confirmation by estradiol level), there were no brain areas that showed greater activity in the periovulatory group compared to the early follicular phase group at either corrected $p < 0.01$ or corrected $p < 0.05$. 87
Brain Activity - Subjective Distress Effects

To compute a group contrast for the effects of subjective distress on brain activation changes, we performed a median split of subjective distress scores; difference in pre vs. post MIST stress score on the Stress and Arousal Checklist (high distress n = 14, low distress n = 14). The subsequent fMRI analysis with the high and low distress groups showed an associated of distress and brain activity during the psychosocial stress task. Women with lower distress scores showed significantly more activity in bilateral hippocampus (Fig5), midbrain, left parietal, and left frontal regions than women with higher distress scores and (Table 4). By contrast, greater subgenual cingulate activity was seen in women with higher distress scores compared to women with lower distress scores (Table 4). These differences indicate that women who respond to the MIST with high subjective distress have a greater change in brain activity during psychosocial stress than women with low subjective distress following the MIST.

Bilateral hippocampal activity during psychosocial stress was inversely associated with distress scores in an analysis of all participants ($p < 0.001$) with lower activity in the hippocampal ROI being associated with greater distress. These correlations were statistically significant bilaterally (left hippocampus $r = 0.51$, $n = 28$, $p = 0.006$, right hippocampus $r = 0.49$, $n = 28$, $p = 0.008$). We re-analyzed the correlation after step-wise removal of percent signal change extreme values and association between left hippocampal percent signal change and distress remained significant ($r = 0.39$, $n = 28$, $p = 0.04$) but the right hippocampus did not ($r = 0.31$, $n = 28$, $p = 0.10$).

There was no significant difference in brain activity between participants run at the two study locations in any of the contrasts of interest at any level of significance including uncorrected $p < 0.05$. 

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Behavioral and Mood Measures - Estradiol Effects

There was no effect of estradiol phase group or distress group on pre-MIST Stress and Arousal Checklist scores, Trait Anxiety Inventory scores, or State Anxiety scores (Table 5), and no effect on any of the Profile of Mood scores, Arousal scores, or any of the scales of the Stress Task Visual Analogue Scale following the MIST (Table 6). Distress scores (difference in SACL Stress score before and after the MIST) were lower in women with high estradiol than in women with low estradiol levels (Table 6). A multiple regression was run to predict stress change from estradiol level, progesterone level, and age, $F(3, 24) = 4.2, p < 0.05, R^2 = .344$. Only estradiol added statistically significantly to the prediction, $p < 0.05$.

Behavioral and Mood Measures - Distress Effects

There was no significant difference between groups on Stress and Arousal Checklist scores, Trait Anxiety Inventory scores, or State Anxiety scores before completing the MIST (Table 5). Women with higher distress scores had higher scores (worse mood) on all negative scales of the post-task Profile of Mood States (including tension, depression, anger, fatigue, confusion, and total mood disturbance) compared to women with lower distress scores, indicating worse mood following the MIST (Table 6). Additionally, women with higher distress scores had significantly lower estradiol levels ($M = 43.47$ pg/mL, $SD = 35.26$) than women with lower distress scores ($M = 74.49$ pg/mL, $SD = 40.93$) ($t(26) = 2.15, p < 0.05$), and there was an inverse correlation between estradiol and distress with lower estradiol levels associated with greater distress ($r = -0.50, n = 29, p < 0.01$). There was no difference in progesterone levels between distress groups - low distress progesterone ($M = 41.04$ pg/mL, $SD = 35.49$), high distress progesterone ($M = 58.96$ pg/mL, $SD = 36.84$), ($t(26) = -1.31, p = 0.20$) (Fig 4).
There was no significant difference in any of the screening or study day subjective or behavioral measures between participants run at the two study locations at $p < 0.05$.

**Cortisol**

All subjects showed an increase in cortisol related to the MIST and cortisol AUC was associated with decreased medial prefrontal activity during psychosocial stress (Fig.6); however there was no significant correlation between estradiol level or distress score group with cortisol response to the MIST. Cortisol AUC showed substantial inter-subject variability with an average AUC for the entire sample of 0.8 and a standard deviation of 2.8.

**Discussion**

Overall, women showed brain activity patterns during psychosocial stress that were similar to the patterns previously seen in studies that included both men and women; deactivation of limbic regions (Pruessner et al., 2008). However, this study showed that higher estradiol levels at periovulation were associated with greater hippocampal activity during psychosocial stress in normally cycling premenopausal women. Menstrual cycle phase and corresponding estradiol levels were directly correlated with hippocampal activity during the stress condition of the MIST; activity in the hippocampus during stress was significantly lower in women in the low estradiol levels compared to women with higher estradiol levels. These results suggest that low estradiol levels during the early follicular phase of the menstrual cycle exaggerate the effect of psychosocial stress on brain activity. Women with higher periovulatory estradiol levels also had lower distress scores following the psychosocial stress task. Group analysis based on distress following the MIST confirmed the relationship between estradiol and both brain and mood
response to stress: women with higher distress had lower left hippocampal activity during the MIST, more negative mood following the MIST, and lower estradiol levels.

Previous work using the MIST has revealed deactivations during the stress condition of the task, in brain areas that are part of the limbic circuit (including hippocampus, hypothalamus, medio-orbitofrontal cortex and anterior cingulate cortex) (Pruessner et al., 2008). These studies have proposed that reduced limbic circuit function is associated, perhaps causally, with the stress response. Additionally, hippocampal deactivation during the stress condition of the MIST has been directly related to cortisol release and inversely correlated with measures of self-esteem, indicating that hippocampal activity is a marker of both the peripheral endocrine response to stress and related to psychological vulnerability to psychosocial stress (Pruessner et al., 2008). These findings are consistent with previous studies showing that the hippocampus is an important inhibitor of the HPA system (Jacobson and Sapolsky, 1991). Hippocampal function is also important in processing emotionally valenced information (Canli et al., 2002), especially in women, and in evaluating the context of social interactions (Berthoz et al., 2002). Our findings suggest that higher circulating estradiol may support continued hippocampal activity during psychosocial stress.

The brain regions that are commonly indicated in studies of major depression and in recent neuroimaging studies of psychosocial stress are important targets for investigating vulnerability to mood disorder and the interaction with stressful life events. That these same regions are responsive to estradiol manipulation (Andreano and Cahill, 2010; Goldstein et al., 2005) is interesting in light of the mood effects of estradiol in both healthy women (Gonda et al., 2008) and mood disorders related to ovarian hormone fluctuations. Estradiol has been shown to modulate activity on brain circuits important for emotional processing; estradiol level changes
across the menstrual cycle are associated with changes in brain activity related to arousal for negative valenced images (Goldstein et al., 2005), and in fear conditioning (Zeidan et al., 2011). These findings provide evidence that estradiol may affect emotional responding through increased top-down modulation of emotional circuitry, including brain areas involved in the stress response, and may be protective against fear and anxiety. Our findings suggest that greater estradiol levels during the periovulatory phase of the menstrual cycle decrease the brain activity change in response to psychosocial stress, and reduce the acute behavioral and mood consequences of the stress experience. This interpretation is further supported by the location of estradiol receptors in the central nervous system; the hippocampus is rich in both estradiol and glucocorticoid receptors, making it an important area of interaction between these hormones.

Estradiol receptors are located in a number of brain areas, including regions important for the autonomic, hormonal, and cognitive-emotional response to psychosocial stress (Love et al., 2010). The relation of stress to depression onset (Frank et al., 1994; Kendler et al., 1999, 2000) and the altered function of the stress system in major depression (Burke et al., 2005; Lupien et al., 2005) suggest that modulation of the psychosocial stress response may be a mechanism through which estradiol fluctuation may contribute to MDD and PTSD risk. The results of this study suggest that estradiol levels may modulate activity in brain areas important for processing emotional information during psychosocial stress. Increased emotional processing and physiological response to psychosocial stress, during low estrogen menstrual phases, may contribute to depressed mood in women with vulnerability to MDD. Indeed, women with MDD have greater HPA axis dysregulation than men with MDD (Young and Ribeiro, 2006; Young et al., 1994), suggesting that the stress system may be particularly important to depression in women. Estradiol may attenuate sympathetic and HPA axis activity to stress (Kajantie and
Phillips, 2006; Roca et al., 2005). Although our study did not include women with mood disorders, the effect of estradiol on brain activity and mood response to psychosocial stress suggest that periods of low estradiol are associated with heightened negative emotional responding. These phases may thus present windows of increased vulnerability to the depressogenic effects of psychosocial stress in women. Further work is needed to determine whether the effects of estradiol on stress responding differ in women with vulnerability to mood and anxiety disorders, and if there is a relation between the occurrence of stressful events during different phases of the menstrual cycle and subsequent MDD or PTSD onset in vulnerable women.

We recognize that menstrual cycle effects are likely not the only, or even the main, determinant of psychosocial stress responding in women; future studies are needed to examine the effects of personality factors, and lifetime trauma or chronic stress. Limitations also include that the separate roles and mechanisms of estradiol and progesterone in emotional processing and response remain to be delineated. Although we did not directly investigate the effect of differing progesterone levels on stress responding, we attempted to isolate the effects of estradiol by having women experience the psychosocial stress task during the early follicular phase or the periovulatory phases when progesterone levels are low. We excluded women whose estradiol levels were not in concordance with their assigned menstrual phase from our menstrual cycle phase/estradiol fMRI analysis, as these women were likely in the mid luteal phase, when both estradiol and progesterone levels are increasing. The periovulatory phase is a very short duration of time (2-4 days) and it is possible that some women in our study were studied after ovulation. Indeed, brain activity during the psychosocial stress task did not show a difference between groups based only on assigned menstrual phase, suggesting that the women who were omitted
from the estradiol level-based analysis may not have been in the correct phase or that estradiol level rather than phase has a stronger effect on neural activity in response to psychosocial stress. Although we believe this approach was appropriate to investigate the effects of estradiol level, it resulted in a reduced sample size for the menstrual cycle phase/estradiol level analysis. As progesterone levels did not appear to have an effect on distress in this study, we included all participants in the analyses by subjective distress. This study was not designed to investigate the separate effects of estradiol and progesterone by menstrual phase, however it did reveal a linear inverse relationship between estradiol levels and subjective distress that did not exist for progesterone.

Additionally, this study did not include women who experience mood disruption directly related to menstrual phase or ovarian hormones (such as Premenstrual Dysphoric Disorder or Polycystic Ovarian Syndrome), nor did this study model these disorders. The effect of ovarian hormone level/menstrual phase on brain activity during emotional processes in women with these disorders likely differs from healthy women, and the effects on stress responding in these populations should be examined separately. Another potential limitation of this study is the difference in block length between stress and control conditions of the MIST. The stress conditions of the task needed to be of sufficient length to allow the stress response to develop, however the limitations in task time (and efficient block length) did not allow for the control conditions to be of similar length. We believe the control condition was of sufficient length and had enough repetitions to use as a comparison to isolate brain activity related to the stress response.

This study is unique in that we used a moderate psychosocial stressor in the MRI environment at different menstrual phases, which allowed us to examine both the subjective
mood response and brain activity response to the stress task. Also our stress task included social evaluative threat- a type of stress that may be particularly relevant for mood in women (Kendler et al., 2001). Our data suggest that estradiol buffers the brain activity changes and negative mood response to psychosocial stress in normally cycling women. This work has important implication for an understanding of the relationship between estradiol levels and the response to stressful life events. Whether this has implications for the development of psychopathology remains to be studied. Future work should extend the investigation of ovarian hormone effects on psychosocial stress responding to women with vulnerability mood or anxiety disorders and further examine the relation of these effects to known risk factors for mood disorders.

**Funding Sources**

This work is supported by NIAR01AG021476, GCRC MO1RR00109, Vanderbilt CTSA grant UL1 TR000445 from NCRR/NIH.

**Acknowledgments**

We would like to thank Dr. Terry Rabinowitz and Dr. Warren Taylor for their help in administering the MIST and suggestions in preparing the manuscript. Thanks are also due to Violet Gau for research assistance, and the research nursing staff at the University of Vermont and Vanderbilt University CRCs for their support of this study. We also thank our participant volunteers for their dedication to clinical research.
References


Table 1: Participant Behavioral Measures at Screening

<table>
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<tr>
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<th>Overall (n=28)</th>
<th>High E2 (n=10)</th>
<th>Low E2 (n=10)</th>
<th>E2 Groups p</th>
<th>High Distress (n=14)</th>
<th>Low Distress (n=14)</th>
<th>Distress Groups p</th>
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<td>Mean  SD</td>
<td>Mean  SD</td>
<td>Mean  SD</td>
<td>p</td>
<td>Mean  SD</td>
<td>Mean  SD</td>
<td>p</td>
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<td>32.10 7.78</td>
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<td>31.71 7.69</td>
<td>28.69 8.86</td>
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<td>4.14 3.74</td>
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<td>4.59 2.70</td>
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<td>5.04 3.49</td>
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<td>1.93 2.04</td>
<td>1.43 2.04</td>
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<td>34.00 5.96</td>
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<td>33.62 7.32</td>
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<td>2.70 3.47</td>
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<tr>
<td>BAI Total</td>
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<td>2.90 2.69</td>
<td>0.65</td>
<td>1.93 2.46</td>
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<td>119.93 12.93</td>
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E2 = Estradiol
MDQ = Menstrual Distress Questionnaire (mean of most recent period and four days before)
FFI = NEO Five Factory Personality Inventory
BDI = Beck Depression Inventory
BAI = Beck Anxiety Inventory
Table 2: Brain Activity during Psychosocial Stress
Stress condition - control condition: All subjects (pooled across menstrual cycle, n = 28), cluster corrected uncorr \( p = 0.005 \), k =58 for \( p < 0.0001 \)

<table>
<thead>
<tr>
<th>Peak MNI coordinates</th>
<th>Peak Description</th>
<th>Cluster extent(k)</th>
<th>Peak t</th>
<th>peak p(uncor)</th>
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<td><strong>Deactivation</strong></td>
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<td>4.94</td>
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<td>0 -18 34</td>
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<td>0 -46 34</td>
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Table 3: Menstrual Cycle/Estradiol Effect on Brain Activity during Psychosocial Stress
Stress condition - control condition: Low estradiol ($n=10$) – High estradiol ($n=10$), cluster corrected uncorr $p = 0.05$, $k = 529$ for $p < 0.005$.

<table>
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<th>Peak MNI coordinates</th>
<th>x</th>
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<th>z</th>
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<th>Peak t</th>
<th>peak p (uncor)</th>
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<tr>
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<tr>
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<td>Precuneus</td>
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<td>5.55E-03</td>
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<td>-24</td>
<td>Left Hippocampus/ Parahippocampus</td>
<td>586</td>
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<td>5.02E-03</td>
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<td>-18</td>
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<td>-4</td>
<td>Left Hippocampus/ Parahippocampus</td>
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<td>6.71E-03</td>
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<tr>
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<td>Activation</td>
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<td>32</td>
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<td>Right Frontal Lobe</td>
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<td></td>
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<td>4</td>
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<td>2.70</td>
<td>7.35E-03</td>
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Table 4: Distress Effect on Brain Activity during Psychosocial Stress.
Stress condition - Control condition: Low distress (n = 14) – High distress (n = 14),
cluster corrected uncorr $p = 0.005$ $k = 58$ for $p < 0.001$

<table>
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<th>Peak MNI coordinates</th>
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<th>Cluster extent(k)</th>
<th>Peak t</th>
<th>peak p (uncor)</th>
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<td>Right Hippocampus/Parahippocampus</td>
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</table>

| Activation           |                                 |                   |         |                |
| -10 0 -10            | Left Subgenual Cingulate        | 1187              | 3.27    | 1.52E-03       |
| -6 22 -10            | Left Anterior Cingulate         |                   | 3.21    | 1.77E-03       |
| -10 10 -14           | Left Subgenual Cingulate        |                   | 2.70    | 6.05E-03       |

Distress = Post SACL Stress – Pre SACL Stress
Table 5: Behavioral Measures at Study Day – Pre MIST

<table>
<thead>
<tr>
<th></th>
<th>High E2 (n=10)</th>
<th>Low E2 (n=10)</th>
<th>E2 Groups</th>
<th>High Distress (n=14)</th>
<th>Low Distress (n=14)</th>
<th>Distress Groups</th>
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<td>SD</td>
<td>Mean</td>
<td>SD</td>
<td>Mean</td>
<td>SD</td>
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<td><strong>STAI - State Anxiety</strong></td>
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<td>7.54</td>
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<td>0.88</td>
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<td></td>
<td><strong>STAI - Trait Anxiety</strong></td>
<td>34.6</td>
<td>10.55</td>
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<td>3.71</td>
<td>8.10</td>
<td>3.31</td>
<td>0.57</td>
<td>8.00</td>
</tr>
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E2 = Estradiol
STAI = State and Trait Anxiety Inventory
SACL = Stress and Arousal Checklist
### Table 6: Behavioral Measures at Study Day – Post MIST

<table>
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<tr>
<th></th>
<th>High E2 (n=10)</th>
<th>Low E2 (n=10)</th>
<th>Menstrual Phase Groups p</th>
<th>Low Distress (n=14)</th>
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<td>SD</td>
<td>Mean</td>
<td>SD</td>
<td>Mean</td>
<td>SD</td>
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<td>Post SACL – Stress</td>
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<td>5.52</td>
<td>13.90</td>
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<td>0.01</td>
<td>5.50</td>
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<tr>
<td>Post SACL – Arousal</td>
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<td>3.01</td>
<td>8.80</td>
<td>2.70</td>
<td>0.64</td>
<td>6.86</td>
</tr>
<tr>
<td>POMS Tension</td>
<td>8.60</td>
<td>7.28</td>
<td>15.10</td>
<td>7.46</td>
<td>0.07</td>
<td>6.57</td>
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<td>POMS Depression</td>
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<td>0.08</td>
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<td>POMS Anger</td>
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<td>0.24</td>
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E2 = Estradiol  
STAI = State and Trait Anxiety Inventory  
SACL = Stress and Arousal Checklist  
POMS = Profile of Mood States  
STVAS = Stress Task Visual Analogue Scale  
Distress = Post SACL Stress – Pre SACL Stress
Figure 1: Overview of study day procedures and timing
Figure 2: Psychosocial Stress Effect fMRI Stress Condition – Control Condition. All participants \((n = 28)\). Greater deactivation in limbic regions during the stress condition compared to the control condition (corrected to \(p < 0.0001\)).
Figure 3: Estradiol and Progesterone Levels for all Participants (n = 28)
Figure 4: Estradiol Effects fMRI.

Stress Condition – Control Condition: High estradiol \((n = 10)\) – Low estradiol \((n = 10)\). Greater activity in left hippocampus during the stress condition in the High estradiol group than the Low estradiol group (cluster-level corrected to \(p < 0.005\)).
Figure 5: Subjective Distress Effects fMRI.

Stress Condition – Control Condition: Low Distress (n = 14) – High Distress (n = 14). Greater activity in bilateral hippocampus during the stress condition in the Low Distress group than the High Distress group (cluster-level corrected to p < 0.0001).
Stress Condition – Control Condition: All (pooled across menstrual phase, n = 28). Greater deactivation in medial prefrontal regions during the stress condition related to salivary cortisol AUC (corrected to $p < 0.0001$).
CHAPTER 3: ATTENTION BIAS IN REMITTED DEPRESSION IS ASSOCIATED
WITH ENHANCED AMYGDALA ACTIVITY AND FUNCTIONAL CONNECTIVITY

Kimberly Albert, Violet Gau, Paul Newhouse

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Abstract

Cognitive bias is a common characteristic of major depression and is posited to remain during remission and contribute to recurrence risk. Attention bias may be related to enhanced amygdala activity or altered functional connectivity to amygdala and within attention networks in depression. The current study examined attention bias, brain activity for emotional images and functional connectivity in participants with and without a history of major depression. Attention bias for negative, positive, and neutral images in an emotion dot probe task during fMRI was examined in 33 postmenopausal women with (n = 12) and without (n = 21) a history of major depression. Additionally, group differences in functional connectivity to amygdala was assessed using a resting state MRI scan. There was a significant group difference for attention facilitation ($t (31) = 2.83, p < 0.01$) and amygdala activity (corr $p < 0.001$) during the emotion dot probe task. There were significant group differences in functional connectivity between the amygdala and hippocampal complex (corr $p < 0.001$). In all participants amygdala activity for negative images was positively correlated with attention facilitation for negative and positive trials. Additionally, amygdala activity and amygdala –hippocampal connectivity were positively correlated with attention facilitation for negative images. These findings provide evidence that differences in activity and functional connectivity in limbic and attentional networks may provide a neurobiological basis for continued cognitive bias in remitted depressed individuals.
Introduction

Cognitive bias for negative emotional information is a common characteristic of mood and anxiety disorders; depressed and dysthymic individuals show enhanced memory and attention (Sears et al., 2011; Wiens and Syrjänen, 2013; Isaac et al., 2014; Duque and Vázquez, 2015) for negative information consistent with cognitive models of depression. Beck’s model (Beck, 2005; Beck and Haigh, 2014) posits that greater attention or memory for negative events contributes to the development, maintenance, and recurrence of depression by influencing schemas about the self and the world. A causative link between cognitive bias and mood is suggested by the finding that methods that modulate attention bias also affect anxiety and depressive symptoms (Hallion and Ruscio, 2011). Experimentally, cognitive bias modification has been shown to be able to both decrease depressive symptoms (by reducing negative bias) and increase depressive symptoms (by increasing negative bias), indicating that bias in attention may contribute directly to depressive symptoms rather than being a consequence of depressed mood.

An important component of the cognitive model of depression is that cognitive bias is a trait marker of depression vulnerability and should remain in euthymic individuals with remitted depression (Sears et al., 2011). Mood congruent cognitive biases have been reliably found in currently depressed individuals and are associated with the severity of depressive symptoms and specific for depression-related information (Gaddy and Ingram, 2014). Cognitive biases in euthymic, remitted depressed individuals may indicate a trait cognitive vulnerability for depression (Joormann and Gotlib, 2007; Sears et al., 2011) that contributes to a high risk of recurrence (Kessler et al., 2003, Solomon et al., 1997; Keller and Berndt, 2002).

Altered activity in fronto-limbic circuits has been associated with cognitive bias, negative mood, and rumination in currently depressed individuals. De Raedt and Koster (2010) posit a
model of depression vulnerability in which decreased dorsal prefrontal activity and increased stress-related amygdala activity contributes to negative mood and risk for future depressive episodes (Raedt and Koster, 2010). This model is supported by studies demonstrating that both currently depressed (Arnone et al., 2012), and at risk populations (Zhong et al., 2011) show greater amygdala activity to negative emotional stimuli than never depressed healthy controls. Attentional bias for negative information in remitted depressed individuals may be related to greater amygdala responses and enhanced attention to negative stimuli (Sears et al., 2011). Whether attentional bias in individuals with remitted depression is associated with parallel alterations in neural activity to emotional stimuli has not been previously examined. Brain activity or functional connectivity differences that remain in remission may represent a neurobiological basis for continued cognitive biases and vulnerability to depression.

To assess attentional bias in euthymic individuals with remitted depression we examined performance and functional brain activity in participants with and without a history of depression during an emotion dot probe (EDP) task. We used fMRI during the EDP task and a resting scan before the task to examine task-related brain activity and functional connectivity differences between participants with and without a history of MDD. We hypothesized that participants with a history of MDD would show attentional bias for negative images that would not be seen in participants without a history of MDD, and that attentional bias would be associated with greater amygdala activity for negative images and altered amygdala functional connectivity.
Methods

Participants

Thirty three right handed postmenopausal (Past MDD: n =12; No MDD: n = 21) women between the ages of 45-75 were included in this study. None of the participants were taking ovarian hormones and were at least one year without such treatment. Participants were recruited for a larger study examining the effects of estradiol replacement on stress responding in postmenopausal women with and without a history of depression, thus all participants in this study were postmenopausal women. No participants received estradiol treatment prior to or during participation in this study. This study was approved by the Vanderbilt University Institutional Review Board and informed consent was obtained from all participants.

Cognitive Screening:

All participants were cognitively assessed using: the Wechsler Abbreviated Scale of Intelligence (WASI) (Wechsler, 1996), the Mini Mental State Exam (MMSE) (Folstein et al., 1975), Brief Cognitive Rating Scale (Reisberg and Ferris, 1988), and the Mattis Dementia Rating Scale (Schmidt et al., 2005) to establish a Global Deterioration Scale score (GDS) (Reisberg et al., 1988). Participants were required to have a GDS score of 1-2 and a MMSE score of greater than 26. No participant scored below 123 on the Mattis scale or below 90 on the WASI; participants were of average or above intelligence with no evidence of dementia or mild cognitive impairment.

MDD History Screening

Participants were screened for current and past depression, mania and dysthymia using the partial Structured Clinical Interview for DSM-IV-TR (SCID) (Spitzer et al., 1992). No
participants met criteria for a history of premenstrual dysphoric disorder on the Composite International Diagnostic Interview for premenstrual dysphoric disorder (CICI-PMDD).

Criteria for never depressed participants were: no current or past episodes that met SCID criteria for MDD, dysthymia, or mania, a current score less than 7 on the Beck Depression Inventory (BDI), and less than 15 on Beck Anxiety Inventory (BAI) (Beck et al., 1961).

Criteria for prior history of MDD were: at least one episode, in the last ten years that met criteria for MDD on the SCID, no MDD episodes in the last year, current BDI score less than 7, and current BAI less than.

**Emotion Dot Probe (EDP) Task**

The EDP task is a spatial attention task that measures attention facilitation (Kimonis et al., 2006). The EDP task used in this study was a picture variant using images from the International Affective Pictures System (Lang et al., 1999) and included neutral, positive, and negative images. Trials of the EDP consisted of a fixation cross presented in the middle of the screen, followed by a brief presentation (500 ms) of a picture pair with one image each on the right and left of the screen. After the picture presentation, a target (asterisk) appeared either on the right or left of the screen (replacing one of the images) and the participant was instructed to indicate by finger button press the side of the screen on which the target appeared as quickly as possible (available response duration was 1750ms). The time between the target’s appearance and the subject’s response was used for the calculations of reaction time; with incorrect trials excluded.

The EDP was adapted for MRI and run as an event related design with three trial types relevant to the presented data: “Neutral” trials: neutral-neutral pair; “Negative” trials: neutral-
negative pair; and “Positive” trials: neutral-positive image. There were no trials in which negative and positive images were presented together and the presentation order of trial types was randomized. In 2/3 of “Negative” or “Positive” trials the target replaced the emotionally valenced image. These trials were used for the measurement of attention facilitation. Attention facilitation for each emotional trial type was defined as the difference in reaction time between “Negative” or “Positive” trials and “Neutral” trials. A shorter reaction time for “Negative” or “Positive” trials indicated attention facilitation for negative or positive images. In 1/3 of “Negative” and “Positive” trials the target replaced the neutral image. These trials were used for the measurement of attentional preference (in other literature referred to as attentional bias). Attentional preferences was measured as the difference in reaction time for trials in which the target replaced the neutral image and trials in which the target replaced the emotionally valenced image. Only correct trials were included in performance measure calculations. ANOVAs were run for group differences in attention facilitation and preference for “Negative” and “Positive” trials. Independent t-tests were run for group differences in attention facilitation for “Negative” and “Positive” trials.

**Self-rated Measures**

Before the EDP, participants completed self-rated measures including: the State – Trait Anxiety Inventory (STAI) (Spielberger, 2010), the Stress Arousal Checklist (SACL) (King et al., 1983), and the Profile of Mood States (POMS) (McNair et al., 1989). Following the EDP, participants completed a second SACL and POMS, and a visual analogue scale (VAS) for task perception including: the extent of their personal involvement, how stressful, new, uncontrollable, and unpredictable the task was, and whether they anticipated negative consequences. These measures have been previously used to assess the stressfulness of
laboratory tasks and procedures (Kirschbaum et al., 1999; Kudielka et al., 2004). Independent t-tests were run for group differences in STAI, VAS, and for difference score on the SACL and POMS (pre to post EDP).

**Imaging Parameters**

Participants were scanned on a Philips 3.0 Tesla Achieva scanner, with an 8 channel head coil. All participants received the following MR sequences:

1) Sagittal T1-weighted 3D Turbo Field Echo Sensitivity Encoding (TFE SENSE) sequence perpendicular to the anterior commissure (AC) -posterior commissure (PC) line, repetition time (TR) of 9.9 ms, echo time (TE) of 4.6 ms, a flip angle of 8°, number signal averages (NSA) 1.0, a field of view (FOV) of 256 mm, a 256 × 256 matrix, and 1.0 mm slice thickness with no gap for 140 contiguous slices.

2) T2- weighted Gradient and Spin Echo (GRASE) sequence parallel to the AC-PC line, TR 2470 ms, TE 80 ms, NSA 3.0, FOV of 230 mm, 0.7 mm slice thickness with 5.0 mm gap for 28 slices.

3) Echoplanar Blood Oxygenation Level Dependent (EpiBOLD) functional resting-state scan with transverse orientation, TR 1500 ms, TE 35 ms, flip angle 90°, 1 NSA for FOV 240 mm, 80X80 matrix, and 5.0 mm slice thickness with no gap, for 24 slices.

4) EpiBOLD functional sequence during the EDP with transverse orientation, TR 2500 ms, TE 35 ms, flip angle 90°, 1 NSA for, FOV 240, 240X128 matrix, and 4.0 mm slice thickness with no gap, with ascending interleaved acquisition, for 35 contiguous slices.
**Imaging Analysis**

fMRI data was processed using Statistical Parametric Mapping (SPM8) (Wellcome Department of Cognitive Neurology, 2008). Preprocessing included: realignment of the functional runs and correction for bulk-head motion, coregistration of functional and anatomical images for each participant, segmentation of the anatomical image, normalization of the anatomical and functional images to the standard MNI template, and spatial smoothing with a Gaussian filter (8 mm at full width, half maximum).

At first level analysis, T-maps were created from linear contrasts for the task conditions “Negative – Neutral” and “Positive – Neutral”; these T-maps were used in the whole-brain (masked for gray matter) second level random effects analysis of participant group effects with two-sample t-tests. The preprocessed functional images had a voxel size of 2X2X2 mm and cluster threshold correction was used to control for multiple comparisons (from voxel wise $p = 0.005$ to corrected $p < 0.001$ with $k = 58$ (corrected voxel-wise $p = 0.000001$) from alpha simulation in REST (Song et al., 2011) toolbox for SPM. Average percent signal change (task condition – fixation time between trials) was calculated for each significant cluster from the second level group comparison using MARSBAR (Brett et al., 2002).

**Functional Connectivity Analysis**

Each participant completed a resting scan (eyes open with fixation cross) before the EDP task scan. Images from the resting scan were preprocessed in SPM8 including: realignment of the functional runs and correction for bulk-head motion, coregistration of functional and anatomical images for each participant, segmentation of the anatomical image, and normalization of the anatomical and functional images to the standard MNI template. To evaluate resting state
functional connectivity to amygdala, we entered right and left amygdala (anatomical ROI from the human AAL atlas defined in WFU PickAtlas) as seeds in a resting state functional connectivity analysis performed using the MATLAB-based functional connectivity toolbox CONN (Whitfield-Gabrieli and Nieto-Castanon, 2011). Each subject’s normalized structural and functional images and T1W tissue maps were used as input into CONN. The resulting BOLD time series were band-pass filtered (0.01-0.1Hz) to further reduce noise and increase sensitivity. The output matrices of SPM movement were entered into Conn as first-level covariates; the mean BOLD time series from each seed was entered as a predictor in a multiple regression general linear model at each voxel.

Individual subject functional connectivity maps (in beta-weight units) were entered into a whole-brain (masked for gray matter) second level random effects analysis of participant group effects with two-sample t-tests. Cluster threshold correction was used to control for multiple comparisons (from voxel wise $p = 0.005$) to corrected $p < 0.001$ with $k = 58$ (corrected voxel-wise $p = 0.000001$) (from alpha simulation in REST (Song et al., 2011) toolbox for SPM).

Results

Participants

Participants in this study were 33 postmenopausal women. Twenty one women had no history of depression (MDD Hx-), and 12 had a past history of depression (MDD Hx+). Depression history was assessed using the SCID: time since first episode ($M = 17.81$ years, $SD = 10.85$), number of episodes ($M = 2.1$, $SD = 0.95$), time since last episode ($M = 4.1$ years, $SD = 2.38$). There was no significant difference in age between the participant groups (Table 1). All participants scored within the normal range for age on the DRS, WASI IQ, and MMSE, and no
participants endorsed significant impairments on the BCRS or GDS. There were no significant difference between groups on these measures nor on the NEO, BDI, or BAI (Table 1), additionally all participants scored within the non-clinical range on these measures consistent with a euthymic state. Two of the participants in the MDD HX+ group (and no participants in the MDD Hx-) were currently taking antidepressants during study participation. Participants taking antidepressant medication were required to have been on the same medication and dosage for at least 3 months. The effect of removing the 2 participants taking antidepressant medication from analysis was examined; all results remained significant and the direction of effects was not changed.

Self-rated Measures

There were no differences between participant groups on any of the subjective measures (Table 2). In an analysis including all participants, SACL arousal significantly decreased after the EDP compared to before the EDP \((t(35) = 4.65, p < 0.001)\); there was no significant change in SACL stress \((t(35) = -0.88, p = 0.38)\) or POMS total mood disturbance \((p = 0.13)\). There was no significant difference between groups in the change in SACL arousal \((t(34) = 0.52, p = 0.61)\), SACL stress \((t(34) = 0.38, p = 0.71)\), or POMS total mood disturbance \((t(34) = 0.80, p = 0.43)\) pre and post EDP. The EDP did not increase subjective stress or negative mood, and there was no difference between the MDD Hx+ and MDD Hx- groups in these self-rated measures.

Emotion Dot Probe Performance

There was a difference between groups in attention facilitation for negative images (reaction time “Neutral” trial – reaction time “Negative” trials) \((F(1) = 5.40, p = 0.03)\); the MDD HX+ group had similar reaction times for “Negative” trials compared to “Neutral” trials \((M = -\)
.30 ms, $SD = 16.08$), while the MDD Hx- group had faster reaction times during “Neutral” trials compared to “Negative” trials ($M = 18.71$ ms, $SD = 13.65$) (Figure 1). Thus the MDD Hx- group showed attention facilitation for neutral images, while the MDD Hx+ group did not.

There was no difference in attention facilitation for positive images compared to neutral images between the groups ($F(1) = 0.58$, $p = 0.45$). Both groups had faster reaction times for “Neutral” trials compared to “Positive” trials: MDD Hx- ($M = 18.70$ ms, $SD = 15.82$) MDD Hx+ ($M = 12.90$, $SD = 17.43$).

There was no difference between groups for attention preference for negative ($F(1) = 0.18$, $p = 0.68$) or positive ($F(1) = 0.00$, $p = 0.97$). Further analysis and discussion will address attention facilitation only. In an analysis of reaction time for each trial type, the MDD HX+ group was slower for all trial types, however there was no significant differences in reaction time between groups for any trial types (negative trials: $t(32) = -0.52$, $p = 0.61$; positive trials; $t(32) = -0.99$, $p = 0.33$; neutral trials; $t(32) = -0.99$, $p = 0.31$).

**Task fMRI**

There was a difference between groups in brain activity during task trials which contained either negative or positive images (corrected $p < 0.001$) (Figure 1). The MDD Hx+ group had significantly greater activity in the left amygdala, right parahippocampal gyrus, and bilateral precuneus during negative image trials (“Negative”- “Neutral”) than the MDD HX- group (Figure 2). During positive image trials (“Positive”- “Neutral”), the MDD Hx+ group had greater activity in the left amygdala and bilateral precentral gyrus than the MDD Hx- group. There was no difference in activity during trials that contained only neutral images.
Left amygdala activity for negative images was positively correlated with attention facilitation for both negative and positive images in an analysis of all participants, with the correlation stronger for negative facilitation (negative facilitation: $r = 0.44$, $p < 0.01$, positive facilitation: $r = 0.34$, $p < 0.05$). However, there was no association between amygdala activity for positive images and attention facilitation for either negative or positive images. Activity in the right parahippocampus and bilateral precuneus was not significantly correlated with negative or positive facilitation.

**Functional Connectivity**

Seeding right or left amygdala in a single t-test for all participants resulted in positive connectivity maps that include bilateral limbic regions (amygdala and hippocampus), temporal pole, inferior and superior temporal regions, basal ganglia, middle cingulum, insula, and inferior frontal regions. A second level independent t-test comparison of participants groups showed greater connectivity in the MDD Hx+ group between left amygdala and right parahippocampus/hippocampus and bilateral precuneus (corrected $p < 0.001$) (Figure 3). In an analysis of all participants, left amygdala resting connectivity to the right parahippocampus/hippocampus was correlated with negative facilitation ($r = 0.36$, $p < 0.05$) and amygdala activity during the EDP task (negative images: $r = 0.47$, $p < 0.01$; positive images: $r = 0.38$, $p < 0.05$; neutral images: $r = 0.47$, $p < 0.01$). Left amygdala connectivity to precuneus did not significantly correlate with amygdala activity or performance during the EDP task. There were no significant differences between groups for right amygdala connectivity.
Discussion

Attention facilitation for negative images differed between the participant groups; participants with a history of MDD showed similar reaction times for negative and neutral trials, while participants without a history of MDD were faster to respond during neutral compared to negative trials. These findings suggest that participants without a history of MDD avoided orienting attention to negative images, while participants with a history of MDD showed attention bias or an inability to suppress preferential orienting to negative images.

The difference in attention for negative images between the participant groups despite euthymic mood supports the hypothesis that cognitive bias is a trait characteristic of depression vulnerability rather than a consequence of negative mood. Beck’s cognitive model and recent studies in individuals with other risk factors for depression (Joormann and Gotlib, 2007; Zhong et al., 2012) suggest that cognitive biases may be present even before MDD onset and contribute to increased risk for mood and anxiety disorders. There were no group differences in attention preferences in this study, however previous studies indicate that attentional bias is modulated by psychosocial stress (Roelofs et al., 2007; Brüne et al., 2013; Sanchez et al., 2013). Stress proximal to EDP performance may reveal greater difference in attention bias between women with and without MDD history. Attentional bias for negative information that remains during depression remission may indicate a susceptibility to the depressogenic effects of negative events and stressors.

In contrast to well accepted findings of bias for positive emotional information in never depressed individuals (Joormann and Gotlib, 2007; Sears et al., 2011; Sanchez et al., 2013; Isaac et al., 2014), this study found that both groups showed attention facilitation away from positive images. This study used shorter presentation durations and more complex visual stimuli than
previous studies that found positivity bias in never depressed participants. It may be that 
attentional avoidance of negative and positive images in never depressed participants represents 
an effect of arousal rather than valence. Both negative and positive images are higher in arousal 
ratings than neutral images in the IAPS. Additionally, because of the short stimuli presentation 
durations, participants may have adopted a strategy of avoiding highly arousing images in an 
attempt to avoid negative images. Electrophysiological studies have shown that voluntarily 
directing attention away from emotional distractors results in decreased amplitudes in signals 
that represents emotional attention (Cesarei et al., 2009; MacNamara and Hajcak, 2009) and 
affects reaction time especially for highly arousing images regardless of valence (Wiens and 
Syrjänen, 2013). Participants in the current study may have avoided directing attention to highly 
arousing images. However, participants with a history of MDD had equivalent reaction times 
during neutral and negative trials suggesting that they were less able to direct attention away 
from the negative images than from positive images.

Participants with a history of MDD also showed greater limbic and visual region activity 
for emotionally valenced images than participants without a history of MDD. In all participants 
left amygdala activity was correlated with attention facilitation for negative images, but not for 
positive images. These results suggest that there is a stronger relation between amygdala activity 
and attention for negative information than positive information. The amygdala has direct 
bidirectional projections to early visual areas and likely enhances attention to emotional 
information through feedback mechanisms to visual regions (Pourtois et al., 2013). Resting 
functional connectivity analyses in the current study indicated stronger connectivity between left 
amygdala and areas important for the integration of visual perception and attention (Cavanna and 
Trimble, 2006) in participants with a history of MDD. Attention networks in individuals with a
history of MDD may be predisposed to attentional gain for negative emotional information through amygdala feedback to visual regions.

The results of this study should be interpreted with consideration to the limitations of the relatively small sample size for the MDD Hx+ group and the unbalanced sample size between the two groups. However the depression history of participants in this study was fairly homogenous with no participants having highly recurrent depressive episodes. Additionally, this study only included postmenopausal women as participants, which limits the interpretation of these results for young women and men. Although sex differences in attention bias in depression have not been previously examined, prior studies suggest sex differences in neural activity for emotional processing that may interact with gonadal hormone levels (Kret and De Gelder, 2012). Future studies should examine whether the association of depression history and cognitive bias differs by sex or hormonal status.

Cognitive models predict that enhanced amygdala sensitivity and attentional processing of negative information increases depression risk by supporting negative cognitive schemas. Greater attention to negative information may contribute to the perception that negative information is more salient or predictive than neutral or positive information, and modulate the depressogenic effects of life events and stressors. The current findings of negative attention facilitation in correlation with amygdala activity and resting connectivity in attention networks provide evidence that differences in activity and functional connectivity in limbic and attentional networks may provide a neurobiological basis for continued cognitive bias in remitted depressed individuals.
Funding Sources

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References


### Table 1: Participant Screening

<table>
<thead>
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<th>MDD Group</th>
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<th>MDD Hx + (n = 12)</th>
<th>p value</th>
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<tr>
<td><strong>Beck Anxiety Inventory</strong></td>
<td>2.68</td>
<td>3.08</td>
<td>0.72</td>
</tr>
</tbody>
</table>
### Table 2: Subjective Measures

<table>
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<tr>
<th></th>
<th>MDD Hx− (n = 21)</th>
<th>MDD Hx+ (n = 12)</th>
<th>p value</th>
</tr>
</thead>
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<tr>
<td>Trait Anxiety</td>
<td>25.23</td>
<td>24.17</td>
<td>0.63</td>
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<tr>
<td>State Anxiety</td>
<td>26.91</td>
<td>28.75</td>
<td>0.43</td>
</tr>
<tr>
<td>Pre SACL - Stress</td>
<td>1.29</td>
<td>1.34</td>
<td>0.45</td>
</tr>
<tr>
<td>Pre SACL - Arousal</td>
<td>10.43</td>
<td>9.25</td>
<td>0.22</td>
</tr>
<tr>
<td>Pre POMS - Total Mood Disturbance</td>
<td>-13.14</td>
<td>-10.50</td>
<td>0.60</td>
</tr>
<tr>
<td>Post SACL - Stress</td>
<td>1.95</td>
<td>1.08</td>
<td>0.47</td>
</tr>
<tr>
<td>Post SACL - Arousal</td>
<td>7.36</td>
<td>6.67</td>
<td>0.60</td>
</tr>
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<td>Post POMS - Total Mood Disturbance</td>
<td>-9.32</td>
<td>-1.75</td>
<td>0.22</td>
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<tr>
<td>VAS - Involved</td>
<td>81.68</td>
<td>83.42</td>
<td>0.81</td>
</tr>
<tr>
<td>VAS - Stressful</td>
<td>19.77</td>
<td>22.42</td>
<td>0.75</td>
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<tr>
<td>VAS - New</td>
<td>75.86</td>
<td>68.58</td>
<td>0.53</td>
</tr>
<tr>
<td>VAS - Uncontrollable</td>
<td>26.41</td>
<td>23.92</td>
<td>0.82</td>
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<td>VAS - Unpredictable</td>
<td>52.00</td>
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<td>VAS - Anticipate</td>
<td>12.05</td>
<td>13.17</td>
<td>0.83</td>
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Figure 1- EDP Performance and Brain Activity: A) MDD HX- group (n=21) showed greater reaction time for both negative and positive images than neutral images during the EDP. The MDD Hx+ (n=12) group did not show a reaction time difference between negative and neutral images. The difference in reaction time between neutral and negative images was significantly greater in the MDD Hx- group compared to the MDD Hx+ group (t (34) =2.78, p < 0.01). B) Amygdala activity during the EDP was greater in the MDD HX+ during both negative positive images than the MDD Hx- group (corr p < 0.001). Error bars: +/- 1 SD.
Figure 2- EDP Brain Activity
Negative images - neutral images: MDD HX+ > -MDD HX- (corr p < 0.001). MDD Hx+ group had greater activity in the left amygdala and right hippocampal complex during negative images than the MDD Hx- group.
Figure 3 - Functional Connectivity with Left Amygdala:

Greater resting functional connectivity between left amygdala and right hippocampal complex and precuneus in MDD HX+ compared to MDD HX- (corr p< 0.001).
CHAPTER 4: CONCLUSIONS AND FUTURE DIRECTIONS

Review of Findings

The main findings of this dissertation provide information about the role of estradiol in the response to psychosocial stress, and amygdala activity and connectivity in attentional facilitation in women with remitted MDD. The findings of Chapter 2 demonstrate that estradiol levels in different menstrual phases modulate the neural and mood responses to psychosocial stress such that young women show greater hippocampal deactivation and negative mood to psychosocial stress during low estradiol phases of the menstrual cycle, while women in high estradiol phases appear to be protected against these effects of stress. Examining cognitive bias during MDD remission in Chapter 3 indicated that postmenopausal women with remitted MDD show enhanced amygdala activity for all emotional image types and attentional facilitation for negative images despite being euthymic. In both remitted and never depressed women amygdala activity for negative images was directly correlated with attention facilitation for negative and positive images. Resting functional connectivity analysis using the amygdala as a seed region demonstrated greater functional connectivity between the left amygdala and right hippocampal complex and visual regions in women with remitted MDD. Additionally, amygdala-hippocampal connectivity was directly correlated with both amygdala activity for all image types and attention facilitation for negative images. Data presented in Appendix A and discussed below indicate that amygdala-hippocampal functional connectivity was reduced following estradiol treatment only in women with remitted MDD and that estradiol treatment had interactive effects with MDD history on mood response to psychosocial stress such that women with remitted MDD benefitted from estradiol treatment while women with no history of MDD were negatively impacted.
Discussion

The core symptoms of MDD represent dysfunction in systems that are necessary for maintaining euthymic mood and regulating endocrine and emotional responses to negative information and stress. One conceptualization of these systems is the dorsal and ventral divisions proposed by Phillips and others (Mayberg, 1997; Phillips et al., 2003, 2008; Price and Drevets, 2012). The ventral system is comprised of brain regions including the amygdala that are involved in early and automatic information processing, which allows for the evaluation of emotional information and quick activation of autonomic and endocrine responses. The dorsal division includes the hippocampus and frontal regions that integrate current information with associations from memory and components of cognitive control (goal maintenance, decision making). These processes serve to place current information within the context of past experiences and future plans and regulate ventral activity. Gold’s model of stress response incorporates the dorsal and ventral divide and demonstrates the importance of maintaining a healthy balance between the systems for adaptive stress responding (Gold, 2015).

The hypothesis that dysregulated stress responding is integral to major depression is supported by consistent findings of endocrine abnormalities in MDD (Gold et al., 1986; Young et al., 1994, 2000) and the commonality of stressful life events as depression antecedents (Caspi et al., 2003; Kendler et al., 1999, 2000). Integrated brain systems for stress responding and emotional cognition provide a mechanism whereby enhanced ventral activity may result in altered cognitive schemas. Unregulated ventral activity results in suppressed function (Conrad et al., 1996; deQuervain et al., 1998; Diamond et al., 1999) and altered structural integrity (Magariños et al., 1996; Watanabe et al., 1992; Woolley et al., 1990) in dorsal regions and may thus perpetuate long-term enhanced emotional reactivity. Such an altered system may become
predisposed to ventral system processes and establish cognitive bias for negative emotional information, especially when efficient use of cognitive resources is necessary as during psychosocial stress.

During normal stress responding dorsal system activity is reduced and ventral activity is released from dorsal inhibition (Arnsten, 2009; Gold, 2015; Simpson et al., 2001). This shift in brain activity initiates autonomic and endocrine responses to the stressor and results in dysphoric mood (Gold, 2015; Phelps and LeDoux, 2005). The brain activity patterns in the presently reported study (Chapter 2) confirm that a similar shift in activity is seen during the stress condition of the MIST; activity is reduced in dorsal regions of the frontal and parietal cortex as well as hippocampus. These findings also replicate those of the task developers (Pruessner et al., 2008), and confirm that a similar activation pattern is seen in young women as mixed-sex studies.

The findings reported in this dissertation support a role for estrogen in maintaining dorsal function during psychosocial stress in young women. Around ovulation, when estradiol levels are high, hippocampal activity was greater during psychosocial stress than in the early follicular phase when estradiol levels are low. These results accord with estrogen’s known effects on hippocampal function and support of hippocampally-mediated cognition. Reduced hippocampal activity during stress in the low estradiol phase may suggests that normal fluctuations in ovarian hormones across the menstrual cycle establish states when the dorsal system exerts less control over ventral activity. During these phases women may be more susceptible to the depressogenic effects of stressful events. Indeed, in the presented study, negative mood or “distress” was greater following the MIST in women during the low estradiol phase and was related to both low estradiol and hippocampal deactivation during the stress condition. Similar to Phillips model,
Pruessner and colleagues posit that hippocampal activity towards evaluation of threat stimuli and automatic regulation of ventral activity is a default and ongoing function (Dedovic et al., 2009; Pruessner et al., 2008). Deactivation of the hippocampus releases ventral activity during the stress response and allows for activation of endocrine and autonomic responses (Dedovic et al., 2009; Jacobson and Sapolsky, 1991; Sapolsky et al., 1984). The hippocampus may act as a gatekeeper for stress responding such that ventral activity must exceed a threshold to reduce hippocampal activity and activate subsequent stress response pathways.

In healthy women the effects of estrogen on hippocampal regulation of stress responding may be subtle and acute, however for women with additional vulnerabilities or preexisting ventral-dorsal dysregulation, loss of estrogen support of dorsal function may contribute to the risk for depression following stressful events. A recent study examining the effect of emergency contraception following sexual assault found that six months after the assault women who received contraception that contained estradiol had less post-traumatic stress symptoms than women who received either contraception containing both estradiol and progesterone or no contraception (Ferree et al., 2012). These findings support the hypothesis that estradiol levels proximal to traumatic or stressful events may impact long term mood and memory for the event. The presented findings similarly demonstrate that high estradiol reduces the negative mood response following stressful events, likely through supporting hippocampal function and restraining ventral system involvement.

The study presented in Chapter 2 did not include women with current or past mood disorder. However preliminary data from a recent study (Appendix A) indicates that stress responsivity and estrogen effects may differ by depression history. Postmenopausal women with a past history of MDD had more negative mood or “distress” following the MIST than women
with no history of MDD (Appendix A). However, 3 months of estradiol treatment appears to reverse the effect of MDD history on mood response, such that estradiol treatment combined with a history of MDD was associated with a less negative mood response to the MIST than estradiol treatment in women without past MDD. Interestingly, in women with no history of MDD estradiol treatment was associated with a more negative mood response to the MIST. While this is the opposite effect as seen in young women (Chapter 2), this result agrees with previous findings in older women. Newhouse and colleagues previously found that estrogen treatment was associated with higher negative mood scores on the POMS following the Trier stress task in healthy postmenopausal women (Newhouse et al., 2008). Although the Trier task shares stressful components with the MIST (performance and social evaluative threat), it is not conducted in the MRI environment suggesting that estrogen has a general effect on the psychosocial stress response that is not specific to the type of stressor or the imaging environment.

The study presented in Chapter 2 and previous work in young women (Andreano and Cahill, 2010; Davydov et al., 2005; Goldstein et al., 2005) demonstrate that estrogen has beneficial effects on emotional processing and mood, however there may be a shift in these effects at menopause or with age such that estrogen has no or negative effect on mood following stress. Enhanced negative emotional response to psychosocial stress in euthymic women with a history of MDD may indicate ongoing vulnerability to depressive symptoms even during remission. However, depression history appears to alter the effects of estrogen, such that women who are vulnerable to depression may remain sensitive to the beneficial effect of estrogen following menopause.
For many MDD is a recurrent disorder. Cognitive models of MDD point to high recurrence rates as an indication that cognitive alterations remain during remission and confer continued vulnerability for depressive episodes (Beck, 2005; Hasselbalch et al., 2011). Bias in allocating cognitive resources such as attention and memory towards negative information may result in negative information appearing more salient than neutral or positive information thus reinforcing negative schemas. The findings in Chapter 3 demonstrate that euthymic postmenopausal women with remitted MDD show both greater amygdala activity and attention to negative information than women with no history of MDD.

Women with no MDD history showed attentional facilitation for neutral images in contrast to previous findings in free viewing tasks that healthy individuals generally show attention bias for positive information (Cotton et al., 2015; Isaac et al., 2014; Joormann and Gotlib, 2007; Sanchez et al., 2013; Sears et al., 2011). Because of the limited image presentation duration, the EDP task simulates a situation in which processing and evaluation of emotional information must occur rapidly. Under these conditions women with no history of MDD appear to avoid attentional allocation to highly arousing information, thereby avoiding attending to all emotionally valenced images compared to neutral images.

Performance on the EDP task suggests that women with past MDD show similar avoidance of positive images as women without such history, attend more to negative images but show no difference in attention between negative and neutral images. It may be that women with a history of MDD are not able to avoid attending to negative information, or alternatively, that they perceive neutral images as negative (as has been found in MDD (Gollan et al., 2008; Leppänen et al., 2004) ) and thus attend equivocally to negative and neutral images. Greater amygdala activity for all image types during the EDP in women with MDD history than without
such history suggests that automatic ventral system processing of emotional information differs between these groups even during remission and euthymia. In addition, amygdala activity was positively correlated with attentional facilitation on the EDP indicating that altered ventral-dorsal function and interactions has consequences for cognitive processes and bias that remains despite remission.

The study presented in Chapter 3 found that amygdala-hippocampal functional connectivity was greater in women with past MDD than in women with no history of MDD and was correlated with attentional bias for negative images. The functional significance of increased amygdala-hippocampal resting connectivity is unclear; better correlated amygdala and hippocampal activity in women with past MDD may represent greater amygdala involvement in hippocampal activity. If this is the case one may expect greater connectivity to be related to better memory for negative information, which was not evident in the preliminary data (Appendix A). Alternatively, greater amygdala-hippocampal resting connectivity may indicate compensatory hippocampal control of enhanced amygdala activity in MDD. Amygdala activity during emotional images and amygdala connectivity to attention and visual processing areas were greater in women with MDD history (Chapter 3) suggesting that attentional bias for negative images may be related to enhanced amygdala activity and ventral-driven gain in attention and visual processes. The women in the current study were euthymic and remitted from MDD but still showed greater amygdala activity for emotional images and greater amygdala-hippocampal functional connectivity than women with no history of MDD; remission from MDD may thus be accompanied by increased hippocampal regulation of amygdala activity. This hypothesis accords with previous studies which have shown that amygdala-hippocampal connectivity in healthy individuals is inversely correlated with cortisol response during
psychosocial stress (Vaisvaser et al., 2013) and with faster recovery from experimental HPA axis
disruption (Kiem et al., 2013). Although greater amygdala-hippocampal functional connectivity
may be associated with MDD remission, tasks such as the EDP may drive amygdala activity
such that hippocampal regulation is overcome as indicated by greater amygdala activity in
women with remitted MDD and related attentional bias.

Preliminary results presented in Appendix A demonstrate that 3 months of estradiol
treatment reduced amygdala-hippocampal functional connectivity specifically in women with
MDD history such that there was no significant difference in connectivity between women with
and without a history of MDD following treatment. Whether the effect of estrogen on functional
connectivity is accompanied by changes in cognitive bias was not investigated in this
dissertation. However, the presented positive effect of estrogen on mood response to
psychosocial stress in women with a history of MDD in combination with reduced amygdala-
hippocampal connectivity may indicate that estrogen normalizes ventral activity and reduces
negative mood.

MDD vulnerability appears to involve aberrant interactions between ventral affective
processing pathways and dorsal regulatory and cognitive association pathways. Enhanced ventral
activity or reduced dorsal regulation may result in altered cognitive processing of emotional
information that biases attention and memory for negative information. The findings of the
studies included in this dissertation suggest a role for estrogen in modulating emotional
processing networks with effects on mood following psychosocial stress and cognitive
processing of emotional information and support the integration of neural system dysregulation
and cognitive models of MDD in women.
Ovarian hormones have long been proposed to have a role in the sex difference in MDD, however the mechanisms through which ovarian hormones affect depression vulnerability have been unclear. The study presented in Appendix A suggests that estrogen enhances dorsal system function and differentially modulates mood responses to psychosocial stress in postmenopausal women with and without a history of MDD. Similarly, Chapter 2 demonstrates that in healthy young women, estrogen fluctuations across the menstrual cycle affect the reduction in dorsal activity that characterizes the central stress response. The effect of low estrogen on the mood response to stress may be related to the negative mood commonly experienced prior to menstruation and create periods of increased risk for depressive symptoms in vulnerable women.

Enhanced ventral activity is a common component in stress response dysregulation (Gold, 2015) and dorsal-ventral dysregulation models of MDD (Phillips et al., 2003; Price and Drevets, 2012) and has a central role in cognitive models as a driver of aberrant allocation of attention and memory for negative information (Beck, 2005; Pourtois et al., 2013). The relation between amygdala activity and attention bias for negative images found in Chapter 3 leads to the hypothesis that enhanced automatic ventral processing of negative emotional information may be a risk for MDD or recurrence. The findings presented in Chapter 3 indicate that amygdala-hippocampal functional integration is stronger in women with past depressive episodes. However, it is unclear whether this enhanced functional connectivity represents aberrant amygdala involvement in hippocampal function that remains in remission and may drive cognitive bias towards negative information, or whether it indicates a compensatory mechanism for restoring dorsal regulation of ventral activity the may be related to successful remission.
Limitations and Future Directions

The results of this dissertation should be interpreted with consideration of a number of limitations. While all participants scored in the non-clinical range for mood symptoms at the study visit, the sample sizes precluded examination of factors that may affect the findings presented in Chapter 3 and the Appendix A, such as current depressive symptoms, number of depressive episodes, length of illness, and treatment history. However, participants in Chapter 3 with past MDD represented a fairly homogenous MDD history with no highly recurrent or treatment resistant depressive episodes. None of the participants reported history of anxiety disorder and there were no group differences in trait or state anxiety, however because of the high comorbidity of depression and anxiety disorders further characterization of anxiety symptoms and history may be useful in determining the separate effects of depression and anxiety. The results presented in the Appendix A should be interpreted as preliminary findings as the group sizes were unbalanced and it is therefore difficult to ascertain the significance of findings concerning all participant groups.

Future work should examine the interactions between estrogen and mood disorder in young women and include participants with current illness to determine whether the beneficial effect of estrogen on mood response to psychosocial stress seen in older women with MDD history extends of young women and active mood disorder. The investigation of young women presented in this dissertation was designed to minimize the effects of progesterone, however pharmacological manipulation of ovarian hormones in young women may be useful in further characterizing the separate effects of estrogen and progesterone.

This dissertation did not investigate the roles of neurotransmitter systems in stress responding or emotional processing and thus cannot provide information about these potential
mechanisms of estrogen’s effects. However, the findings support hippocampal function as a promising target of further investigation. Examining neurotransmitter systems that impact hippocampal function (such as the cholinergic system) may be of particular interest in future studies of estrogen’s effect on mood, stress response, and attention in relation to MDD. Similarly the effects of estrogen on cognitive bias should be further examined; the present study (Chapter 3 and Appendix A) did not investigate attentional bias following estradiol treatment and found no evidence of memory bias, but did find an estradiol effect on amygdala-hippocampal functional connectivity which suggest that estrogen may alter the functional relationship between brain regions involved in cognitive processing of emotional information. Further, the addition of eye tracking during the EDP task may aide in distinguishing between attentional facilitation and avoidance.

Conclusions

The findings presented in this dissertation suggest that fluctuations of estrogen across the menstrual cycle and at other reproductive events may contribute to negative mood alterations through effects on brain systems integral to emotional evaluation and response with potential cognitive consequences. Estrogen may thus protect against the depressogenic effects of stressful life events in women. For women with current or past MDD, estrogen supplementation in young women or replacement in older women may be a successful strategy for preventing recurrence following stressful events. The association between negative mood response to psychosocial stress and ovarian hormone fluctuation in women supports stronger consideration of MDD in women as a reproductive phase-associated mood disorder and of hormone therapy as an adjunctive treatment similar to the standard ovarian hormone therapy treatment of premenstrual dysphoric disorder, post-partum depression, and polycystic ovarian syndrome. The findings in
this dissertation indicate that cognitive bias for negative information remains during remission in women and that estrogen affects brain networks involved in the cognitive processing of such emotional information.

This work should be expanded to investigate the effects of menstrual cycle phase and estradiol level on psychosocial stress response in women who are currently experiencing depressive episodes or other mood and anxiety disorders such as post-traumatic stress disorder. Additionally, whether estradiol treatment or menstrual phase affects attention facilitation for negative images in women with current or past mood disorder should be examined. Determining the efficacy of cognitive bias modification in reducing depressive symptoms and perhaps reducing recurrence risk requires further investigation. However, ovarian hormone therapy may be a potential augmentative strategy in women by supporting new learning and modulate ventral-dorsal interactions integral to successful cognitive training approaches.
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APPENDIX A: PRELIMINARY DATA
Methods

Two groups of postmenopausal women were recruited, with and without a personal history of MDD. Of each MDD history group a portion of women received three months of open-label estradiol (E2) and a portion received no treatment. Mood response to psychosocial stress was assessed during a laboratory psychosocial stress task. We hypothesized that E2 treatment would be associated with less negative mood response to the stress task only in women with a previous history of MDD. Recognition memory for emotionally-valenced words was tested after the stress task. We hypothesized that E2 treatment would decrease recognition memory for negative words following the stress task. Women who received E2 treatment completed both a baseline visit before treatment and a study day visit following treatment. The effects of E2 treatment on amygdala-hippocampal functional connectivity was analyzed using resting state scans from the baseline and study day visits. We hypothesized that E2 treatment would reduce amygdala-hippocampal connectivity specifically in women with MDD history (Figure 1).

Participants

Fifty three postmenopausal women between the ages of 45-75 were enrolled in this study. None of the participants were taking ovarian hormones and were at least one year without such treatment. Participants were recruited through mass mailing letters, fliers at clinical offices, Research Match, and email announcements to Vanderbilt faculty and staff. This study was approved by the Vanderbilt University Institutional Review Board and informed consent was obtained from all participants.
Participants were assigned to open-label oral E2 for 3 months (1mg per day for the first month, and 2mg per day for the second and third months) or to no treatment. Generally, women who were willing and medically able to take estradiol were placed in the E2+ group, while women who were not willing or had medical contraindications to estradiol were placed in the E2- group. One participant’s data was excluded from analysis as an outlier in MRI signal change, so that a total of 52 participants’ data are included in the data reported here. There were four participant groups: 1) women who received estradiol treatment with no history of MDD (E2+/MDD Hx-, n = 17; 2) women who received estradiol treatment with a history of MDD (E2+/MDD Hx+, n = 10); 3) women who did not receive estradiol treatment with no history of MDD (E2-/MDD Hx-, n = 20); 4) women who did not receive estradiol treatment with a history of MDD (E2-/MDD Hx+, n = 5).

Cognitive Screening

All participants were cognitively assessed using: the Wechsler Abbreviated Scale of Intelligence (WASI) (Wechsler, 1996), the Mini Mental State Exam (MMSE) (Folstein et al., 1975), Brief Cognitive Rating Scale (Reisberg and Ferris, 1988), and the Mattis Dementia Rating Scale (Schmidt et al., 2005) to establish a Global Deterioration Scale score (GDS) (Reisberg et al., 1988). Participants were required to have a GDS score of 1-2 and a MMSE score of greater than 26. No participant scored below 123 on the Mattis scale or below 90 on the WASI; participants were of average or above intelligence with no evidence of dementia or mild cognitive impairment.
MDD History Screening

Participants were screened for current and past depression, mania and dysthymia using the partial Structured Clinical Interview for DSM-IV-TR (SCID) (Spitzer et al., 1992). No participants met criteria for a history of premenstrual dysphoric disorder on the Composite International Diagnostic Interview for premenstrual dysphoric disorder (CICI-PMDD).

Criteria for never depressed participants were: no current or past episodes that met SCID criteria for MDD, dysthymia, or mania, a current score less than 7 on the Beck Depression Inventory (BDI), and a score less than 15 on Beck Anxiety Inventory (BAI) (Beck et al., 1961).

Criteria for prior history of MDD were: at least one episode, in the last ten years that met criteria for MDD on the SCID, no MDD episodes in the last year, current BDI score less than 7, and current BAI less than 15 (Santesso et al., 2008).

Participants in the estradiol treatment group completed an additional study day prior to estradiol treatment, the results of that baseline day are presented in Chapter 3, however resting functional connectivity comparison between the baseline and study day for the estradiol treatment group will be presented below.

Stress Task

We employed the Montreal Imaging Stress Task (MIST) for psychosocial stress induction (Pruessner et al., 2008). The MIST produces moderate psychosocial stress through a combination of motivated performance and social-evaluative threat. We presented the MIST as an arithmetic task in the MRI scanner, and instructed participants that they should achieve an 80-90% correct performance for their data to be usable in the context of this experiment. Unbeknownst to the subjects, the MIST contains an algorithm producing script that
automatically adjusts the difficulty of the math tasks to the aptitude of the participant, this way maintaining a low performance rate (between 40-50%) by changing either the problem difficulty or the allotted time. A “control” condition, in which the participants solve arithmetic problems with no time limit and no performance limit, serves as a contrast to control for brain activity changes induced by arithmetic task demands (visual stimuli, motor response, and mental arithmetic). Social evaluative threat comes from scripted experimenter interaction; the experimenters enter the MRI room between runs and inform the participants that they are not doing well enough and that they need to improve their performance for the experiment to be successful.

In this study, participants completed three runs of the MIST. After the first run, experimenter 1 entered the scanner room and provided the scripted feedback and asked the participant to complete a second run of the task. After the second run the experimenter 1 entered the scanner room and told the participant that the experimenter 2 (“doctor”) would like to speak with them about their performance, at which point the experimenter 2 entered the scanner room and provided the scripted feedback and asked the participant to complete a third run of the task. This protocol was designed to maintain both performance and social evaluative threat throughout the MIST and to prevent participants from habituating to the performance challenge or giving up on completing the task. Specifically the interaction of the participants and the experimenters was structured to generate psychosocial stress. To habituate the participants to the scanning environment and decrease scanner-related stress at the study day, participants entered an MRI simulator during the screening visit; in the MRI simulator, participants watched a nature video while being exposed to the MRI environment (simulated MRI sounds, head coil, and being placed in the MRI bore) and the stimulus presentation system.
The MIST was presented in a block design; each condition was presented twice per 3 runs ("stress": 100 seconds, "control": 50 seconds, "rest": 30 seconds). Participants practiced the MIST task (control trials only) in the MRI simulator, before the MRI session.

**Imaging Parameters**

Participants were scanned on a Philips 3.0 Tesla Achieva scanner, with an 8 channel head coil. All participants received the following MR sequences:

1) Sagittal T1-weighted 3D Turbo Field Echo Sensitivity Encoding (TFE SENSE) sequence perpendicular to the anterior commissure (AC) -posterior commissure (PC) line, repetition time (TR) of 9.9 ms, echo time (TE) of 4.6 ms, a flip angle of 8°, number signal averages (NSA) 1.0, a field of view (FOV) of 256 mm, a 256 × 256 matrix, and 1.0 mm slice thickness with no gap for 140 contiguous slices.

2) T2- weighted Gradient and Spin Echo (GRASE) sequence parallel to the AC-PC line, TR 2470 ms, TE 80 ms, NSA 3.0, FOV of 230 mm, 0.7 mm slice thickness with 5.0 mm gap for 28 slices

3) Echoplanar Blood Oxygenation Level Dependent (EpiBOLD) functional resting-state scan with transverse orientation, TR 1500 ms, TE 35 ms, flip angle 90°, 1 NSA for FOV 240 mm, 80X80 matrix, and 5.0 mm slice thickness with no gap, for 24 slices.

4) Three EpiBOLD functional sequences during the 3 runs of the MIST with transverse orientation, TR 2500 ms, TE 35 ms, flip angle 90°, 1 NSA for, FOV 240, 240X128 matrix, and 4.0 mm slice thickness with no gap, with ascending interleaved acquisition, for 35 contiguous slices (task-related fMRI data will not be presented).
**Functional Connectivity Analysis**

Participants who received estradiol treatment completed resting scans (eyes open with fixation point) before task scans during the MRI session at both the baseline (pre estradiol) and study day (post estradiol). Images from the resting scan were preprocessed in SPM8 including: realignment of the functional runs and correction for bulk-head motion, coregistration of functional and anatomical images for each participant, segmentation of the anatomical image, and normalization of the anatomical and functional images to the standard MNI template.

Each subject’s normalized structural and functional images and T1W tissue maps were used as input into CONN. The resulting BOLD time series were band-pass filtered (0.01-0.1Hz) to further reduce noise and increase sensitivity. The output matrices of SPM movement were entered into Conn as first-level covariates; the mean BOLD time series from each region-of-interest was entered as a predictor in a multiple regression general linear model at each voxel.

To evaluate whether estradiol treatment affected the difference in amygdala-hippocampal functional connectivity between MDD history groups (presented in Chapter 3) a mask created from the significant hippocampal cluster in Chapter 3 was used as an ROI in a 2nd level resting state functional connectivity analysis with the left amygdala as a seed region (anatomical ROI from the human AAL atlas defined in WFU PickAtlas42). Cluster threshold correction was used to control for multiple comparisons within the hippocampal ROI (from voxel wise $p = 0.05$) to corrected $p < 0.001$ with $k = 7$ (corrected voxel-wise $p < 0.001$) (from alpha simulation in REST toolbox for SPM (Song et al., 2011)). Independent t tests were run to compare mean correlations between activity time series in the left amygdala seed and right hippocampal ROI between groups for baseline, study day, and change values between the E2+/ MDD Hx- and E2+/MDD Hx+ groups.
Subjective Measures

Before the MIST, participants completed self-rated measures including: the State – Trait Anxiety Inventory (STAI) (Spielberger, 2010), the Stress Arousal Checklist (SACL) (King et al., 1983), and the Profile of Mood States (POMS) (McNair et al., 1989). Following the EDP, participants completed a second SACL and POMS, and a visual analogue scale (VAS) for task perception including: the extent of their personal involvement, how stressful, new, uncontrollable, and unpredictable the task was, and whether they anticipated negative consequences. These measures have been previously used to assess the stressfulness of laboratory tasks and procedures (Kirschbaum et al., 1999; Kudielka et al., 2004). A 2X2 (E2 treatment X MDD Hx) multivariate ANOVA, with alpha = 0.05 was run for all self-rated measures, and for difference score on the SACL and POMS (pre to post MIST). Change in SACL-Stress score was termed “Distress” for analysis. Independent T test with alpha = 0.05 were run for groups comparisons for measures that had significant effects of the interactions E2 X MDD Hx.

Emotional Words Memory Task

At the study day participants completed an emotional episodic memory task that consisted of an encoding (before the MIST) and recognition phase (after the MIST). During the encoding phase, subjects were presented with 30 words for study (10 neutral, 10 positive, and 10 negative). The study words were taken from the Affective Norms for English Words (ANEW) list (Bradley and Lang, 1999), which provides a set of normative emotional ratings, including valence and arousal, for a large number of words in the English language. Words used were of medium frequency of occurrence, and high concreteness. During the encoding task, subjects
were instructed to read the words out loud, to ensure that they read each word, and to try to remember the words for later testing.

During the recognition phase, subjects were presented with a combination of the 30 words that were presented during the encoding phase, and 30 new words that were not presented during the encoding task. Subjects were instructed to press a button to indicate whether the word was an old word that they had seen previously or a new word they had not seen prior to the fMRI session. The new words were matched on valence, frequency, and concreteness to the studied words. Measures of sensitivity and response bias were obtained from the behavioral performance of this task. Performance on the emotional memory task was calculated by d’, a measure of sensitivity in detecting hits and false alarms, response bias was calculated as C, a measure of bias to respond to a binary choice either towards “yes” (liberal bias) or “no” (conservative bias). Similar protocols have been used to study that effects of cortisol administration and acute laboratory stress on emotional memory encoding and consolidation (Buchanan and Lovallo, 2001; Kuhlmann and Wolf, 2006; Kuhlmann et al., 2005; Roozendaal, 2002; Roozendaal et al., 2008).

Results

Functional Connectivity

A resting functional connectivity analysis for the left amygdala seed with the hippocampal ROI created from the significant cluster in the results of Chapter 3 was completed only for participants who received estradiol treatment and thus had both a baseline pre estradiol treatment resting scan and a study day post estradiol treatment resting scan. Comparison of
functional connectivity at the baseline visit showed greater connectivity between the left amygdala and right hippocampus in the MDD Hx+ group compared to the MDD Hx- group ($t(26) = 4.68, p < 0.01$), confirming the results in Chapter 3 in this subset of participants. There was no significant difference in left amygdala-right hippocampal functional connectivity between MDD Hx+ group and MDD HX-group at the study day following estradiol treatment ($t(26) = -0.26, p = 0.80$). Comparison of change in functional connectivity between left amygdala and right hippocampus (estradiol effect) showed a significant difference between groups ($t(26) = 2.50, p = 0.02$). Paired t test showed that the MDD Hx+ group had a significant decrease in left amygdala right hippocampal function connectivity from baseline to study day ($t(10) = 3.09, p = 0.01$), while the MDD Hx- group had no significant change ($t(16) = -0.50, p = 0.62$).

**Subjective Measures**

In a 2X2 (E2 treatment X MDD Hx) multivariate ANOVA, there was a main effect of E2 treatment for a number of pre MIST measures with the E2+ groups having significantly lower scores: STAI – state, BDI, BAI, Pre POMS-Depression (Table 1). There was an effect of the interaction E2 X MDD Hx for VAS- Unpredictable ($F(1) = 8.45, p = 0.01$) and change in SACL-Stress “Distress” ($F(1) = 8.00, p = 0.01$). In independent T-tests for group comparisons there was a trend ($t(25) = 1.37, p = 0.06$) for greater VAS-Unpredictable score in the E2+/MDD+ group ($M = 85.80, SD = 19.22$) compared to the E2+/MDD Hx- group ($M = 65.12, SD = 30.10$); there were no significant differences in VAS-unpredictable for all other group comparisons. There was a significant difference in “distress” in the group comparisons: 1) The E2+/ MDD Hx- group had greater ($t(25) = 2.80, p = 0.01$) “distress” than the E2+/MDD Hx+ group; 2) The E2+/MDD Hx- group also had greater ($t(35) = 2.15, p = 0.04$) “distress” than the E2-/MDD Hx- group; 3) The E2-/MDD Hx – group had greater ($t(35) = -1.47, p = 0.16$)
“distress” than the E2-/MDD Hx+ group (Table 2). In women who did not receive estradiol, women with MDD history had a more negative mood response to the MIST than women without MDD history. However, in women who received estradiol treatment this was reversed; women with MDD history had a less negative mood response than women without MDD history.

**Emotional Words Memory Task**

In a 2X2 (E2 treatment X MDD Hx) ANOVA of emotional words memory task performance there were no main effects of E2 or MDD history, and no significant interaction between E2 treatment and MDD history (Table 2 and Table 3).
References


Table 1: Subjective Measures – Pre MIST

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<th></th>
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<th></th>
<th></th>
<th></th>
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E2 = Estradiol
STAI = State and Trait Anxiety Inventory
BDI = Beck Depression Inventory
BAI = Beck Anxiety inventory
SACL = Stress and Arousal Checklist
POMS = Profile of Mood States
Table 2: Subjective Measure Changes

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Distress = Stress Arousal Checklist – Stress Change
Arousal Change = Stress Arousal Checklist – Arousal Change
POMS TMD = Profile of Mood States Total Mood Disturbance
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E2 = Estradiol
MDD = Major Depression Disorder
Table 4: Emotion Words Recognition –ANOVA

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E2 = Estradiol  
MDD = Major Depression Disorder
Figure 1: Study Overview
APPENDIX B: OTHER PUBLISHED WORK

Other primary research articles have been published in the following form:


Review articles have been published in the following form:
